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ARTICLE *in* ARCHIVES OF BIOCHEMISTRY AND BIOPHYSICS · SEPTEMBER 2004

Impact Factor: 3.02 · DOI: 10.1016/j.abb.2004.03.037 · Source: PubMed

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Quinohemoprotein alcohol dehydrogenases: structure, function, and physiology

Hirohide Toyama, F. Scott Mathews, Osao Adachi, and Kazunobu Matsushita*

Department of Biological Chemistry, Faculty of Agriculture, Yamaguchi University, Yamaguchi, Yamaguchi 753-8515, Japan
Department of Biochemistry and Molecular Biophysics, Washington University of School of Medicine, St. Louise, MO 63110, USA

Abstract

Quino(hemo)protein alcohol dehydrogenases (ADH) that have pyrroloquinoline quinone (PQQ) as the prosthetic group are classified into 3 groups, types I, II, and III. Type I ADH is a simple quinoprotein having PQQ as the only prosthetic group, while type II and type III ADHs are quinohemoprotein having heme *c* as well as PQQ in the catalytic polypeptide. Type II ADH is a soluble periplasmic enzyme and is widely distributed in Proteobacteria such as *Pseudomonas*, *Ralstonia*, *Comamonas*, etc. In contrast, type III ADH is a membrane-bound enzyme working on the periplasmic surface solely in acetic acid bacteria. It consists of three subunits that comprise a quinohemoprotein catalytic subunit, a triheme cytochrome *c* subunit, and a third subunit of unknown function. The catalytic subunits of all the quino(hemo)protein ADHs have a common structural motif, a quinoprotein-specific superbarrel domain, where PQQ is deeply embedded in the center. In addition, in the type II and type III ADHs this subunit contains a unique heme *c* domain. Various type II ADHs each have a unique substrate specificity, accepting a wide variety of alcohols, as is discussed on the basis of recent X-ray crystallographic analyses. Electron transfer within both type II and III ADHs is discussed in terms of the intramolecular reaction from PQQ to heme *c* and also from heme to heme, and in terms of the intermolecular reaction with azurin and ubiquinone, respectively. Unique physiological functions of both types of quinohemoprotein ADHs are also discussed.

Keywords: Alcohol dehydrogenase; PQQ; Quinoprotein; Quinohemoprotein; Cytochrome *c*; Ubiquione; Azurin; Proteobacteria; Intramolecular electron transfer; Intermolecular electron transfer; Acetic acid bacteria; *Pseudomonas putida*

Pyrroloquinoline quinone (PQQ)[1] has been found in as many as 10 different species of enzymes working mainly on the dehydrogenation of the primary or secondary hydroxyl group in alcohols or sugars [1]. In these enzymes, PQQ is non-covalently bound to the apoenzyme. These enzymes also contain 1 mol Ca^{2+}/mol of PQQ that is coordinated both by PQQ and by several amino acid side chain atoms and is required for activity [1,5]. Some of these quinoproteins have, together with PQQ, an additional prosthetic group, heme *c*, within a single polypeptide; these are called quinohemoproteins to distinguish them from the quinoproteins which contain only the quinone cofactor as the prosthetic group [2].

Alcohol dehydrogenase (ADH) is widely distributed in many different types of organisms ranging from bacteria to mammals. It is most often an NAD(P)-dependent enzyme present in the cytoplasm. In contrast, PQQ-dependent ADHs are rather unique and are found in only a narrow range of species of bacteria, the α, β, and γ-proteobacteria, and are localized only in the periplasmic fraction. PQQ-dependent ADH includes

* Corresponding author. Fax: +81-83-933-5858.
E-mail address: kazunobu@yamaguchi-u.ac.jp (K. Matsushita).

[1] *Abbreviations used*: ADH, alcohol dehydrogenase; ADH I, quinoprotein alcohol dehydrogenase of *Psudomonas putida* HK5; ADH IIB/IIG, two different quinohemoprotein alcohol dehydrogenases of *P. putida* HK5; BDH, 1-butanol dehydrogenase (type II); BOH, 1-butanol dehydrogenase (type I); GLDH, glycerol dehydrogenase; MDH, quinoprotein methanol dehydrogenase; PEGDH, polyetheylene-glycol dehydrogenase; PPGDH, polypropyleneglycol dehydrogenase; PQQ, pyrrolo-quinoline quinone; PVADH, polyvinylalcohol dehydrogenase; Q_2, ubiquinone-2; Q_{10}, ubiquinone-10; qEDH, quinoprotein ethanol dehydrogenase; qhEDH, quinohemoprotein ethanol dehydrogenase; THFADH, tetrahydrofurfurylalcohol dehydrogenase; UQ, ubiquinone.

both quinoprotein- and quinohemoprotein-type enzymes as described above. Some are soluble in the periplasm and the others are bound to the outer surface of the cytoplasmic membrane. Thus, like other quinoprotein dehydrogenases, quino(hemo)protein ADH forms a so-called ''periplasmic alcohol oxidase system'' together with the membrane-bound respiratory chain. They function as primary dehydrogenases, which transfer reducing equivalents directly to the bacterial aerobic respiratory chain, in the periplasm. As such they have a truncated, and thus less energy-efficient, respiratory chain that leads to a direct oxidation of substrate without any energy-consuming uptake of the substrate or excretion of the oxidized products [2].

PQQ-dependent ADH is the largest enzyme group within the quinoprotein family. Its members consist of 18 different enzymes (see Table 1) and are classified into 3 groups, type I, II, and III ADHs. Type I ADH found in a limited number of Proteobacteria are very similar to quinoprotein methanol dehydrogenase (MDH) in methylotrophs, and are simple quinoproteins having PQQ as the only prosthetic group; they can be differentiated with respect to substrate specificity (see C. Anthony in this volume). Unlike type I ADH, type II and III ADHs are quinohemoproteins or contain a quinohemoprotein as one of its subunits, respectively. Type II ADH is an enzyme soluble in the periplasm and has a relatively wide distribution among several Proteobacteria. Type III ADH is membrane-bound, working on the periplasmic surface and is unique to acetic acid bacteria. The latter consists of three subunits, two of which comprise a quinohemoprotein catalytic subunit and a triheme cytochrome *c* subunit.

In this article, the main foci among these PQQ-dependent ADHs are the quinohemoproteins ADH II and ADH III. Their structural features are summarized and discussed, based on the genetic information and the recent X-ray crystallographic analyses, and are compared with MDH and type I ADH. Furthermore, their electron transfer properties are discussed in terms of intramolecular transfer from PQQ to heme *c* or from one heme *c* to another heme *c*, and in terms of the intermolecular electron transfer to a blue copper protein or ubiquinone (UQ).

