

## Nomenclature Committee of the International Union of Biochemistry (NC-IUB)

### Nomenclature of electron-transfer proteins

#### Recommendations 1989

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#### 1. GENERAL INTRODUCTION

The processes of oxidation in living cells are catalyzed by the cooperation of a number of enzyme and coenzymes that transfer reducing equivalents, either hydrogen atoms or electrons, in successive steps from an initial donor to a final acceptor. The enzymes concerned can be named according to the general principles laid down in *Enzyme nomenclature* [1].

At the end of that book a survey, written in 1978, about electron transfer proteins is given. Since that time not only have many new electron transfer proteins been reported, but

also practices in many subfields have changed, so that an update and extension of these rules has become opportune.

Electron transfers are e.g. the oxidation of intermediary metabolites by molecular oxygen in the mitochondria of animal, plant and protist cells, and in the protoplasmic membranes of those protists that lack mitochondria; these often require the successive transfer of hydrogen atoms or electrons, first to  $\text{NAD}^+$ , then from  $\text{NADH}$  to an ubiquinone (Q), next from  $\text{QH}_2$  to ferricytochrome *c* and finally from ferrocyclochrome *c* to oxygen. These reactions are catalyzed, e.g., by an oxidoreductase using  $\text{NAD}^+$  or  $\text{NADP}^+$  as acceptor,  $\text{NADH}:\text{Q}$  oxidoreductase (EC 1.6.5.3)<sup>1</sup>,  $\text{QH}_2$ :cytochrome *c* oxidoreductase (EC 1.10.2.2), and ferrocyclochrome *c*: $\text{O}_2$  oxidoreductase (EC 1.9.3.1), respectively. In some instances,  $\text{NADP}^+$  is used as the first hydrogen acceptor and an additional enzyme,  $\text{NAD(P)}^+$  transhydrogenase (EC 1.6.1.1-2), is required for the initial reduction of  $\text{NAD}^+$ . In other cases, particularly with substrates of higher redox potential, neither  $\text{NAD}^+$  nor  $\text{NADP}^+$  is required and appropriate enzymes catalyze either the direct reduction of Q (for example, succinate dehydrogenase, EC 1.3.99.1) or introduce electrons into the sequence *via* the so-called electron-transfer flavoprotein (see below). Similarly, in the light-driven oxidation of water

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<sup>1</sup> Also called  $\text{NADH}$  dehydrogenase (ubiquinone), formerly given the systematic name  $\text{NADH}:(\text{acceptor})$  oxidoreductase.

that occurs in the chloroplasts of green plants, hydrogen atoms or electrons are transferred successively to plastoquinone (PQ), thence to plastocyanin and finally to  $\text{NADP}^+$  in reactions catalyzed by photosystem II,  $\text{PQH}_2$ :plastocyanin oxidoreductase (EC: see<sup>2</sup>, photosystem I (EC: see<sup>2</sup>), and ferredoxin: $\text{NADP}^+$  reductase (EC 1.18.1.2), respectively. Although the photosystems II and I are not usually classified as enzymes, they could be designated  $\text{H}_2\text{O}$ :PQ oxidoreductase (light-driven) and plastocyanin:ferredoxin oxidoreductase (light-driven), respectively.

Often the metal-containing electron transport proteins are closely associated with, or a part of, an enzyme and in these cases the EC numbers — as far as unambiguously known — are also listed. Many of these enzymes are in fact large protein complexes and consist of a number of subunits; some of these subunits are electron carriers that were discovered before it was recognized that they are indeed subunits of their respective enzymes. For example, ubiquinol/cytochrome-*c* reductase (EC 1.10.2.2) contains a diheme cytochrome *b* (previously considered to be separate cytochromes, i.e. *b*-562 and *b*-566, respectively), cytochrome *c*<sub>1</sub> and an iron-sulfur cluster. These electron carriers catalyze electron transfers within the enzyme, and participate in overall reaction mechanisms which may be quite complex and are still subject to clarification. Although each of these electron-transfer carriers, being a catalytically active protein, satisfies the all-embracing definition of an enzyme, for example, cytochrome *c*<sub>1</sub> in  $\text{QH}_2$ :ferricytochrome *c* oxidoreductase, it is undesirable to give the subunit of an enzyme a separate enzyme name. Moreover, since much is known about the electron-carrying centers of these enzymes, it is more appropriate to classify them on the basis of the chemical structure of the prosthetic group and the manner of their attachment to the protein. Finally, many redox enzymes occur for which the electron transfer function of the protein has not yet been proven, such as in the vanadium proteins; these have nevertheless been included in the present document.

Several groups of electron-transfer proteins are classified below: flavoproteins, proteins containing reducible disulfide groups, cytochromes, iron proteins, copper proteins, molybdenum proteins, nickel proteins, vanadium proteins and quinoproteins.

When the electron transfer protein contains more than one redox-active prosthetic group, this should be indicated as follows: the group reacting with the physiological electron donor should be listed first and the group reacting with the physiological electron acceptor should be listed last. Thus, yeast L-lactate dehydrogenase (cytochrome) (EC 1.1.2.3) would be indicated as a flavohemoprotein. If additional groups are present they should be indicated as illustrated for xanthine oxidase (EC 1.1.3.22): molybdenum-(2[2Fe-2S])-flavoprotein. If the physiological electron donor and acceptor are not known, then all the prosthetic groups should be enclosed in parentheses.

In many cases rather recent examples from the literature have been included, although this might have some disadvantages<sup>3</sup>.

Finally, spectroscopists often use metal proteins in which the natural metal has been replaced by another metal ion (e.g. Fe replaced by Co or Zn). These proteins are briefly discussed in chapter 11.

## 2. FLAVOPROTEINS

Flavoproteins commonly contain one of two prosthetic groups, FMN (e.g. NADH dehydrogenase, EC 1.6.99.1) and FAD. The FMN is non-covalently bound in all known cases. FAD may be non-covalently bound [e.g. in dihydrolipoamide dehydrogenase (NADH), EC 1.8.1.4] or covalently bound by a methylene bridge between the benzene ring of the benzo[g]pteridine-2,4-dione and an amino acid residue, such as cysteine, histidine or tyrosine, in the protein (e.g. succinate dehydrogenase, EC 1.3.99.1), or directly at ring position 6. 8-Hydroxypyrimidino[4,5-*b*]quinoline-2,4-dione functions as prosthetic group in methanogens and in deoxyribo-dipyrimidine photolyase (EC 4.1.99.3).

Apart from a few exceptions where the role of the flavin is not clear, e.g. tartronate-semialdehyde synthase (EC 4.1.1.47), flavoproteins carry out oxidation-reductions, in which one substrate is oxidized and a second is reduced. For all these enzymes each catalytic cycle consists of two distinct processes, the acceptance of redox equivalents from a reducing substrate and the transfer of these equivalents to an oxidized acceptor. Accordingly, the catalysed reactions consist of two separate half-reactions: a reductive half-reaction in which the flavin is reduced and an oxidative half-reaction, in which the reduced flavin is reoxidized.

The nature of the substrate involved in the two separate half-reactions has been used as the basis for a scheme in which five broad classes of flavoenzymes are defined [2]:

a) Transhydrogenase, where two-electron equivalents are transferred, along with the appropriate hydrogen ions, from one organic substrate to another.

b) Dehydrogenase-oxidase, where two-electron equivalents are transferred to the flavin from an organic substrate, where molecular oxygen is the oxidizing substrate, being reduced to  $\text{H}_2\text{O}_2$ .

c) Dehydrogenase-monooxygenase, where the flavin is reduced, generally by a reduced pyridine nucleotide, and where on oxidation with  $\text{O}_2$  in the presence of a co-substrate one atom of oxygen is inserted into the co-substrate, while the other is reduced to  $\text{H}_2\text{O}$ .

d) Dehydrogenase-electron transferase, where the flavin is reduced by 2-electron transfer from a reduced substrate and then reoxidized in sequential single electron transfers to acceptors, such as cytochromes and iron-sulfur proteins. An example is the NADPH-cytochrome *b*<sub>5</sub> reductase (EC 1.6.2.2). This class might be further subdivided to distinguish those enzymes which are functioning in the reverse sense, i.e., those which receive electrons one at a time and then transmit them in a two-electron step in the reduction of a pyridine nucleotide. An example is ferredoxin- $\text{NADP}^+$  reductase (EC 1.18.1.2).

e) Electron transferase, where the flavin is reduced and reoxidized in one-electron steps. There are two examples. The first is the so-called electron-transfer flavoprotein that catalyzes the transfer of electrons from another enzyme, namely butyryl-CoA dehydrogenase (EC 1.3.99.2), acyl-CoA dehydrogenase (EC 1.3.99.3), sarcosine dehydrogenase (EC 1.5.99.1) or dimethylglycine dehydrogenase (EC 1.5.99.2), to the respiratory chain. The second is flavodoxin, a group of flavoproteins of low potential that catalyze electron transfer between two other redox proteins as part of photosynthetic, nitrogen- or sulfate-reducing or hydrogen-evolving systems.