Table 1
Quinoprotein and quinohemoprotein alcohol dehydrogenases and the related enzymes

Enzymes	Location[a] S or M	Sources (Reference)
Quinoproteins able to oxidize alcohols		
Methanol dehydrogenase (MDH)	S	Methylotrophs [40]
Type I ADH (PQQ)		
Ethanol dehydrogenase (qEDH)	S	*Pseudomonas aeruginosa* [50]
Alcohol dehydrogenase (ADH I)	S	*Pseudomomas putida* [11]
1-Butanol dehydrogenase (BOH)	S	*Pseudomonas butanovora* [13]
Polypropylenegycol dehydrogenase (PPGDH)	S	*Stenotrophomonas maltophilia* [3]
Other Quinoproteins able to oxidize alcohols (PQQ)		
Sorbose/Sorbosone dehydrogenase	S	*Gluconobacter* species [4] *Pseudogluconobacter saccharoketogenes* [5]
Glycerol dehydrogenase (GLDH)	M	*Gluconobacter* species [6,7]
Quinohemoproteins able to oxidize alcohols		
Type II ADH (PQQ/heme *c*)		
Vanillyl alcohol or polyethyleneglycol dehydrogenase (PEGDH)	S	*Rhodopseudomonas acidophila* [8–10]
Ethanol dehydrogenase (qhEDH)	S	*Comamonas testosteroni* [47]
Alcohol dehydrogenase (ADH IIB)	S	*Pseudomonas putida* [11]
Alcohol dehydrogenase (ADH IIG)	S	*Pseudomonas putida* [11]
Polyvinylalcohol dehydrogenase (PVADH)	S	*Pseudomonas* sp. [26]
Tetrahydrofurfuryl alcohol dehydrogenase (THFADH)	S	*Ralstonia eutropha* [46]
1-Butanol dehydrogenase (BDH)	S	*Pseudomonas butanovora* [12]
Type III ADH (PQQ/heme *c* /3 hemes *c*)		
Alcohol dehydrogenase	M	*Acetobacter aceti* [20,21]
Alcohol dehydrogenase	M	*Acetobacter pasteurianus* [22]
Alcohol dehydrogenase	M	*Gluconoactobacter polyoxygens* [23]
Alcohol dehydrogenase	M	*Acidomonas methanolicus* [24]
Alcohol dehydrogenase	M	*Gluconobacter suboxydans* [25]
Other related quinohemoproteins		
Lupanine hydroxylase (PQQ/heme *c*)	S	*Pseudomonas* sp. [15]
Amine dehydrogenase (CTQ/heme *c*)	S	*Pseudomonas putida* [16] *Paraccocus denitrificans* [17]

[a] Soluble (S) or Membrane-bound (M).

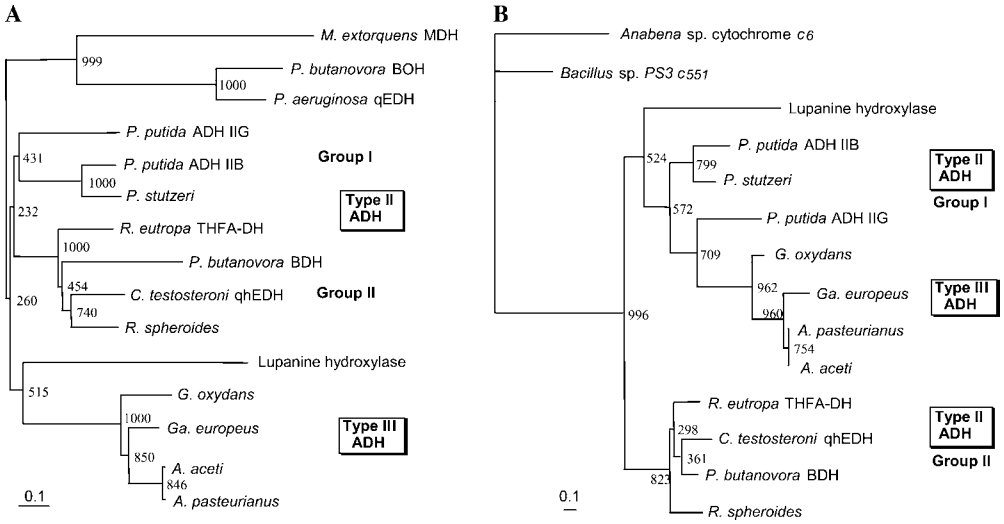

Fig. 1. Phylogenetic tree for the PQQ domain (A) and the heme *c* domain (B) of quinoprotein and quinohemoprotein alcohol dehydrogenases.

Species and distribution of quinoprotein and quinohemoprotein alcohol dehydrogenases

As shown in Table 1, four different type I quinoprotein ADHs have been isolated and identified so far from several *Pseudomonas* species and from a single *Stenotrophomonas* species, both belonging to γ-Proteobacteria. These are all soluble enzymes and seemingly work in the periplasm. Of these enzymes, polypropylene glycol dehydrogenase (PPGDH) [3] is somewhat exceptional in terms of substrate specificity. Two additional enzymes, soluble sorbose/sorbosone dehydrogenase [4,5] and membrane-bound glycerol dehydrogenase (GLDH) [6,7], can also be regarded as a type I ADH, even though their physiological substrates seem not to be alcohol, since these enzymes are also capable of oxidizing several alcohols with high efficiency. Of type II ADHs, seven different quinohemoproteins have been isolated from several Proteobacteria such as *Rhodopseudomonas*, *Pseudomonas*, *Ralstonia*, and *Comamonas* species (Table 1). In addition, several quinohemoprotein genes exhibit sequence similarity to type II ADH, and are found in genome sequence such as *Pseudomonas stutzeri* and *Rhodopseudomonas palustris* (not shown). Although quinohemoprotein polyethylene glycol dehydrogenase (PEGDH) and vanillyl alcohol dehydrogenase were isolated from the same strain, it seems that they are a single enzyme because both isolated enzymes have similar structural and functional properties [8–10]. Two type II ADHs, ADH IIB and ADH IIG, are produced in the same bacterial strain, *Pseudomonas putida* HK5, together with another type I ADH, ADH I, depending on the nature of the substrate alcohols added to the culture medium as the carbon and energy sources [11]. 1-Butanol dehydrogenase (BDH) is also produced simultaneously with another 1-butanol dehydrogenase (BOH) which is a type I ADH in a single strain, *Pseudomonas butanovora* [12–14]. In this case, both type I and type II enzymes are induced together with the same alcohol.

Included in Table 1 are two additional related quinohemoproteins, lupanine hydroxylase and amine dehydrogenase, isolated from *Pseudomonas* and *Paracoccus* species, even though neither have any alcohol oxidizing ability at all. Lupanine hydroxylase contains 1 mol each of PQQ and heme *c*, and the amino acid sequence has a high degree of similarity to the type II ADHs (Fig. 1). However, instead of the dehydrogenation of a hydroxyl group, it catalyzes dehydrogenation

Fig. 2. Alignment of amino acid sequences of quinohemoprotein ADHs. Residue numbers are presented without signal sequence and start at the N-terminal amino acid in the mature protein as 1. W-motifs (W1 to W8) are indicated as black boxes. Segments S1 and S2 are shown as blue boxes, and loop 4 (L4) as a red box. Amino acid residues involved in PQQ binding are colored red, and those involved in heme *c* binding are colored pink. The residues found to make contact with the substrate/product in the crystal structures are shown in green. 2ClEtDH is 2-chloroethanol dehydrogenase from *Pseudomonas stutzeri* (AF176640_2), and ADH2_Rhod. is a hypothetical protein in *Rhodoseudomonas palustris* (ZP_00012705). ADH3_Apoly, ADH3_Geuro, ADH3_Apast, ADH3_Aacet, and ADH3_Goxyd are the sequences of the dehydrogenase subunit of ADH from *Acetobacter polyoxogenes* (DHET_ACEPO), *Gluconacetobacter europaeus* (DHET_ACEEU), *Acetobacter pasteurianus* (BAA40252), *Acetobacter aceti* (DHET_ACEAC), and *Gluconobacter oxydans* (DHET_GLUOX), respectively.

Fig. 3. The equatorial interactions of PQQ with nearby amino acid residues and the coordination of the calcium ion in the active site of ADH IIB, qhEDH, qEDH, and MDH (from *M. extorquens* AM1) as determined in the crystal structures.

②

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ADH IIB:      -22 MKKPLRTSLLMLCLATPLAALA----------------------------------AGVDEA-
2ClEtDH:      -23 MPNLTQSRRLVCLLTALLVAAGA----------------------------------QAAKVDEA-
THFA-DH:      -30 MHSNKLRLLVCVMAAASVALPAPMAPGANA---------------------------AARVDGA-
ADH2_Rhod.:   -42 MRKPADAVRTAIRPVQQPAIRLVARRLGAAALMAALLTPAVA-AAQDSKGSAAHIRAVTGAVDSA-
qhEDH:        -31 MERLIDNSHGWPGRMVWLLAACLGSAAAFAQ----------------TGPAAQAAAAVQRVDGD-
ADH3_Geuro:   -35 MISAVFGKRRSLSRTLTAGTICAALISGYATMASA-------------------DDGQGATGEA-
ADH3_Apast:   -35 MTRPASAKRRSLLGILAAGTICAAALPYAAVPARA--------------------DQGGNTGEA-
ADH3_Goxyd:   -24 MTSGLLTPIKVTKKRLLSCAAALA-----------------------------FSAAVPVAP
qEDH_Paeru:   -34 MTTRTSPAPAGLLRPSLHCLAFAVALGSAGAALA-----------------------KDVTWE-
MDH_Mextor:   -27 MSRFVTSVSALAMLALAPAALSSGAYA-------------------------------ND-

                  W8                                                    L4                                                  S1                                                  W6
  7 -------AIRATEQAG--GEWLSHGRTYAEQRFSPLKQIDASNVDGLGLAWVMDL--DNTRGLEATPLFHDGVIYTSMSN
  9 -------AIRASEQDG--SEWLSHGRTYAEQRFSPLKQIDAGNVCKLGLAWVLDL--ENNRGLEATPLVSDGVLYASLSW
  8 -------AIRANEAGT--PNWPSYGLDYAETRFSKLEQVNAGNVFNLGLAWSYDL--ESTRGVEATPLVVDGVMYVSAPW
 23 -------AIVANVKTT--KDWPSYGLDYAETRFSKLDQINTENVKQLGLQWSYSL--GSERGVEATPVVVDGIMYVTASW
 18 -------FIRANAART--PDWPTIGVDYAETRYSRLDQINAANVKDLGLAWSYNL--ESTRGVEATPVVVDGIMYVSASW
 11 -------IIHADDHP---GNWMTYGRTYSEQRYSPLDQINRSNVGNLKLAWYLDL--DTNRGQEGTPLVIDGVMYATTNW
 10 -------IIHADDHP---ENWLSYGRTYSEQRYSPLDQINRSNVGDLKLAWYTYL--DTNRGQEATPLVVDGIMYATTNW
 10 AQEDTGTAITSSDNGGHPGDWLSYGRSYSEQRYSPLDQIINTENVGKLKLAWHYDL--DTNRGQEGTPLIVNGVMYATTNW
  7 -------DIANDDKTT--GDVLQYGMGTHAQRWSPLKQVNADNVFKLTPAWSYSFGDEKQRGQESSQAIVSDGVIYVTASY
  3 -------KLVELSKSD--DNWVMPGKNYDSNNFSDLKQINKGNVKQLRPAMTFST--GLLNGHEGAPLVVDGKMYIHTSF

                  W1                                                    W2                                                  W7
 76 SR---VIAVDAASGKELWRYDPEVAKVKARTSCCDAVNRGVALW-GDK-----VYVGTLDGRLIALDAKTGKAIWSQQT-
 78 SR---VMAVDLRSGKRLWQFDPQVDRGHSRYTCCDAVNRGVALW-NGK-----VYVGALDGRLIALDAKTGRELWSEQT-
 77 SV---VHAIDARTGKRLWTYDPQVPRDQAYKGCCDVVNRGVALY-QGK-----VFVGAFDGRLVAIDAATGKKVWEQDTI
 92 SV---VHAIDTRTGKKLWTFDPKVDHSKGYRGCCDVVNRGVALY-KGK-----VFVGAYDGRLIALDAATGSKAWEIDT-
 87 SV---VHAIDTRTGNRIWTYDPQIDRSTGFKGCCDVVNRGVALW-KGK-----VYVGAWDGRLIALDAATGKEVWHQNT-
 79 SM---MKAVDAATGKLLWSYDPRVPGNIADKGCCDTVNRGAAYW-NGK-----VYFGTFDGRLIALDAKTGKLVWSVNT-
 78 SK---MEALDAATGKLLWQYDPKVPDNIADKGCCDTVNRGAGYW-NGK-----VFWGTFDGRLVAADAKTGKKVW-EVN-
 88 SK---MKALDAATGKLLWSYDPKVPGNIADRGCCDTVSRGAAYW-NGK-----VYFGTFDGRLIALDAKTGKLVWSVYT-
 78 SR---LFALDAKTGKRLWTYNHRLP--DDIRPCCDVVNRGAAIY-GDK-----VFFGTLDASVVALNKNTGKVVWKKKF-
 72 PNNTFALGLD-DPGTILWQDKPKQNPAARAVACCDLVNRGLAYWPGDGKTPALILKTQLDGNVAALNAETGETVWKVEN-