It should be noted that flavoproteins can act in sequence. The most extreme case is found in the pathway for the  $\beta$ -oxidation of fatty acids. A flavoprotein dehydrogenase first oxidizes the saturated fatty acyl CoA. The dehydrogenase the

<sup>2</sup> Not yet listed in *Enzyme nomenclature* [1].

<sup>3</sup> It has to be realized that many systematic names for proteins quite often have to be adjusted because they — later — appear to have a different or additional function.

transfers its electron *via* a second flavoprotein, the electron-transferring flavoprotein, to the membrane-bound iron-sulfur flavoprotein, electron-transferring protein ubiquinone oxidoreductase. This last protein reduces coenzyme Q, thus delivering electrons to the respiratory chain. Similar sequences of reactions are found in the catabolic pathways of several amino acids and in the operation of the mitochondrial one-carbon cycle.

### 3. PROTEINS CONTAINING REDUCIBLE DISULFIDE

Lipoylproteins containing lipoic acid covalently bound by an amide link between its carboxyl group and the 6-amino group of a lysine residue in the protein are involved in the oxidation of both pyruvate and 2-oxoglutarate. The disulfide group of the lipoic acid is both reduced and acylated by pyruvate dehydrogenase (lipoamide) (EC 1.2.4.1) and oxoglutarate dehydrogenase (lipoamide) (EC 1.2.4.2). Thus, lipoylproteins act as both hydrogen and acyl acceptors. They are sub-units of the pyruvate and 2-oxoglutarate dehydrogenase complexes.

The flavoproteins catalyzing the reduction of lipoamide, oxidized glutathione, thioredoxin, and glutaredoxin by reduced nicotinamide-adenine nucleotides (EC 1.8.1.4, 1.6.4.2 and 1.6.4.5, respectively) contain reducible cystine residues that are involved in the electron-transfer reaction; NADPH-mercury(II) reductase (EC 1.16.1.1) also contains a reducible cystine. Thioredoxin, which is required for the reduction of cytidine diphosphate to deoxycytidine diphosphate, is itself an electron-transferring protein with a cystine residue as the electron-transferring center; glutaredoxin is similar. Thioredoxins of higher plants are involved in the regulation of enzymes in the Calvin cycle; they are reduced by ferredoxin, not NAD(P)H, but the enzyme(s) have not been characterized.

## 4. CYTOCHROMES

### 4.1. INTRODUCTION AND DEFINITIONS

Hemeproteins<sup>4</sup> that transfer electrons belong to the family of the cytochromes. The name 'cytochrome' was introduced by Keilin [3] in 1925 to describe a group of intracellular hemeproteins that undergo oxidation-reduction and, upon reduction, exhibit intense absorption bands between 510 and 615 nm. As currently used, the name appears to include all intracellular hemeproteins with the exception of hemoglobin, myoglobin, the peroxidases, catalase, tryptophan 2,3-dioxygenase, heme-thiolate proteins (*P*-450) and the nitrite and sulfite reductases. Consequently, proteins of markedly different function are found in this family.<sup>5</sup>

It has been customary to assign the cytochromes to the groups *a*, *b* and *c* according to the nature and mode of binding of the heme prosthetic group: group *d* was introduced by the IUB Enzyme Commission in 1961. Recommendations for the further classification within the four groups were issued in 1961 [4], 1964 [5], 1972 [6], and 1978 [7], and have helped to develop widely accepted designations for the various

cytochromes. The groups are indicated by lower-case italic letters.

Since the publication of the 1978 report [7], knowledge of the primary and tertiary structure, and of the physical properties, of the cytochromes has continued to accumulate at a substantial rate. It has become apparent that the cytochromes represent such a diverse family of compounds that no simple, complete and self-consistent basis for classification currently exists, although comprehensive suggestions have been made for a system of classification within the *c*-family of proteins from both sequence (pp. 263–279 in [8]) and crystallographic data [9]. Consequently, as was the conclusion in earlier recommendations, 'it is premature to do more than to monitor and, hopefully, to coordinate present practices in classification and nomenclature of cytochromes', to present guidelines for the future reporting of the properties of newly discovered proteins, and to revise aspects of earlier recommendations in the light of more recent knowledge. The present recommendations follow the same lines as the 1978 report [7].

The term 'heme' is usually understood as any tetrapyrrolic chelate of iron [10]. The terms 'ferroheme' and 'ferriheme'<sup>6</sup> still refer to the Fe(II) and Fe(III) oxidation states in heme; however, the Fe(IV) oxidation level of heme iron is found as a catalytic intermediate in some systems. A hemochrome is defined as a low-spin compound of heme in which fifth and sixth coordination places are occupied by strong field ligands regardless of the oxidation state of the iron.<sup>7</sup> Finally, the terms 'hemoprotein' or, preferably, 'hemeprotein' refer to a protein containing a heme as a prosthetic group.

The classical definition of cytochrome is retained: a cytochrome is a hemeprotein whose characteristic mode of action involves transfer of reducing equivalents associated with a reversible change in oxidation state of the prosthetic group. Formally, this redox change involves a single-electron, reversible equilibrium between the Fe(II) and Fe(III) states of the central iron atom.

### 4.2 CYTOCHROME GROUPS

Four major groups of cytochromes are currently recognized:

- Cytochromes *a*. Cytochromes in which the heme prosthetic group is heme *a*, *i.e.* the iron chelate of cytoporphyrin IX [10].
- Cytochromes *b*. Cytochromes with protoheme (the iron chelate of protoporphyrin IX, see [10]) as prosthetic group but which lack a covalent bond between the porphyrin and the protein.
- Cytochromes *c*. Cytochromes with covalent thioether linkages between either or both of the vinyl side chains of protoheme side chains and the protein.
- Cytochromes *d*. Cytochromes with a tetrapyrrolic chelate of iron as prosthetic group in which the degree of conjugation of double bonds is less than in porphyrin, *e.g.* dihydroporphyrin (chlorin; heme *d* [12]), tetrahydroporphyrin

<sup>4</sup> The American spelling 'heme', as opposed to 'haem', is used here although both are acceptable and widely used.

<sup>5</sup> Thus a number of enzymes are also referred to as cytochromes. These include cytochrome-*c* oxidase (EC 1.9.3.1), L-lactate dehydrogenase (cytochrome) (yeast cytochrome *b*<sub>2</sub>, EC 1.1.2.3) and cytochrome *P*-450 (EC 1.4.14.1).

<sup>6</sup> Ferriheme is sometimes referred to as 'hematin', a usage still sanctioned by tradition. However, this term should not be used for the compound crystallized as the chloride or other salt; such compounds are customarily termed 'hemin'.

<sup>7</sup> The traditional definition that the fifth and sixth coordination positions are occupied by nitrogen atoms was obviously too restrictive as borne out by the case of cytochrome *c*, wherein one of these sites is occupied by the sulfur atom of methionine.

Table 1. Practical criteria for determining the cytochrome group

| Cytochrome     | $\alpha$ -Band of pyridine ferrohemochrome in alkaline solution    | Solubility of product treatment of cytochrome with acetone-HCl in ether |
|----------------|--|---|
| Group <i>a</i> | 580–590 nm   | soluble   |
| Group <i>b</i> | 556–558 nm   | soluble   |
| Group <i>c</i> | 549–551 nm (two thioether links)<br>553 nm (single thioether link) | insoluble <sup>a</sup>  |
| Group <i>d</i> | 600–620 nm   | <i>d</i> is soluble <sup>b</sup><br><i>d</i> <sub>1</sub> is insoluble  |

<sup>a</sup> While still attached to the peptide.

<sup>b</sup> Requires elaborate precautions to prevent decomposition to an insoluble product.

(isobacteriochlorins; heme *d*<sub>1</sub> [13], siroheme [14]). Heme *d* has also been known as heme *a*<sub>2</sub>.

**Comment.** It was originally suggested that the heme component of cytochromes *a* could be called heme *a*. However, the hemes present in groups *b–d* were not classified. Heme *b* is acceptable for noncovalently bound protoheme. However, the various alternative forms for hemes '*c*' and '*d*' preclude a simple classification.

Use of the small unprimed italicized letter implies that the heme prosthetic group is in a hemochrome linkage. To indicate that in both the oxidized and reduced forms the heme prosthetic group is not in a hemochrome linkage, a primed lower-case italicized letter, e.g. '*c*', should be used.

In the case of a cytochrome having two or more differing heme groups attached to a specific protein, each different heme may be indicated, e.g. in *Pseudomonas* cytochrome oxidase (*cd*<sub>1</sub>, EC 1.9.3.2). In the case of a cytochrome having two or more of the same heme groups attached to a specific protein but in different environments, so that one or more is in a hemochrome linkage and one or more in a non-hemochrome linkage, both types of linkage should be indicated by using both the unprimed and primed lower-case italicized letter, appropriate for the heme in question. As an example, cytochrome-*c* oxidase (EC 1.9.3.1) is considered to contain both a heme *a* in hemochrome-type linkage (called 'cytochrome *a*') and a heme *a* in a non-hemochrome-type linkage (called 'cytochrome *a*<sub>3</sub>'). By the suggested convention, this cytochrome should be called 'cytochrome *aa*', although the current usage of 'cytochrome *aa*<sub>3</sub>' is unlikely to be abandoned. The recommended notation should be adopted for future cases. In general, the name of a cytochrome will not necessarily indicate the number of identical heme centers per molecule of protein.