                                                     W3                                                  W8                                                  S2
146 T-DPA-------K-PYSITGAPRVVK----GKVII--GNGGAEYGVRGFVSAYDADTGKLAW-------------RFYT
148 T-DPA-------K-PYSITGAPRVVK----GKVII--GNGGAEYGVRGFPSAYDAETGKMAW-------------RFYT
148 V-DRS-------K-SYTITGAPRVYN----GKVII--GNGGAEYGVRGYITAYDAETGKQQW-------------RWYT
162 LIDHE-------H-SYTITGAPRVFN----GKVVI--GNGGAEYGARGYVTAYDAETGKQAW-------------RWFT
157 F-EGQ-------KGSLTITGAPRVFK----GKVII--GKRGAEYGVRGYITAYDAETGERKW-------------RWFS
149 I-PPEAELGKQ-R-SYTVDGAPRIAK----GRVII--GNGGSEFGARGFVTAFDAETGKVDW-------------RFFT
147 T-IPADASLGKQR-SYTVDGAVRVAK----GLVLI--GNGGAEFGARGFVSAFGAETGKLKW-------------RFYT
158 I-PKEAQLGHQ-R-SYTVDGAPRIAK----GKVLI--GNGGAEFGARGFVSAFDAETSKLDW-------------RFFT
146 A-DHG-------A-GYTMTGAPTIVKDGKTGKVLLIHGSSGDEFGVVGRLPARDPDTGEEIWMRPFVEGHMGRLNGKDST
150 S-DIK-------V-GSTLTIAPYVVK----DKVII--GSSGAELGVRGYLTAYDVKTGEQVW-------------RAYA

197 VPG---D---PAL------PYEHPE--------LRE---AAKTWQ-GDQYW-KL-GGGGTVWDSMAYDPELDLLYVGTGN
199 VPG---D---PAQ------PYEHPE--------LAE---AAKTWK-GDQYW-KL-GGGGTVWDSMAYDPELDLLYIGTGN
199 VPG---D---PAR------PFENEA--------MAK---AAATWDPSGKYW-IN-GGGGTVWNTMAFDPELNLMYIGTGN
214 VPG---D---PSK------PFEDES--------MEK---AAKTWDPAGKWW-LN-GGGGTAWDTITFDPDLNLIYVGTGN
209 VPG---D---PSK------PFEDES--------MKR---AARTWDPSGKWW-EA-GGGGTMWDSMTFDAELNTMYVGTGN
206 APN---------------PKNEPDHTASDSVLMNK---AYQTWS-PTGAWTRQ-GGGGTVWDSIVYDPVADLVYLGVGN
205 VPN---N---KN-------ETDHAASDNIL---MKK---AYKTWG-PKGAWERQ-GGGGTVWDFLVYDPVSDLIYLAVGN
215 VPN---------------PENKPDGAASDDILMSK---AYPTWG-KNGAW-KQGGGGTVWDSLVYDPVTDLVYLGVGN
217 VTG---DVKAPSW------PDDRNS--------PTG---KVESWS----------HGGGAPWQSASFDAETNTIIVGAGN
201 TGP---D---KDLLLASDFNIKNPH--------YGQKGLGTGTWE-GDA-W-KI-G-GGTNWGWYAYDPGTNLIYFGTGN

                  W4
251 GSPWNREVRSPGGG-----DNLYLSSIIALRPDTGKLAWHVQVTPGDSWDFTATQQITLAEL-NI-DGKPRKVLMQAPKN
253 GSPWNREIRSPGGG-----DNLYLSSIIALRPDSGKLLWHYQTTPGETWDFTATQQITLATL-EL-DGKPRKVLMQAPKN
254 AGPWSRKLRSPKGG-----DNLYAASVVALNPDTGEYVWHYQETPGDNWDYTSTQDIILADL-KI-DGQPRKVILHAPKN
269 GSPWNRHLRSPAGG-----DNLYLASIVALNADTGKYVWHYQETPGDNWDYTSTQPMILADI-AI-DGKPRKVILHAPKN
264 GSPWSHKVRSPKGG-----DNLYLASIVALDPDTGKYKWHYQETPGDNWDYTSTQPMILADI-KI-AGKPRKVILHAPKN
265 GSPWNYKYRSEGKG-----DNLFLGSIVALKPETGEYVWHFQETPMDQWDFTSVQQIMTLDL-PI-NGETRHVIVHAPKN
264 GFPWNYKYRSEGIG-----SNLFLGSIVALKPETGEYVWHFQATPMDQWDYTSVQQIMTLDM-PV-NGEMRHVIVHAPKN
274 GSPWNYKFRSEGKG-----DNLFLGSIVAINPDTGKYVWHFQETPMDEWDYTSVQQIMTLDM-PV-NGEMRHVIVHAPKN
267 PGPWNTWARTAKGGNPHDYDSLYTSGQVGVDPSSGEVKWFYQHTPNDAWDFSGNNELVLFDY-KAKDGKIVKATAHADRN
262 PAPWNETMRP---G-----DNKWTMTIHGRDADTGEAKFGYQKTPHDEWDYAGVNVMMLSEQKDK-DGKARKLLTHPDRN