The main practical tests to be adopted as criteria in determining the group to which a cytochrome belongs should be (a) the position of the  $\alpha$ -band of the pyridine Fe(II) hemochrome and (b) the ether solubility of the hemin after treatment of the cytochrome with acidified acetone, or acidified methylethylketone, as shown in Table 1.

For the moment it is recommended that for groups *a*, *b* and *d* the position of the  $\alpha$ -band of the pyridine Fe(II) hemochrome should be determined after the acidified acetone-cleaved hemin has been extracted into ether and the extracted from the ether by dilute sodium hydroxide.<sup>8</sup>

Once the nature of the prosthetic heme group and its mode of linkage have been determined, so that the group to which a cytochrome belongs can be stated, the procedure for naming it can follow the steps recommended below:

a) At the first mention in a publication, the name should be expanded to the systemic name that will include the source

in parentheses, e.g. cytochrome *b*<sub>1</sub> (*Bacterium X*). Since the need has arisen to refer to different *b* and *c*-type cytochromes present in the same cell or in the same organelle of a given cell type, it will often be necessary to add distinguishing characteristics, such as 'microsomal', 'tetraheme', 'high potential', etc.

b) The names of the already well-established cytochromes with consecutive subscript numbering, listed below, are retained. All cytochromes not fitting in this category should be given a name based upon the  $\alpha$ -band wavelength (nm) and written thus: cytochrome *c*-554.

c) The  $\alpha$ -band wavelength used should be determined at room temperature, not liquid-nitrogen temperature, and should, if possible, be obtained from absolute absorption spectra of the purified protein under carefully defined conditions. Since an error of 1 nm in assigning the position of the band alters the name, care should be taken to standardize the spectrophotometer with standard lines, e.g. with those given by Nd(III). Failing this, the absorption maximum can be determined by calibration against horse heart mitochondrial cytochrome *c* using 550.25 nm for the wavelength maximum when dissolved in phosphate buffer, pH 7.

In application of the naming procedures based on location of the  $\alpha$ -wavelength maximum, any asymmetry or splitting of the  $\alpha$ -peak should be noted, e.g. cytochrome *c*-555 (550) indicates a minor peak or shoulder (in parenthesis) at 550 nm. It is urged that assignments should not be made solely on the location of the  $\alpha$ -band maximum, and reviewers and editors of reports dealing with discovery and description of cytochromes should insist that, whenever possible, the above procedures, based on chemical characterization, be followed. However, these rules need elaboration whenever solubilization of cytochromes is not possible without denaturation, as in some membrane-bound proteins. The rules given are applied readily

<sup>8</sup> In addition, a number of chemical reactions that can be carried out with small amounts can also be used for the establishment of the group (the original literature should be consulted for details). Oxime formation of the isolated hemin with hydroxylamine combined with conversion of the oxime into a nitrile, and a number of spectroscopically observable reactions of the hematin or porphyrin moiety with sodium hydrogen sulfate, or with dimedone, prove the presence of a heme with a formyl side-chain (group *a*). The reduction of an unsaturated side-chain with hydrazine-HI, or Pt-H<sub>2</sub>, together with the criterion of  $\alpha$ -band position shows the presence of protoheme (group *b*). The splitting of the thioether bond with silver or Hg(II) sulfate is a test for group *c*. Group *d* diagnosed from the presence of four bands between 500–700 nm in the optical spectrum of the reduced heme; the assignment should be verified by preparation of the pyridine hemochrome (Table 1). It is desirable that the result should be verified with authentic samples of the pertinent heme.

only to whole cell systems that have a simple composition, e.g. *c*-type proteins only. Problems arise when bound cytochromes *c* occur together with frequently encountered *b*-type complexes whose  $\alpha$ -band maxima overlap those of the *c*-type proteins. In such cases, attempts should be made to separate adequately the membrane from the soluble fractions in a whole-cell system. Electron-paramagnetic resonance spectroscopy (EPR, formerly also called electron spin resonance, ESR) is of considerable value in resolving complex cytochrome *a* and heme protein systems which include mixture of high- and low-spin components. Use of redox buffers combined with spectroscopy can also be used occasionally to characterize cytochrome components but redox potentials measured at ambient temperature should not be applied to spectral moieties determined at the low temperature associated with EPR spectroscopy.

Purification of soluble proteins to homogeneity after separation from membrane-bound components should be effected by the necessary chromatographic, gel-filtration and electrophoretic procedures, together with conventional salting-out techniques and, if feasible, crystallization, before assay of physico-chemical properties.

Cytochromes should not be assigned on the basis of spectral data obtained with whole cells or other complex systems, unless it can be unequivocally demonstrated that the spectral features arise from a single species.

#### 4.3. VARIABILITY IN CYTOCHROME GROUPS

The variations in spectroscopic and functional character of cytochromes, found especially as a result of work with prokaryotic systems, has led to an undesirable proliferation of subscripts to distinguish subgroups of uncertain character. It appears that this unfortunate tendency has been halted, but adequate characterization of subgroups remains a problem. Presumably, the primary sequence will eventually provide a rational basis for proper assignments to subgroups and some attempts along these lines have been made for the *c*-family [8], but the suggestion that a phylogenetic basis for such classification based on sequence similarities may exist is still premature. However, sequence similarity can be invoked to reclassify a number of prokaryotic cytochromes *c*, as is indicated in the revised list of cytochrome given below.

Certain variant cytochromes remain difficult to classify. Cytochromes '*o*', a type of protoheme oxidase found in prokaryotes, and heliocorubin, should be listed as *b*-type cytochromes.

Likewise, cytochrome '*P*-450', which originally received its name in a casual manner (location of Soret peak of the reduced CO compound) not in conformity with any of the recommendations given herewith or previously, has been characterized as a class of proteins with activity as a monooxygenase involved in hydroxylation associated with electron transfer. Its chemical nature as a *b*-type cytochrome with atypical non-nitrogenous ligands is well established in a number of systems, notably that of the pseudomonads that effect hydroxylation of terpenes.

This group of monooxygenases known as cytochrome *P*-450 enzymes could, however, easily be included in the cytochrome *b* class, since they are enzymes in which protoheme is non-covalently attached to the protein. On the other hand, since the characteristic mode of action of these enzymes is not electron transfer [some *P*-450 enzymes probably do not even involve the reversible Fe(II)/Fe(III) equilibrium], but rather oxygen atom transfer, the name 'cytochrome' did not

seem appropriate. Based on the fact that a thiolate ligand at the heme is responsible for the unusual spectral and catalytic properties of these hemoproteins the name 'heme-thiolate proteins' is now recommended.

The characteristic feature of the heme-thiolate prosthetic group is its activating power on an oxygen species as verified in e.g. monooxygenases (such as EC 1.14.14.1), prostacyclin synthase (EC 5.3.99.4), thromboxane synthase (EC 5.3.99.5), leukotriene-B<sub>4</sub> 20-monooxygenase (EC 1.14.13.30) and probably chloride peroxidase (EC 1.11.1.10). However, it is noted that this example appears to have a similar ligand environment; consequently the ascription heme-thiolate is not exclusive to the *P*-450 class of monooxygenases.

#### 4.4. LIST OF CYTOCHROMES

##### 4.4.1. Cytochrome-*a* group

Cytochrome *aa*<sub>3</sub> is probably identical with one of the subunits of cytochrome-*c* oxidase of eukaryotes and some prokaryotes. The protein complex contains two hemes *A*, a low-spin component called cytochrome *a* and a high-spin component called cytochrome *a*<sub>3</sub>. The  $\alpha$ -band of the reduced cytochromes is at about 605 nm, the  $\gamma$ -band (Soret band) at around 445 nm. The reduced cytochrome *a*<sub>3</sub> combines with CO with a shift of the  $\alpha$ -band to 590 nm and the  $\gamma$ -band to approximately 430 nm; it also combines with cyanide with a shift of the  $\alpha$ -band to 590 nm with little effect on the  $\gamma$ -band. The reduced form is autoxidizable. In the presence of cyanide, one-half of the heme content is autoxidizable. The enzyme complex also contains two copper atoms (see section 6.5; cytochrome *aa*<sub>3</sub> is membrane bound and catalyzes the oxidation of mitochondrial cytochrome *c* and some related bacterial proteins by O<sub>2</sub>.

Cytochrome *a*<sub>1</sub> has been isolated from *Nitrobacter agilis*. It has an absorption maximum at 587 nm and is not autoxidizable. Autoxidizable proteins with absorption maxima have been reported for *Acetobacter pasteurianum* and *Escherichia coli*. However, no heme *a* has been detected in *E. coli* and thus the existence of this cytochrome in these latter organisms must be considered speculative until such time as a pure protein has been isolated and shown to contain heme *a*.