652 VMSREYEDYMARHKAAP 668
655 VMSREYEDHLKAQQAARP 672
656 IQGT--ADAVRPRKP 668
671 IQGT--ADAIRPKK 682
666 IQGT--ADAI-RPKP 677
678 LIKRANETYQREVDARKNADGIPEQLP 704
676 LIKRANDTYQREVDARKNDKNIPENPTLGINP 707
691 IVKRANESYDDEVKARENSTGVPNDQFLNVPQSTADVPTADHP 733
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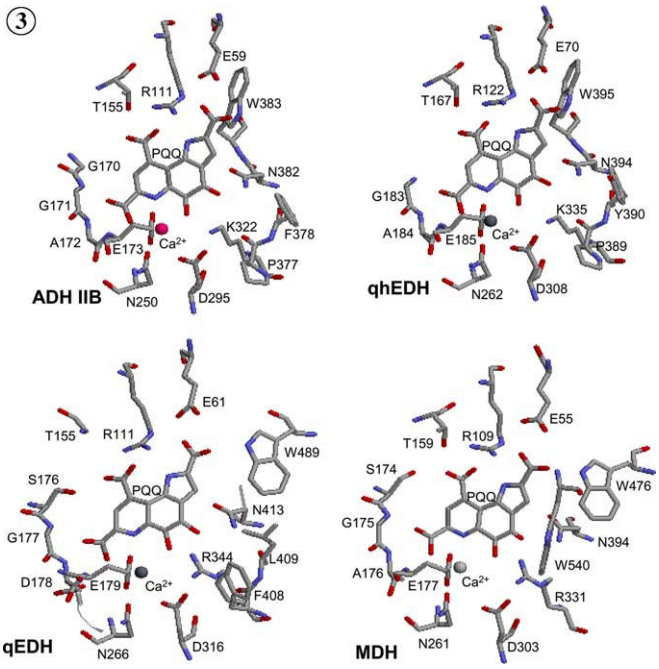

of an imine group, followed by hydration of the product to yield 17-hydroxylupanine [15]. The amine dehydrogenase that was recently isolated and crystallized is a completely different quinohemoprotein, containing a covalently bound cysteine tryptophyl quinone cofactor and two heme *c* moieties in an αβγ heterotrimer [16–19]. Although this amine dehydrogenase is dissimilar to quinohemoprotein ADH, it has similar characteristics to type II and III ADHs in terms of the intramolecular and intermolecular electron transfer steps.

Type III ADH consists of three subunits, a quinohemoprotein subunit I, a triheme *c*-type cytochrome subunit II, and subunit III that contains no cofactor; it is found only in acetic acid bacteria, a single group of α-Proteobacteria. Five different ADHs of this type have been isolated from *Acetobacter*, *Gluconacetobacter*, *Acidomonas*, and *Gluconobacter* species [20–25].

The quinoprotein and quinohemoprotein ADHs listed in Table 1, including MDH, have a relatively high degree of sequence similarity, with over 30% sequence identity overall within nearly 600 amino acid residues, and thus form a large superfamily (Fig. 2). Type II ADHs have higher sequence similarity, with over 50% identity to each other in about 670 residues. They also display about 44% sequence identity in about 715 amino acids with the quinohemoprotein subunit of the membrane-bound type III ADHs. The type III ADHs of acetic acid bacteria exhibit even higher similarity to each other, rising to over 70% identity. The one exception to the high sequence similarity among quinohemoproteins is polyvinylalcohol dehydrogenase (PVADH) [26], which exhibits only about 24% sequence identity with any other types of quinohemoprotein ADH and of quinoprotein ADHs. PVADH differs from the others in another respect because its heme *c* domain is contained in the N-terminal portion of the amino acid sequence, instead of the C-terminal portion, as in all the other quinohemoprotein ADHs [27]. Thus, PVADH is exceptional among these quinoproteins.

A phylogenetic relation of the PQQ-domain among these PQQ-dependent ADHs can be drawn as shown in Fig. 1A. Type I ADH is closely related to MDH, and thus both are evolutionary related. Although a little distant from type I ADH, the PQQ-domain of type II ADHs and the quinohemoprotein subunit of type III ADHs are closely related to each other, and seem to be derived from type I ADH or MDH. Type III ADHs are clearly more closely related to each other, while type II ADH is more divergent and can be separated into two groups. Group I enzymes are from *P. putida* and *P. stutzeri*, both of which are γ-Proteobacteria. Group II enzymes are from *Ralstonia eutropha*, *P. butanovora* (seemingly in the genus *Azoarcus*), *Comamonas testosteroni*, and *R. palustris*, all of which are classified as α, β-Proteobacteria. As described above, lupanine hydroxylase, surprisingly, has a relatively high homology to type II ADH with ~37% identity and to type III ADH with 34–36% identity.

In quinohemoprotein ADHs, the sequence conservation within the PQQ domains is considerably greater than within the cytochrome domain. The cytochrome domains are more divergent, having approximately 30% identity while the respective quinoprotein domains retain roughly 55–60% identity. Since the heme *c* domains are equally well conserved within the groups I and II of type II ADH, discrimination is based on their PQQ-domain. Among the type III ADHs (Fig. 1B), however, both the heme *c* domain and the PQQ-domain seem to have a strong evolutionary relatedness among them. The heme *c* domains of quinohemoproteins also have some sequence homology to a cytochrome *c* subunit of nitrite reductase of *P. aeruginosa* (30% to that of ADH IIB), to a cytochrome c_6 of *Anabaena* sp. PCC7120 (26%), and to cytochrome c_{551} of *Bacillus* sp. PS3 (29%). When the phylogenetic tree of the heme *c* domains of quinohemoproteins is drawn and compared with the cyanobacteria and bacillus cytochromes, the heme domain is divided into two groups, each containing several type II ADHs; the cytochrome domain of type III ADH thus seems to have evolved from those type II ADHs from *P. putida* and *P. stutzeri* (Fig. 1B).

Structural characteristics and alcohol reacting site of quinohemoprotein alcohol dehydrogenases

All the PQQ-containing quinoproteins have a common basic structure, a "propeller fold" superbarrel made up of eight 4-stranded anti-parallel β-sheets arranged with radial symmetry like the blades of a propeller. The anti-parallel β-sheets have the shape of the letter "W" and are called "W motifs." When the amino acid sequences of the W motifs among type I to type III ADHs are compared, as shown in Fig. 2, there are 11 amino acid residues in each of the β-sheets that usually retain Ala (A) at the first position, Asp (D) at the 3rd, Gly (G) at the 7th, Lys or Glu (K or E) at 8th, and Trp (W) at the last position. This consensus sequence, Ax-DxxxGK(E)xxW, is relatively well conserved in W1, W2, W3, W4, W6, and W7, but not so in W5 and W8. X-ray crystallographic analyses clearly have shown that the consensus sequence is important in stabilizing the anti-parallel β-sheets of the W-motif [28–30]. Besides the W-motifs, several portions of sequence are well conserved among type I to type III ADHs, as shown in Fig. 2, which include several important residues for PQQ binding and for catalysis (Fig. 3). About 100 amino acids located between the consensus sequences of W5 and W6 have a relatively low degree of sequence homology; this segment contains one of the important structural differences between type I and type II ADHs [31], and appears to define the likely site of substrate

entry into those ADHs having a broad substrate specificity (see below). Type II and III ADHs have an additional stretch in the C-terminal of about 100 amino acids, which conserve the **CXX**C**H**G and G**M** signature sequence involved in heme *c* ligation.

Six structures have been determined by X-ray crystallography: three MDHs from methylotrophs [28,29, 32], the quinoprotein ethanol dehydrogenase (qEDH) from *P. aeruginosa* [30], the quinohemoprotein ethanol dehydrogenase (qhEDH) from *C. testosteroni* [33], and ADH IIB from *P. putida* HK5 [31]. Amino acid residues involved in the binding of PQQ and the calcium ion in the active site are well conserved (Figs. 2 and 3). The volume of the active site cavity, where substrates bind and react on the top of PQQ, can be calculated from the crystal structures of MDH, qEDH, and ADH IIB (Fig. 4), yielding 18, 62, and 120 $Å^3$, respectively. Recently, we succeeded in obtaining the crystal structure of ADH IIG (manuscript in preparation), and its active site cavity volume was calculated to be 150 $Å^3$. These numbers are well correlated with the substrate specificity. MDH and type III ADH have a rather narrow substrate specificity, while type I and especially type II ADHs have a broad substrate specificity and are thus able to carry out a variety of alcohol oxidation reactions [1]. Although type I ADHs, qEDH and ADH I, react well with a relatively larger alcohols as well as ethanol, type II ADHs accommodate alcohol substrates of much larger size and variety in the active site cavity; ADH IIG has broader substrate specificity than ADH IIB.

The side chains of Leu556 or Leu547 in MDH from *Methylobacterium extorquens* AM1 or *Methylophilus methylophilus*, respectively, and Trp557 in qEDH, form a lid-like cover (Fig. 4) to shield the substrate from bulk solvent during the reaction; this cover could flip away in order to provide substrate access to the hydrophobic active site cavity in these enzymes, as is also predicted by docking calculation [30]. In ADH IIB and qhEDH, similar lid-like structures are also formed, in these cases by the side chains of residues Phe419 and Phe425, and by residues Trp440 and Phe606, respectively (Fig. 4). These residues lie on the top of the reacted products found in the crystal structures (see below). However, the heme-domain is located above these residues, and it seems unlikely that the substrate gains access to the active site in a similar manner to qEDH and MDH. Instead, the substrates would probably enter through the hydrophobic mouth of a channel leading to the active site cavity and located on the side of the PQQ-domain in the case of ADH IIB, and between PQQ- and heme-domains in the case of qhEDH (see below). In the case of qhEDH, one amino acid residue (Phe606) that helps to form a hydrophobic wall for the active site cavity is located in the heme-domain, and is part of the heme *c*-binding signature motif, -**C**VF**CH**-. The corresponding amino acid residue in ADH IIB is Val593, which is located just above Phe419 in the crystal structure. Met597 in ADH IIB and Val610 in qhEDH are each residues in the heme-domain and also appear in the hydrophobic channel leading to the active site cavity; the former side chain makes close contact with Phe425. These structural features suggest that the heme-domain would move up and down a little during turnover, which would be caused by the movement of the residues in the active site during catalysis.

Among MDH and qEDH, and the PQQ-domains of ADH IIB and qhEDH, relatively high identity in amino acid sequence is observed, as described above (Fig. 2). The overall three-dimensional structures are also quite similar among them. However, one segment (S1, blue box in Fig. 2) is quite variable in conformation among the structures of PQQ domains (red strands in Fig. 5). This segment is located in the loop region (L4, red box in Fig. 2: residues 398–437 and 410–457 in ADH IIB and qh-EDH, respectively) between strands 2 and 3 of propeller blade 6, and the sequence corresponds to that preceding W6 (Fig. 5). The S1 region of ADH IIB, particularly Phe419 and Phe425, was shown to be close to the bound acetone ligand, the reaction product of 2-propanol (an additive needed to grow the crystals). The residues Trp440 and Phe446 in the *C. testosteroni* enzyme were likewise found to be close to the product, tetrahydrofuran-2-carboxylic acid (Fig. 4). Moreover, the L4 region is open like a 'mouth' in the case of ADH IIB, whereas the two corresponding segments in L4 are closed in the case of qhEDH (Fig. 5), and the 'mouth' accepting the substrate appears to be provided by the S1 portion of L4 and the cytochrome *c* domain, since these entrances are surrounded by hydrophobic amino acid residues. The other segment, S2 (residues 528–538 and 544–553 of ADH IIB and qhEDH, respectively), also shows structural variation (blue strands in Fig. 5). These S1 and S2 segments are also variable in qEDH and MDH (Fig. 5). When the active site is viewed down onto PQQ as in Fig. 4, the residues of the left-side wall in the active-site cavity are well conserved among the quinoproteins (Cys105, Cys106, Glu173, Trp254, and Asp295 in the case of ADH IIB, see Fig. 2). In contrast, other residues making up the active-site wall are variable (Phe419 and Phe425 in S1, Val525 and Thr529 around S2, and Pro377 and Phe378 in the case of ADH IIB), which should lead to determining the substrate specificity.

The three-dimensional structures of ADH IIB and qhEDH were solved by X-ray crystallography with the reacted product, acetone and tetrahydrofuran-2-carboxylic acid, respectively, bound in the active site. These structures provide much valuable information about substrate-binding and the recognition mechanism in quinoprotein enzymes. Although quinohemoprotein ADHs show relatively broad substrate specificity, they also show high enantioselectivity to chirally active