##### 4.4.2. Cytochrome-*b* group

Cytochrome-*b* is present in mitochondria of eukaryotes and in chloroplasts. It is an integral membrane protein which contains two hemes in a protein of molecular mass of about 40 kDa. The two heme centers can be distinguished by the position of the  $\alpha$ -band in the optical spectrum of the reduced protein, the location of the low-field *g*-value in the EPR spectrum of the oxidized protein and by differences in redox potential. A strongly related protein, cytochrome *b*-563, also known as cytochrome *b*<sub>6</sub>, functions in the *bc* complex present between photosystems I and II of green plant photosynthesis. Related *bc* complexes can be found in some photosynthetic bacteria. Cytochrome *b*-559 is present between the center for water cleavage and photosystem II of green plants. White blood cells contain a cytochrome *b*-562.

Cytochrome *b*<sub>1</sub> has been detected in *E. coli* by the presence of an absorption maximum at 560 nm. However, it seems that this band reflects contributions from several different cytochromes of slightly differing optical properties and redox potentials. Thus the existence of this group should be considered moot.

Cytochrome  $b_2$  is present in yeast. It contains one mole each of heme and FMN as prosthetic groups and acts as an L-lactate dehydrogenase (cytochrome) (EC 1.1.2.3). It is also called flavocytochrome  $b$ . The cytochrome is part of the membrane-bound NADPH-oxidoreductase, which reduces  $O_2$  to  $H_2O_2$  and  $O_2^{\cdot-}$ .

Cytochrome  $b_3$  is present in microsomal material from non-photosynthetic plant tissues. Its characteristic absorption band is at 559 nm. This category is not sufficiently defined to warrant further use.

Cytochrome  $b_5$  is present in animal microsomes and the cytoplasm of the erythrocyte. It is reduced by NADH in the presence of cytochrome- $b_5$  reductase (EC 1.6.2.2) or NADH ferrihemoglobin reductase (EC: see<sup>2</sup>). It contains one heme per polypeptide of 137 residues; the heme iron has bis-histidine coordination and is low-spin. An X-ray structure is available for the proteolytically degraded form from microsomes; the erythrocyte protein is analogous to this short form. Its characteristic band is the  $\alpha$ -band at 557 nm with a shoulder at about 560 nm. It functions in electron transfer associated with desaturation and elongation of higher fatty acids, hydroxylation (detoxification) and maintaining hemoglobin in the ferrous state.

Cytochrome  $b_7$  is present in the spadice of various *Arum* species. Its characteristic absorption is at 560 nm. It is autoxidizable. This category is not sufficiently defined to warrant further use.

Cytochrome  $b_8$  is a monomeric, monoheme protein, typified by cytochrome  $b$ -562 from *E. coli*. It contains one heme per 12-kDa molecule with iron coordination provided by methionine and histidine. The tertiary structure is similar to that of cytochrome  $c'$ . The midpoint potential is 190 mV (pH 7) and is pH dependent.

Cytochrome  $b'$  was formerly known as cytochrome  $o$ . It is a prokaryotic terminal oxidase with protoheme as prosthetic group. (It has also been reported in some protozoans.) The reduced CO compound has absorption peaks at 557–567 nm ( $\alpha$ ) and 532–537 nm ( $\beta$ ). Its absorption spectrum indicates it is a high-spin heme protein. It exists in two forms: the soluble (s) form appears to transport oxygen, the membrane (m) form is a terminal oxidase. The term cytochrome  $o$  is discouraged, but for continuity it can be indicated as 'cytochrome  $b'$ ' (formerly cytochrome  $o$ ). Some autoxidizable low-spin cytochromes  $o$  have been reported.

#### 4.4.3. Cytochrome- $c$ group (see [15])

Cytochrome- $c$  is present in eukaryotic mitochondria where it functions as the substrate for the terminal oxidase (EC 1.9.3.1) in oxidative phosphorylation. It is a soluble, low-spin, monohemeprotein with 103–112 residues. Its midpoint redox potential over most of the physiological pH range is about 250 mV. In its reduced form, the  $\alpha$ -band maximum is at 550 nm, the  $\beta$ -band at 520 nm and the Soret peak at 415 nm. Both vinyl groups are present in thioether bonds and the heme iron is coordinated by histidine and methionine. It is the prototypic  $c$ -type cytochrome.

Cytochrome  $c_1$  is the 30-kDa membrane-bound  $c$ -type protein of mitochondria with  $\alpha$ -band maximum at 553 nm in the reduced form. On solubilization with detergents this  $\alpha$ -maximum remains unchanged. It is a low-spin monoheme protein with the same axial ligands as cytochrome  $c$ . The midpoint potential is about 270 mV. It functions as electron donor to cytochrome  $c$  in the mitochondrial and bacterial respiratory chain. The related protein present in the  $bc$  complex

of green plants is also called cytochrome  $f$ ; it has histidine and lysine residues as axial ligands and a midpoint potential of about 360 mV. Cytochrome  $f$  transfers electrons to the copper protein, plastocyanin.

Cytochrome  $c_2$  is a small, soluble, low-spin cytochrome with the same tertiary folding and binding of heme as in mitochondrial cytochrome  $c$ , but with limited ability to replace it as a substrate for cytochrome- $c$  oxidase (EC 1.9.3.1). It is a monoheme protein with midpoint redox potential usually some 100 mV higher than that of mitochondrial cytochrome  $c$ , and with much different pH redox profile. In *Rhodospseudomonas viridis* (and probably *Chromatium vinosum*) it functions to transport electrons from the  $bc$  complex to the membrane bound  $c$ -type cytochrome which functions as electron donor to the bacterial photosystem. In *Rhodobacter sphaeroides* it reacts directly to reduce the photooxidized special pair of bacteriochlorophyll.

Cytochrome  $c_3$  is a low-potential (much less than 0 mV), low-spin cytochrome with the same thioether binding to heme as in mitochondrial  $c$ , but with very different tertiary structure and no sequence similarity to the eukaryotic proteins. The monomeric form is about 13 kDa; it can exist as a dimer. Each iron atom has bis-histidine coordination. It exists in triheme and tetraheme forms as a monomer with the same 68–115 residues and is found in the strictly anaerobic sulfate- and sulfur-reducing bacteria where it participates in sulfate respiration coupled to phosphorylation. It exhibits no reactivity with mitochondrial reductase or oxidase. The triheme form was formerly called cytochrome  $c_7$ . Cytochrome  $c$ -552 from *E. coli* may be related.

Cytochrome  $c_4$  is a diheme, high-potential protein with about 190 residues found in *Azotobacter vinelandii* and some *Pseudomonas* species. The heme attachment and tertiary structure resemble eukaryotic cytochrome.

Cytochrome  $c_5$  is a low-spin dimeric monoheme protein containing a single heme in each of two subunits, usually with 80–90 residues per monomer exhibiting the same thioether binding and extraplanar ligands as in mitochondrial cytochrome  $c$ . It is unreactive with cytochrome- $c$  reductase and oxidase. Its midpoint redox potential is somewhat higher than that of mitochondrial  $c$  and its  $\alpha$ -peak is red-shifted to about 554–555 nm. It may be proteolytically modified as isolated. It appears to be present also in the strictly aerobic nitrogen-fixing azotobacter.

Cytochrome  $c_6$  is a soluble monoheme, monomeric low-spin cytochrome which in algae has a molecular mass of about 10 kDa and the same binding and extraplanar ligands as in mitochondrial cytochrome  $c$ . Its midpoint redox potential is usually about 100 mV higher than that of the mitochondrial protein. Its reduced  $\alpha$ -peak is asymmetric and red-shifted to about 552–554 nm. It functions like cytochrome  $c$  in that it mediates electron transfer at the high potential terminus of the photophosphorylation chain in chloroplasts and algae. It is unreactive with mitochondrial cytochrome- $c$  reductase or oxidase but reactive with *Pseudomonas* cytochrome  $cd_1$ . It is the functional equivalent to plastocyanin. It should not be confused with the membrane-bound cytochrome  $c$ -553 (of the  $bc$  complex).

'*Pseudomonas*' cytochrome  $c$ -551 is another prokaryotic, monomeric monoheme cytochrome with 80–90 residues, unreactive with mitochondrial cytochrome- $c$  reductase or oxidase, although possessing similar midpoint redox potential, the same extraplanar ligands and heme thioether binding. It apparently functions in nitrite and nitrate reduction in

pseudomonads, but it is also found in other bacteria. It is functionally analogous to cytochrome *c*.

Bacterial photosystem cytochrome *c* is a subunit of the photosynthetic reaction center of the purple non-sulfur bacterium *Rhodospseudomonas viridis*. The subunit contains four heme-*c* centers in a polypeptide of about 330 residues and mediates electron transfer from cytochrome *c*<sub>2</sub> to the photo-oxidized bacteriochlorophylls *b*. The cytochrome exhibits  $\alpha$ -band maxima at 552 and 558 nm. Two of the hemes have a low potential (about 0 mV); the other two have a high potential (about 300 mV). It is structurally unrelated to cytochromes *c*<sub>2</sub> and *c*<sub>3</sub>. Similar proteins appear to be present in *Thiocapsulata pfenigii*, *Chromatium vinosum* and *Rhodospseudomonas gelatinosa*.

Cytochrome *c*-555 from green bacteria is a low-spin monoheme cytochrome of 80–90 residues found exclusively in the green photosynthetic bacteria (chlorobium) with intermediate midpoint redox potential (about 150 mV) and variable reactivities with mitochondrial cytochrome-*c* reductase and oxidase and *Pseudomonas* cytochrome *cd*<sub>1</sub>. Like other prokaryotic cytochromes *c* mentioned above, it possesses the characteristic thioether heme binding, the same extraplanar ligands, and a tertiary structure distinctly related to mitochondrial cytochrome *c*. Its reduced  $\alpha$ -peak is asymmetric and red-shifted (to about 555 nm) as in cytochrome *c*<sub>6</sub>.

Cytochrome *c*' is a high-spin variant cytochrome *c* (originally known as 'RHP') with heme binding through side-chain thioether linkage as in mitochondrial cytochrome *c* but with unrelated tertiary structure. It is unreactive with the mitochondrial cytochrome-*c* reductase or oxidase. It occurs usually as a dimer, with monomer molecular masses of about 14 kDa, and has a midpoint redox potential at pH 7 close to zero. The heme is 5-coordinated with a single histidine axial ligand but exhibits a low-spin hemochrome EPR spectrum at pH values higher than 12. It is found in purple photosynthetic bacteria as well as in nitrate-reducing pseudomonads. When soluble, it reacts only with NO and CO in its reduced form, and with NO in its oxidized form. It is unreactive with the usual ligands (CN<sup>-</sup>, F<sup>-</sup>, N<sub>3</sub><sup>-</sup>, etc.) for high-spin heme proteins. The reduced heme exhibits a broad absorption band centered between 540 and 560 nm. It is the most widely distributed bacterial cytochrome known.

Various other forms are as yet insufficiently characterized to be placed in sub-groups. An example is flavocytochrome *c*, found in purple sulfur photosynthetic bacteria.

#### 4.4.4. Cytochrome-d group

Cytochrome *d* was earlier known as cytochrome/heme *a*<sub>2</sub>. It is present in many aerobic bacteria, especially when grown with a limited oxygen supply. Typical species include *Escherichia coli* and *Aerobacter aerogenes*. In protein complexes, it typically gives an absorption band at about 636 nm (oxidized) or 628 nm (reduced). It is found associated with other prosthetic groups in multi-subunit complexes. It is difficult to detect as a Fe(II) pyridine alkaline hemochrome, because of limited stability under these conditions. If it is extracted from the protein complex and placed in ether containing 1 to 5% HCl, it gives a distinctive band at 603 nm (oxidized).

Cytochrome *bd* functions as a terminal oxidase in *Photobacterium phosphoreum* and *E. coli*.

Cytochrome *cd*<sub>1</sub> is found in a common dissimilatory nitrite reductase present in many denitrifying bacteria.

Typical species include *Pseudomonas aeruginosa*, *Paracoccus denitrificans* and *Thiobacillus denitrificans*. Heme *d* is non-covalently associated while the heme *c* is covalently bound to polypeptide. The band maximum of the  $\alpha$ -peak in the Fe(II) state is broad and very sensitive to pH and the reductant used, appearing at 625–630 nm with dithionite reduction, and 655 nm with ascorbate reduction. The *d*<sub>1</sub>-chromophore is typically found to auto-reduce under a carbon monoxide atmosphere. The Fe(II) alkaline pyridine hemochrome band maximum is at 618 nm. The heme is very hydrophilic and is readily water soluble at neutral pH.

## 5. NON-HEME IRON PROTEINS

### 5.1. INTRODUCTION

Most but not all of the proteins in this group are iron-sulfur proteins. Recommendations for the nomenclature of iron-sulfur proteins were first formulated in 1971 and revised in 1978 [16]. Since that time, a number of major contributions to the field have reported structure determination by X-ray crystallography, elaboration of a series of model compounds, and the discovery of novel examples of these proteins. On the basis of the new information, it became desirable to adjust the nomenclature of the iron-sulfur proteins and thereby take into account the more precise description of known species while retaining flexibility for future discoveries.

Whenever possible, terms that have gained with acceptance have been retained and particular attention has been given to the development of a useful shorthand notation. However, in order to comply with the established rules for the nomenclature of inorganic compounds [17], it was decided in 1978 to make some changes from usage in the biochemical literature that had developed in the early 1970s.<sup>9</sup>

Proteins containing iron can be divided into three groups: hemeproteins, iron-sulfur proteins, and other iron-containing proteins (see Scheme). The last group includes ferritin, transferrin and the oxygenases; of these, the oxygenases also have electron-transfer functions. The term 'iron-sulfur proteins' refers only to those proteins in which a non-heme iron is ligated with inorganic sulfur or cysteine sulfur.

The iron-sulfur proteins (abbreviation: Fe-S proteins) fall into two major categories: 'simple' iron-sulfur proteins and 'complex' iron-sulfur proteins. 'Simple' need only be used when

<sup>9</sup> The group of 'high potential iron-sulfur proteins' has been deleted on the following basis. (a) The three-dimensional structure of the active center of this protein has been found to be essentially the same as that of ferredoxins [18, 19]. The magnetic properties differ because the Fe-S clusters may assume three different oxidation levels [18, 20]. Thus, an oxidized ferredoxin (diamagnetic) corresponds to the reduced *Chromatium vinosum* high-potential iron-sulfur protein (diamagnetic); the extreme oxidation levels, i.e., those of reduced ferredoxin and oxidized *C. vinosum* high-potential iron-sulfur protein, have one electron more or more electron less, respectively, than this oxidation level and are paramagnetic. (b) After the original study [21] with the *C. vinosum* high-potential iron-sulfur protein, it became apparent that other iron-sulfur proteins may have the unusual oxidation level of the *C. vinosum* protein without a high midpoint oxidation-reduction potential [22]. Conversely, ferredoxins with the usual oxidation level and magnetic properties may have high midpoint potentials [23]. The expression, 'high-potential Fe-S protein' (or HiPIP) has been used increasingly in the literature to indicate the ability to form the higher oxidation level of the *C. vinosum* protein. This practice is inappropriate since it uses a term applying to a redox potential for the description of a magnetic property or an oxidation level.



the difference from complex iron-sulfur proteins is emphasized. Simple iron-sulfur proteins contain only one or more Fe-S clusters whereas the complex proteins bear such additional active redox centers as flavin, molybdenum or heme. Often EPR spectra are used for classification and identification of Fe-S clusters in proteins.

## 5.2. TYPES OF SIMPLE IRON-SULFUR PROTEIN

Simple iron-sulfur proteins fall into three groups: rubredoxins, ferredoxins and other simple iron-sulfur proteins (see Scheme).

### 5.2.1. Rubredoxins

Rubredoxins (abbreviation: Rd) comprise those iron-sulfur proteins without acid-labile sulfur that are characterized by having iron in a typical thiolate coordination, i.e. an iron center surrounded by four cysteine residues or sulfur-containing ligands. More than one iron center of this type may exist in the molecule. Oxidized rubredoxin usually exhibits the characteristic EPR spectrum of high-spin Fe(III) ion in a rhombic ligand field,  $g = 4.3$ ; the reduced form gives no discernible EPR signal. Only negative redox potentials at pH 7 have been noted for those rubredoxins presently characterized. Desulfuredoxin is a variant rubredoxin with a somewhat higher symmetry and distinctive EPR spectrum with  $g$ -values of 7.7, 4.1 and 1.8.

The full name should be listed as follows: (source) rubredoxin, (function), e.g. *Pseudomonas oleovorans* rubredoxin, alkane  $\omega$ -hydroxylation.

### 5.2.2. Ferredoxin

Ferredoxins (abbreviation: Fd) comprise those iron-sulfur proteins that contain more than one iron and labile sulfur atoms and that exclusively display electron-carrier activity but not classical enzyme function. Thus, since hydrogenases (iron-sulfur proteins containing one or more Fe-S clusters) are enzymes, they are not classified as ferredoxins.

The criterion that ferredoxins must have negative oxidation-reduction midpoint potential at pH 7 has been abandoned; there is no need to distinguish between ferredoxins and the previously designated 'high-potential iron-sulfur proteins'<sup>9</sup> as exemplified by an iron-sulfur protein from *Chromatium vinosum* [20]. Ferredoxins may contain one or more clusters of two or four iron and labile sulfur atoms. In addition to these clusters, more recently both in proteins and in synthetic analogs, several other clusters have been reported.<sup>10</sup>

### 5.2.3. Other iron-sulfur protein

All simple iron-sulfur proteins that are neither rubredoxins nor ferredoxins will fall into the category of 'other iron-sulfur proteins'. At this point it should be noted that some iron-sulfur proteins are known that do not appear to have a redox role; examples are endonuclease III and aconitase.