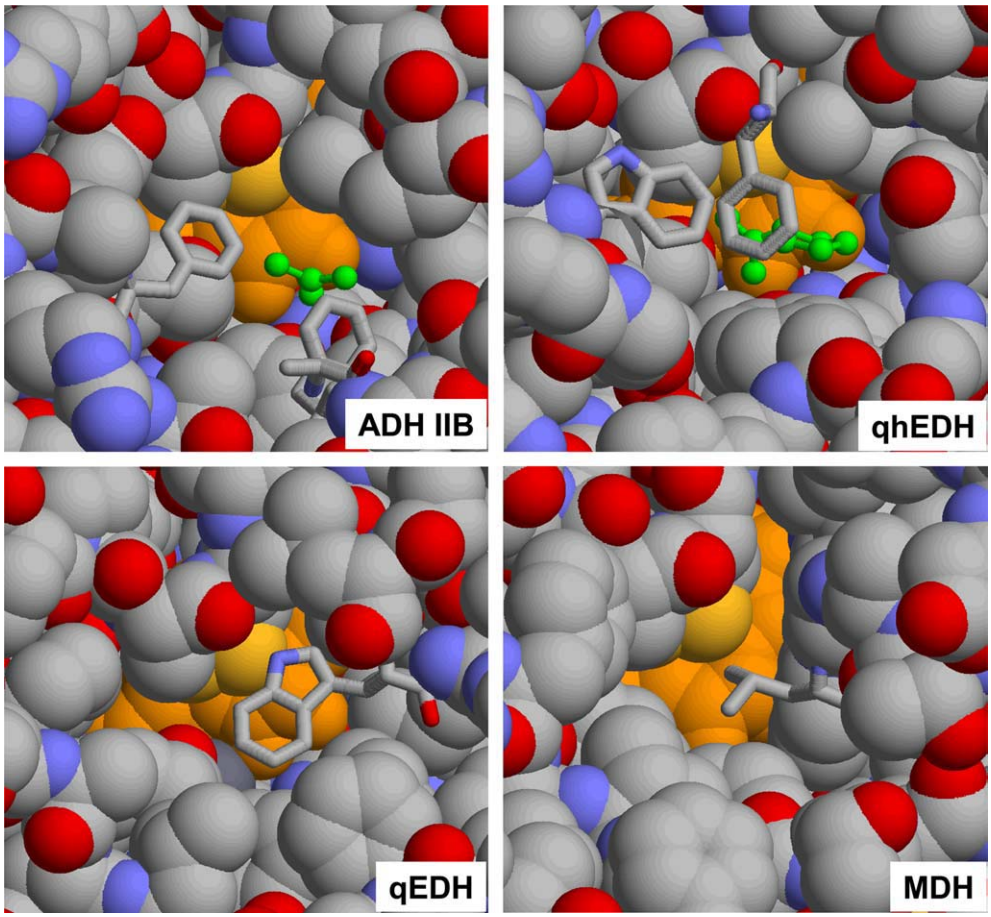

Fig. 4. Active site cavity of quinoprotein ADHs. PQQ and the side chains of the quinoprotein domain that surround the cavity are represented as spacefilling models. PQQ is colored orange. The reaction products found in the cavity of ADH IIB and qhEDH are shown as green ball-stick models. The residues (Phe419 and Phe425, Trp440 and Phe606, and Trp557 and Leu556 in ADH IIB, qhEDH, qEDH, and MDH, respectively) that cover on the top of the substrate/product-binding pocket are drawn as stick models, to show the cavity well.

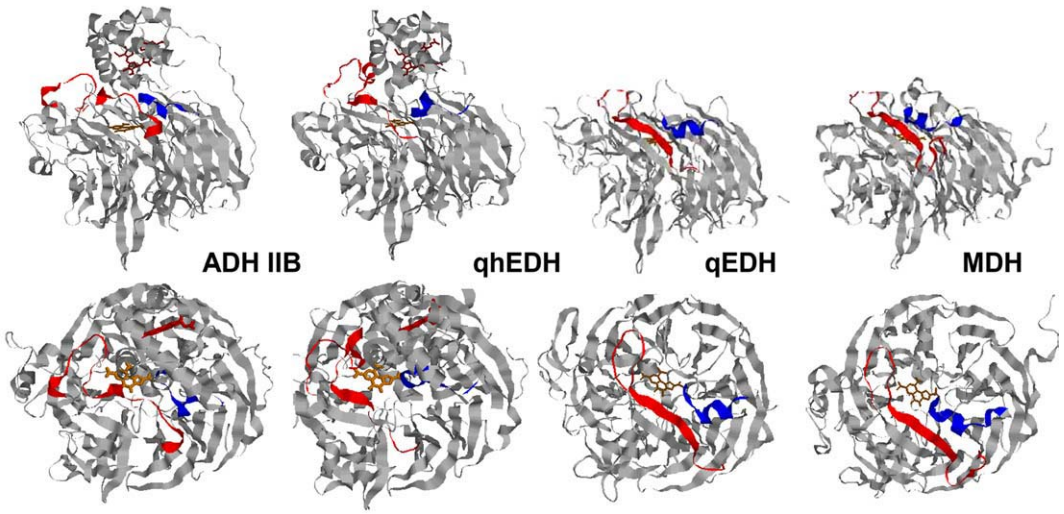

Fig. 5. The overall structures of quinoprotein ADHs are drawn as ribbon models. The PQQ and heme *c* moieties are shown as stick models, colored orange and red, respectively. The S1 and S2 regions described in Fig. 2 are colored red and blue, respectively. The top panel shows a side view of the superbarrel of the PQQ-domain/subunit, and the bottom panel shows a view looking down the pseudo 8-fold axis to PQQ.

secondary alcohols and to primary alcohols with chiral center such as solketal [34], an important building block for synthesis of homochiral pharmaceuticals. In qhEDH, the tetrahydrofuran-ring of the product is directed toward residue Tyr390 and there is enough space to accommodate a bigger organic group, while in the case of qEDH and MDH of *M. extorquens* AM1, Leu409 and Trp540, respectively, occupy the corresponding space and makes the active site cavity smaller (Fig. 4). Recently, we successfully obtained the crystal structures of the complexes of ADH IIB with the alcohol substrates (manuscript in preparation). It is found that the hydroxyl group of the alcohols points toward the calcium ion, and the hexanyl group of 1-hexanol and butyl group of *S*-(+)-2-hexanol are directed toward Phe378, which corresponds to residue Tyr390 in qhEDH. In the complex with 2-hexanol, the methyl group is directed upward from PQQ, and no side chain bigger than a methyl group could be accommodated in the active site in that direction. Thus, *S*-(+)-2-hexanol is preferred over the *R*-(−)-form by ADH IIB, and also by qhEDH.

Electron transfer reactions of quinohemoprotein alcohol dehydrogenasaes

Electron transfer during the oxidative half reaction differs among the three types of ADHs. Type I ADH, like MDH, has been shown to donate electrons directly to a soluble cytochrome *c* [35,36]. A model for such an interaction has been proposed for MDH from *P. denitrificans* [32]. Intermolecular electron transfer for type II ADH appears to involve a soluble copper protein, at least in the case of ADH IIB [37]. On the other hand, the membrane-bound type III ADHs donate electrons directly into the membranous UQ pool [2].