## 5.3. RECOMMENDATIONS FOR DESIGNATING IRON-SULFUR PROTEINS

### 5.3.1. General recommendations

a) If any iron-sulfur protein has been given another name previously, this should be stated to minimize confusion.

b) The term 'high-potential iron-sulfur protein' (abbreviated HiPIP), may continue to be used for the original iron-sulfur protein of photosynthetic bacteria that had been given this name initially. Otherwise, the use of the terms HiPIP or 'high-potential Fe-S protein' is discouraged; there are ferredoxins occurring naturally at the oxidation level of oxidized and reduced spinach ferredoxin that have oxidation-reduction potentials as high as the original HiPIP of bacteria (see footnote 9).

c) At least once in a report, the source of the protein should precede the term rubredoxin, ferredoxin, or iron sulfur protein. Similarly, for iron-sulfur cluster, or 'centers' of complex iron-sulfur proteins, the proper designation of the parent protein or enzyme should be given, preferably along with the source, e.g. beef-heart NADH-dehydrogenase Fe-S cluster 1. Thereafter, the designation 'cluster 1' may be used. The proliferation of such other abbreviations as center N-1, S-1, or bc-1 is discouraged. With regard to more complex system, including the complex iron-sulfur proteins, it appears to be neither suitable nor desirable to present designations for Fe-S clusters that are inadequately characterized.

d) Iron-sulfur proteins from the same source that have the same type of Fe-S cluster are numbered sequentially with Roman numerals. A newly isolated iron-sulfur protein that is not fully characterized should be called 'iron-sulfur protein' and given the lowest unused numeral. By analogy, the various iron-sulfur clusters, or 'centers' of an iron-sulfur protein, should be designated Fe-S cluster 1, Fe-S cluster 2, and so on in the order of discovery. Arabic numerals should be used for the different clusters of the same protein or complex, since iron-sulfur proteins and the complexes of the respiratory chain are designated by Roman numerals.

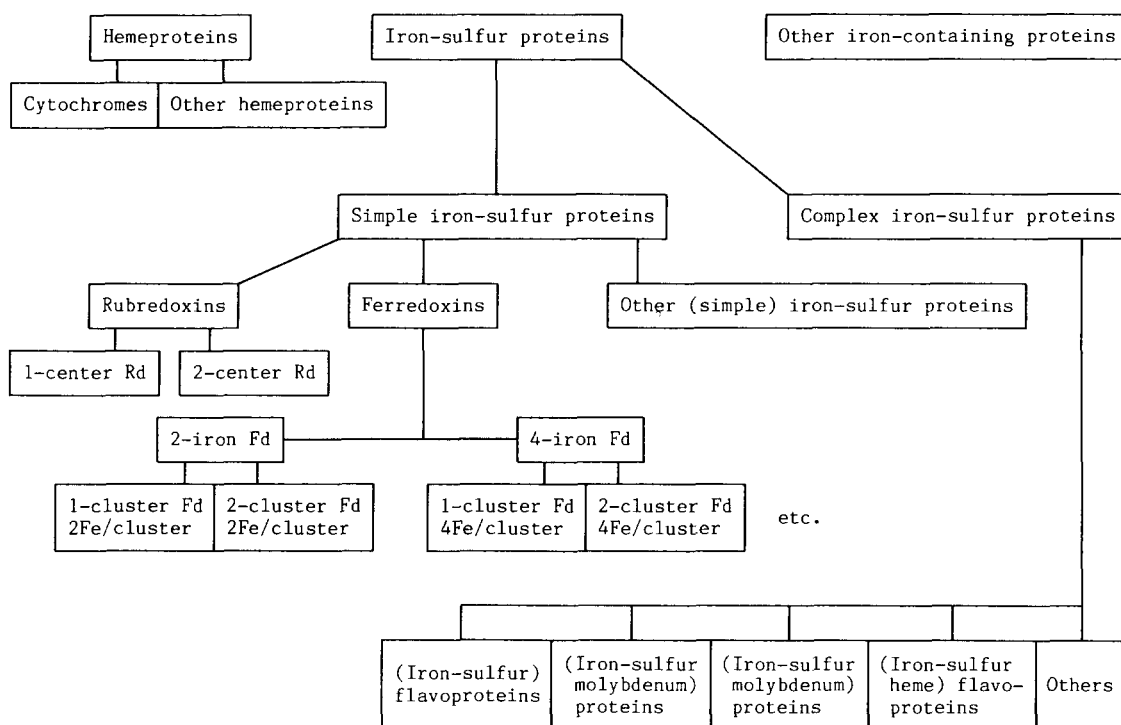
e) It is useful to present midpoint redox potentials, light absorption and EPR characteristics, particularly when an iron-sulfur protein is first mentioned in a publication.

### 5.3.2. Designation of clusters

a) The designation of a cluster in an iron-sulfur protein containing labile sulfur atoms should consist of square brackets about the number of iron and labile sulfur atoms. Thus, [2Fe-2S] represents a two-iron, two-labile-sulfur cluster, and [4Fe-4S] a four-iron-labile-sulfur cluster. The protein incorporating such a cluster may be called a 'two-iron-two-sulfur' or 'four-iron-four-sulfur' ferredoxin or iron-sulfur pro-

<sup>10</sup> The existence of Fe-S clusters of novel and unusual stoichiometry has now been proven. The identity of the [3Fe-4S] cluster has been established by chemical analysis, EXAFS and X-ray diffraction [24, 25] methods. In particular there has been a reevaluation of the X-ray structure [26] of the FdI of *Azotobacter vinelandii* both by the original investigators and by a second group. Both groups concur that the original conclusion of the presence of a 3Fe-3S cluster in this protein was in error and that this cluster is actually of the 3Fe-4S type, comprising a cubane structure with one iron atom missing. Chemical model studies have also indicated that linear structures are possible [25]; a linear structure can also be produced in aconitase. When the Fe/S stoichiometry of a given structure is known, the notation [3Fe-xS] has been introduced; a general form of this notation would be [nFe-xS]. It should be noted that in the absence of precise stoichiometries no formal charge can be assigned to the cluster. Ligands for such clusters are not necessarily cysteine thiolate groups for all metal ions. Evidence has been obtained for some *A. vinelandii* ferredoxins that 3Fe clusters may have a physiological role; the [3Fe-4S] type certainly has a role as intermediate during assembly and degradation.





Scheme. Iron-containing proteins

tein. The more recently found [3Fe-4S] clusters, in some cases derived from [4Fe-4S] clusters by loss of Fe, may be called 'three-iron-four-sulfur'. See also footnote 10.

**Comment.** The use of hyphens and parentheses (including square brackets) is firmly codified in the nomenclature of inorganic chemistry [17]. In order to avoid confusion with established practices in coordination chemistry, a short hyphen is used to write Fe-S and the term is placed between square brackets instead of parentheses.

b) The presence of several clusters, as in clostridial ferredoxin, is indicated as follows: 2[4Fe-4S]. This may be called a two-cluster four-iron-four-sulfur ferredoxin or iron-sulfur protein.

c) When the formal charge of the cluster is calculated, the sulfur atoms of the bound cysteine residues are not included in the calculation, contrary to the widespread practice in the earlier literature; thus, for the oxidized and reduced forms, respectively, we have:

|  |   |
|--|---|
| Spinach ferredoxin                         | $[2\text{Fe-2S}]^{2+} : [2\text{Fe-2S}]^{1+}$   |
| <i>Bacillus polymyxa</i> ferredoxin        | $[4\text{Fe-4S}]^{2+} : [4\text{Fe-4S}]^{1+}$   |
| <i>Clostridium pasteurianum</i> ferredoxin | $2[4\text{Fe-4S}]^{2+} : 2[4\text{Fe-4S}]^{1+}$ |

**Comment.** The charges referred to are those within the entire cluster. This may seem in contrast with usage in inorganic chemistry where a charge shown in such a formula often refers to the whole coordination entity, i.e. including the other ligands [17]. There is also possibility for misunderstanding the codified use of the term, oxidation state, which usually characterizes a single atom but never a group of atoms [17]. In order to avoid confusion, the term 'oxidation level' is used to refer to the cluster as such, and indicated in the square brackets.

d) For ferredoxins at the oxidation level typical of oxidized *C. vinosum* HiPIP, the expression  $[4\text{Fe-4S}]^{n+}$  ( $n = 1, 2, 3$ ), may differentiate between what was formerly called HiPIP and a ferredoxin.

e) If the oxidation levels in which a ferredoxin can occur are known, this may be indicated as follows for a [4Fe-4S] ferredoxin that is generally obtained on isolation at the (2+) level:  $[4\text{Fe-4S}]^{2+(3+:2+:1+)}$ . This designation implies that the ferredoxin can occur at all three possible oxidation levels. However, these designations should only be used to refer to oxidation levels that can be reached in a biological milieu, i.e. in the absence of agents denaturing the protein, even though artificial oxidants may be used to attain such oxidation levels. By this designation, the highest oxidation levels normally found in *C. vinosum* HiPIP and the protein from *Bacillus polymyxa*, both [4Fe-4S] ferredoxins, may be differentiated:

|                                     |                               |
|-------------------------------------|-------------------------------|
| <i>Chromatium vinosum</i> HiPIP     | $[4\text{Fe-4S}]^{2+(3+:2+)}$ |
| <i>Bacillus polymyxa</i> ferredoxin | $[4\text{Fe-4S}]^{2+(+:1+)}$  |

This shorthand denotes that *C. vinosum* ferredoxin occurs in the reduced (2+) state but also can be found at the (3+) state whereas *B. polymyxa* ferredoxin occurs in the oxidized (2+) state, has not been found in the (3+), but can exist in the (1+) state. The (2+) state is diamagnetic whereas both the (3+) and (1+) states are paramagnetic and detectable by the EPR measurements. When the EPR arises from a net spin ( $S'$ ) of 1/2 the EPR signal is readily detected. However, the (1+) oxidation state can also be obtained from clusters in which  $S' = 3/2$ . In these instances, detection of the EPR signal may require concentrated samples and high instrumental sensitivity. It is recommended that the designation for the oxidation level, whether (3+), (2+) or (1+), when used in a publication, should be that occurring in the experiments described. Terms such as 'reduced', 'oxidized', 'super-reduced' or 'super-oxidized' have been and still are being used. These should be phased out and replaced with the precise cluster- and charge-designations whenever possible.

f) Rubredoxins are treated in an analogous fashion, except that the basic center is designated [Rd] since there is no ambiguity concerning numbers of metal atoms involved.

Table 2. Designation of iron-sulfur proteins as generally obtained on isolation

| Previous designation   | Recommended designation  |
|--|--|
| Spinach chloroplast ferredoxin                               | Spinach chloroplast $[2\text{Fe-2S}]^{2+}$ Fd or, in a specific context, $[2\text{Fe-2S}]^{2+(2+;1+)} \text{Fd}$               |
| <i>Azotobacter vinelandii</i> iron-sulfur protein I          | <i>Azotobacter vinelandii</i> $[2\text{Fe-2S}]^{2+}$ Fe-S protein I  |
| <i>Chromatium vinosum</i> high-potential iron-sulfur protein | <i>Chromatium vinosum</i> $[4\text{Fe-4S}]^{2+}$ Fd or, in a specific context, $[4\text{Fe-4S}]^{2+(3+;2+)} \text{Fd}$         |
| <i>Clostridium pasteurianum</i> ferredoxin                   | <i>Clostridium pasteurianum</i> $2[4\text{Fe-4S}]^{2+}$ Fd or, in a specific context, $2[4\text{Fe-4S}]^{2+(2+;1+)} \text{Fd}$ |

Rubredoxins with multiple centers are denoted as  $n[\text{Rd}]$ . The formal charges of rubredoxins are  $[\text{Rd}]^{3+}$  and  $[\text{Rd}]^{2+}$  for the oxidized and reduced forms, respectively.

### 5.3.3. Examples

The examples given in Table 2 illustrate and contrast the new designations with those applied previously.

### 5.3.4. Clusters of different types

Iron-sulfur proteins with clusters of different types may be designated in a similar manner as for the above examples.

It seems appropriate to have a nomenclature system that allows for other clusters. According to model work [27] and Mössbauer spectroscopy on a Fe-S protein [28], it has become clear that changing the terminal ligands of iron atoms in a cluster to groups that do not contain sulfur, does not greatly change the EPR spectra and other properties. The tacit assumption that the terminal ligands are thiolates therefore no longer seems justified for Fe-S clusters in proteins.

In those cases of ligands other than thiolates, it is needed to have a notation for these systems. In the presently available shorthand notation, e.g.  $[4\text{Fe-4S}]$ , the terminal ligands are not considered and no changes are therefore necessary. We now recommend, however, that, in cases where the terminal ligands are to be specifically defined, the same notation be used as for coordination compounds in general and in particular for chemicals models, e.g.  $[\text{Fe}_4\text{S}_4(\text{RS})_2(\text{L})_2]^{2-}$  [29] for the diamagnetic state of a  $[4\text{Fe-4S}]$  cluster with two different sets of terminal ligands; in this case the charge of terminal ligands (*i.e.* both for the thiolates and the other ligands), must be included in the cluster charge, outside the square brackets.

*Comment.* In biochemistry many authors still use the designation  $\text{Fe}_2\text{S}_2$  or  $\text{Fe}_4\text{S}_4$  for  $[2\text{Fe-2S}]$  or  $[4\text{Fe-4S}]$  clusters, respectively. Within the framework of the nomenclature recommended above, where the notations  $[2\text{Fe-2S}]$ , etc., are merely to serve as convenient shorthand, it would be appropriate to use designations such as  $\text{Fe}_2\text{S}_2$  or  $\text{Fe}_4\text{S}_4$  when reference is made specifically to the core of clusters in proteins, or as indicated above, when the terminal ligands are to be specified.

## 5.4. OTHER IRON PROTEINS

During the past decade many iron proteins have been isolated and characterized that have electron-transfer properties but are neither iron-sulfur nor iron-heme proteins. Known examples are: (a) the lipoygenases (EC 1.13.11.12); (b) tyrosine 3-monooxygenase (EC 1.14.16.2); (c) purple acid phosphatase (EC: see footnote 2); (d) uteroferrin (EC: see footnote 2); (e) catechol 2,3-dioxygenase (EC 1.13.11.2); (f) mandelate 4-monooxygenase (EC 1.14.16.6); (g) methane monooxy-

genase (EC 1.14.13.25); (h) anthranilate 3-monooxygenase (deaminating) from *Aspergillus niger* (EC 1.14.13.35).

Often these proteins are divided into low classes, *i.e.* those containing mononuclear iron sites, such as in the lipoygenase, and those containing dinuclear iron sites, such as in uteroferrin and in purple acid phosphatase [30], which are believed to be similar. In these last proteins the two iron sites are assumed to contain the bridge  $[\text{Fe}(\text{O})(\text{RCO}_2)_2\text{Fe}]$ , as based upon spectroscopy and comparison with synthetic analogs. However, other bridges, like a phosphate, cannot be excluded.

## 6. COPPER PROTEINS

### 6.1. TYPE-1 COPPER PROTEINS

The Cu(II) state of this category has an intense blue color due to a thiolate ligand to Cu(II) charge transfer, and unusual EPR properties arising from the asymmetrical Cu site (distorted trigonal-pyramidal). The proteins all have a low molecular mass and have, so far, rather arbitrarily been divided into sub-groups, such as azurins, plastocyanins, pseudoazurins, amicyanins and various other blue proteins. Of these the azurins, amicyanins, pseudo-azurins and plastocyanins apparently have similar copper coordination by two histidine, one cysteine and one methionine residue. Where the function of type-1 copper proteins is known, it is invariably electron transfer. As yet the names for these proteins are all trivial and are often derived from source, function or color. The different classes are usually discerned on the basis of their primary and tertiary structure.

The first bacterial blue proteins to be described were called azurins. Rusticyanin is another example of a bacterial protein. It has unusual properties with a reduction potential of 680 mV, and is functional at pH 2. The azurins have well-defined electron transfer functions.

The so-called pseudo-azurins differ from the azurins in the N-terminal amino acid sequence and the optical spectra, which resemble those of plastocyanins.

The blue proteins known as plastocyanins occur in plants, blue-green and green algae. Their electron transfer role is well defined, *i.e.* from the  $bc_1$  complex (EC 1.10.2.2) to the photooxidized *P*-700.

Amicyanins are electron carriers between methylamine dehydrogenase and cytochrome *c*, with a characteristic amino acid sequence.

Of the remaining blue proteins stellacyanin is a well-known example. Unmecyanin, plantacyanin and mavicyanin are also considered to belong to this group. Although these proteins undergo redox reactions *in vitro*, their true biological functions remains unknown. Most of these proteins exhibit an unusual EPR signal in which the copper hyperfine splitting pattern

is poorly resolved. There is good evidence that at least for stellacyanin, methionine does not function as a ligand for copper.

## 6.2. TYPE-2 COPPER PROTEINS

The copper centers in these proteins are spectroscopically consistent with square planar or pyramidal coordination, containing oxygen and/or nitrogen ligation. The Cu(II) is EPR active, with a 'normal' signal. There is no intense blue color. This group includes the copper/zinc superoxide dismutase (EC 1.15.1.1), dopamine  $\beta$ -monooxygenase (EC 1.14.17.1), galactose oxidase (EC 1.1.3.9) and the various copper-containing amine oxidases. Some members of this last group may also contain an organic prosthetic group, such as PQQ (see section 10) or a modified amino-acid residue.

## 6.3. TYPE-3 COPPER PROTEINS

In this group a pair of copper atoms comprise a dinuclear center, with no EPR activity as for single Cu's. The best known example of an enzyme containing a single type-3 center is tyrosinase (catechol oxidase, EC 1.10.3.1). This protein contains a metal center which is a structural analog of the dinuclear copper center in hemocyanin [31].

## 6.4. MULTI-COPPER OXIDASES

In addition to the above, there are several proteins with catalytic activity that contain types 1, 2 and 3 centers in various stoichiometric ratios. These include L-ascorbate oxidase (EC 1.10.3.3), laccase (EC 1.10.3.2) and ceruloplasmin (ferro-oxidase, EC 1.16.3.1), the latter two having aromatic diamine and diphenol oxidase activity. There is growing evidence that in these proteins the type-2 and type-3 copper centers are juxtaposed. Recently it has been shown that in L-ascorbate oxidase, a trinuclear copper site is present, consisting of a type-3 copper site, very close (3.9 Å) and possibly bridged to a type-2 copper site [32a]. There is a view that ceruloplasmin functions as a ferro-oxidase and the Fe(III) produced in this reaction can then oxidize the same substrates as laccase.

## 6.5. COPPER CENTERS IN CYTOCHROME OXIDASE

There are two copper centers that appear to be unique. Both are present in cytochrome-*c* oxidase (EC 1.9.3.1). The first appears to be an isolated metal ion and has been referred to as Cu<sub>d</sub> and Cu<sub>A</sub>. The second appears to be part of a dinuclear center with cytochrome *a*<sub>3</sub>. It has been referred to as Cu<sub>u</sub>, Cu<sub>a3</sub> and Cu<sub>B</sub>. At the moment the ascriptions Cu<sub>A</sub> and Cu<sub>B</sub> are most frequently used; however, the recent discovery [32b] of a cytochrome oxidase in which cytochrome *a* has been replaced by cytochrome *b*, leads to the recommendation that Cu<sub>B</sub> shall be referred to as Cu<sub>a3</sub>.

There is a striking similarity between two of the Cu centers of N<sub>2</sub>O reductase and Cu<sub>A</sub> [32c, 32d].

## 7. MOLYBDENUM PROTEINS<sup>11</sup>

### 7.1. MOLYBDENUM ENZYMES (GENERAL)

Molybdenum enzymes are enzymes that contain molybdenum and in which it is involved in the reaction with a

substrate. They may be divided into those that contain the iron-molybdenum cofactor and those that contain the pterin-molybdenum cofactor.

### 7.2. ADDITIONAL CENTERS

If a molybdenum enzyme contains flavin, it may be called either a molybdenum flavoprotein or a flavomolybdenum protein, as indicated above. Other centres should be treated similarly, e.g. an iron-sulfur molybdenum protein.

### 7.3. MOLYBDENUM ENZYMES CONTAINING THE IRON-MOLYBDENUM COFACTOR

The only enzymes at present known to belong to this group are the nitrogenases (EC 1.18.6.1; and EC 1.19.6.1); see pp. 89–116 in [33] and pp. 91–100 in [34].

### 7.4. MOLYBDENUM ENZYMES CONTAINING THE PTERIN-MOLYBDENUM COFACTOR

These enzymes (see pp. 411–415 in [33], and [36]) may be divided into those in which the molybdenum bears a cyanide-labile sulfido (or thio<sup>12</sup>) ligand (i.e. containing the S<sup>2-</sup> ligand as Mo=S) and those lacking this ligand. The former group includes xanthine oxidase (EC 1.1.3.22), xanthine dehydrogenase (EC 1.1.1.204), aldehyde oxidase (EC 1.2.3.1) and purine hydroxylase (EC: see footnote 2)<sup>11</sup>. These may be called 'molybdenum-containing hydroxylase' as is widely done. Molybdenum enzymes lacking the sulfide (thio) ligand include sulfite oxidase (EC 1.8.3.1), NAD(P)<sup>+</sup>-independent aldehyde dehydrogenase and nitrate reductase (assimilatory and dissimilatory) (EC 1.6.6.1–3).

### 7.5. OTHER MOLYBDENUM ENZYMES

Molybdenum enzymes not yet sufficiently characterized for them to be assigned to classes include formate dehydrogenase (EC 1.2.1.2), carbon-monoxide oxidase (EC 1.2.3.10), tetrathionate reductase (EC 1.8.2.2), chlorate reductase (EC 1.97.1.1), trimethylamine-*N*-oxide reductase (EC 1.6.6.9) and biotin-*S*-oxide reductase.

## 8. NICKEL PROTEINS

Nickel has now been shown to be an integral component of a number of redox enzymes. Many but not all hydrogenases contain nickel in addition to iron-sulfur clusters, and it appears that it is a common constituent of those hydrogenases whose function is to oxidize rather than to evolve hydrogen. The nickel center can undergo facile changes in oxidation number. Evidence has been presented that hydrogen, as well as the competitive inhibitor carbon monoxide, can bind directly to nickel [37, 38], implying that the nickel center might be the active site of these enzymes.

A nickel-tetrapyrrole coenzyme, Co-F430, is present in the methyl CoM reductase and in methanogenic bacteria; the tetrapyrrole is classed as a tetrahydrocorphinoid, being inter-

<sup>11</sup> An expert panel is advising NC-IUB on the listing of these enzymes.

<sup>12</sup> Inorganic chemists also use 'thio' for the S<sup>2-</sup> ligand, especially when this ligand is bound to one metal only, forming a M=S unit. The fully systematic name 'sulfido' is to be preferred, since this applies to both the M=S unit and the bridging S<sup>2-</sup> ligand.

mediate in structure between porphyrin and corrin. Changes in redox state, as well as changes in nickel coordination, have recently been observed with enzymes of bacteria [39].

The nickel-containing carbon monoxide dehydrogenase (EC 1.2.99.2) is now recognized to be a CO-dependent acetyl-CoA synthase. Little is known about the structure of the nickel site but there is some evidence that it is a component of an iron-sulfur cluster.

## 9. VANADIUM PROTEINS

Vanadium has a role in a number of biological systems. Bromoperoxidases from marine algae contain vanadium as a prosthetic group essential for enzymic activity [40]. These enzymes appear to be involved in the formation of organohalo species secreted by seaweeds. The vanadium ion is present in these enzymes in the 5+ oxidation state (in the resting state); data have not yet been provided that the vanadium undergoes redox changes during catalysis. The vanadium ion seems to serve as the binding site for hydrogen peroxide and bromide.

Evidence [41] has been presented that in bacteria two nitrogen fixing systems are present: the conventional molybdenum nitrogenase and an alternative nitrogen-reducing enzyme complex. The alternative nitrogenase complexes were shown to contain a vanadium-iron cofactor, analogous to the MoFe cofactor in the conventional enzymes. These VFe nitrogenases differ from the conventional nitrogenases in substrate specificity.

## 10. QUINOPROTEINS

Quinoproteins contain pyrroloquinoline quinone (PQQ; also called methoxatin), or an amino-acid-derived quinone as a cofactor. PQQ, which has been shown to be present in some bacterial dehydrogenases, is non-covalently bound. Other quinoproteins contain an amino-acid residue in their protein chain which is modified to a quinone prosthetic group. Some quinoproteins also contain a redox-active metal ion, and as such some of them may already have been mentioned in previous sections. Two subgroups have already been classified:

a) *Dehydrogenases*. Many gram-negative bacteria contain quinoprotein dehydrogenases [42], such as methanol dehydrogenases (EC 1.1.99.8) which occurs in all gram-negative methanol utilizers investigated so far. Two different types of quinoprotein alcohol dehydrogenases occur in alkane or alcohol-grown *Pseudomonas* species. Glucose dehydrogenase also occurs in two quite different types, the soluble (EC 1.1.99.17) and the membrane-bound ones. Amine dehydrogenase (EC 1.4.99.3) is the first quinoprotein for which the 3-dimensional structure has been elucidated by X-ray diffraction [43]. Its cofactor consists of a tryptophan dimer [44] of which one contains the *o*-quinone group.

b) *Oxidases*. The only bacterial quinoprotein oxidase is methylamine oxidase [45]. Mammalian examples are pig kidney diamine oxidase and bovine serum amine oxidase (both EC 1.4.3.6). All these oxidases contain also copper as a second redox center and they all form hydrogen peroxide as a product. The organic prosthetic group is one of the tyrosine residues in the protein chain, which is modified to topaquinone or to 6-hydroxydopaquinone [46].

When the presence of such a quinone has been established, it is recommended that the name of the protein be prefixed with: 'quinone-containing'.

## 11. METAL-SUBSTITUTED METALLOPROTEINS

Scientists from several areas, dealing with spectroscopy and electron-transfer mechanisms, often use metalloproteins in which a metal at the site has been substituted by another metal ion, like Co, Zn, Hg, Cd. Examples are zinc-substituted cytochromes and cobalt-substituted ferredoxins.

The names for such modified proteins are easily given by using indications like 'zinc-substituted...'. In case of multi-metal proteins, where ambiguity might arise about which metal has been substituted, one could easily add in parentheses the name of the metal that has been replaced, such as: cobalt-substituted [Fe]nitrogenase.

In formulae fragments or short names one could use the following notation:  $[3\text{Fe}1\text{Co-4S}]^{2+}$ , cytochrome  $c'[\text{Fe} \rightarrow \text{CoFe}]$ , plastocyanin $[\text{Cu} \rightarrow \text{Hg}]$ . When needed the particular isotope of the new metal can be also mentioned, as in plastocyanin $[\text{Cu} \rightarrow ^{113}\text{Cd}]$ .

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