Before these intermolecular electron transfer events occur, in type II and III ADHs, an intramolecular electron transfer step is believed to occur first from PQQ to the covalently bound heme, although this reaction has not been thoroughly characterized as yet. The redox potential of the heme *c* of ADH IIB is +188 mV [37], which is similar to that of another quinohemoprotein, lupanine hydroxylase (+193 mV) [15]. Although the redox potential of PQQ in type II ADH has not been measured, it is expected to be sufficiently lower than that of the heme *c* for electron transfer to take place. Its potential has been measured in soluble glucose dehydrogenase giving values of ~+30 and −10 mV for the reduced/semiquinone and semiquinone/oxidized couples, respectively [38]. A much lower value of −167 mV for the 2-electron potential has been obtained in type III ADH of *Gluconoobacter suboxydans* [39]. Thus, on binding to the enzyme, substrate alcohol would deliver a pair of electrons to the PQQ to generate $PQQH_2$, then one of the two electrons in the cofactor would be expected to be transferred to the heme *c*, leaving the PQQ as the semiquinone form. In fact, ADH IIB, and also type III ADH of acetic acid bacteria, is always isolated with cytochrome *c* in the reduced state, due to the presence of trace amounts of alcohols contaminating in the buffer solution. Therefore, similar to MDH, which is usually isolated in the PQQ semiquinone state [40], ADH IIB and other quinohemoprotein ADHs would have both cofactors, PQQ and heme *c*, each being isolated in the single electron-reduced state. Recently, ADH IIB has been shown to display an intense EPR signal of its PQQ semiquinone; its X-band spectrum exhibited *g* value of 2.0046, and its W-band EPR showed a rhombic type spectrum with *g* value of 2.00513 (unpublished observations). The observation of such intense EPR signals for the PQQ radical is reasonable, because the PQQ semiquinone radical has been shown to strongly interact with and be stabilized by Ca^{2+} ligated to the C-5 carbonyl of PQQ in soluble glucose dehydrogenase [38]. Such an interaction of PQQ and Ca^{2+} has been shown in the crystals of ADH IIB and qhEDH, as well as in type I ADH and MDH (Fig. 3). In addition, the planar nature of the PQQ ring in the crystal structure of ADH IIB is also consistent with the semiquinone form as predicted on the basis of quantum mechanical calculations [31]. Although an electronic interaction between PQQ and heme *c* has not been shown in ADH IIB, there is some evidence to show such an interaction in qhEDH, which can be isolated as the PQQ-free apo-form. The addition of PQQ to this apoenzyme causes an increase of the redox potential of the heme by +60 mV; changes in the spectroscopic properties of the heme *c* in qhEDH suggest that binding of PQQ to apo-qhEDH leads to a rotation of the methionine ligand of heme *c* and also to a compact enzyme conformation that enables it to catalyze a rapid intramolecular electron transfer [41,42].

Recently, more substantial evidence about intramolecular electron transfer has been obtained from the crystal structures of both ADH IIB [31] and qhEDH [33], and of ADH IIG (unpublished). Since the heme *c* domain is structurally separated from, and lies covering, the PQQ domain, both prosthetic groups are reasonably close to each other (Fig. 5). Although, the shortest edge to edge distance between PQQ and heme *c* is slightly different among them, ~13, ~14, and ~15 Å in qhEDH, ADH IIG, and ADH IIB, respectively, the distance from the iron atom of the heme and the C-5 atom of PQQ, in the case of ADH IIB, is about 20 Å and the planes of both cofactors are tilted to each other by about 70°. This arrangement seems to be a little far for the direct electron transfer reaction to occur (Fig. 6). Therefore, the most probable way for electrons to pass from PQQ to heme *c* is along a path of chemical bonds connecting both prosthetic groups. There is a conserved

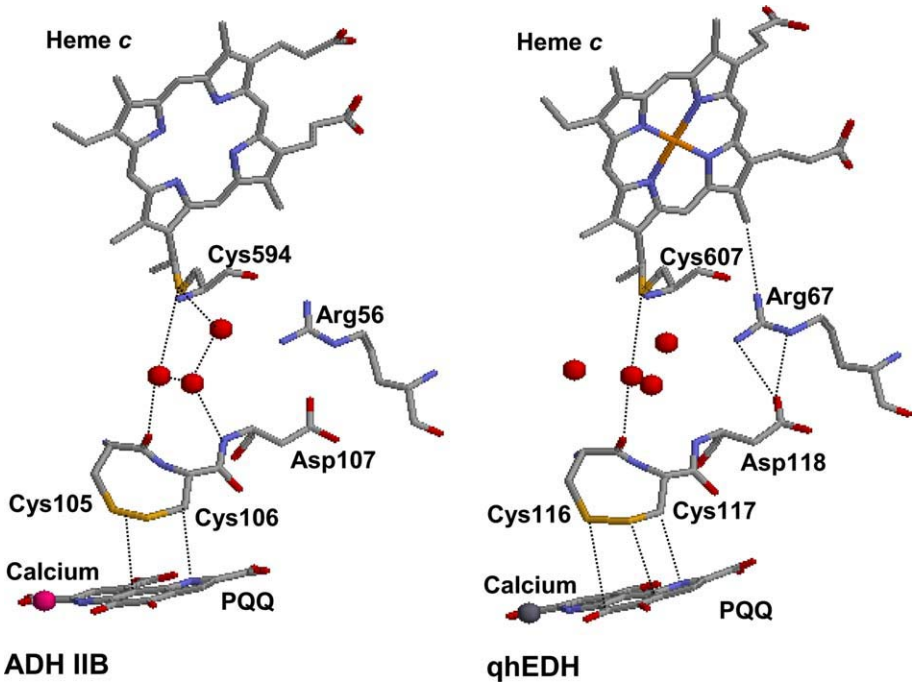

Fig. 6. Possible electron pathway from PQQ to heme *c* in ADH IIB and qhEDH. The PQQ, heme *c*, and amino acid residues which could be involved in the electron transfer process are shown as stick models. Water molecules stabilized in the crystal structure, which could be involved in the electron transfer, are drawn as red spheres. The optimal pathways predicted are drawn as dotted lines.

sequence, **CCD**xVNRG (situated between W1 and W2), between PQQ and heme *c*, which may be involved in the electron transfer. In ADH IIB, the most efficient path for electron flow from the C-5 of PQQ to the heme iron is calculated to involve a through-space jump from PQQ to Cys106 of the conserved sequence, then travel through the amide nitrogen of Asp107 and along hydrogen bonds through enzyme-bound water molecules followed by a through-space jump to Cys594 covalently linked to the heme ring. Alternatively, however, it remains possible to directly transfer electrons from PQQ to heme iron over the intervening distance, although this distance seems a little far as described above. A third possibility involves a conformational change. In several redox enzymes such as cytochrome cd_1 [43] or cytochrome bc_1 [44], large redox-dependent conformational changes have been observed in their crystal structures, that could enable direct electron transfer between two prosthetic groups such as from heme *c* to *d*, or from the iron–sulfur cluster to heme *c*, respectively. Since the

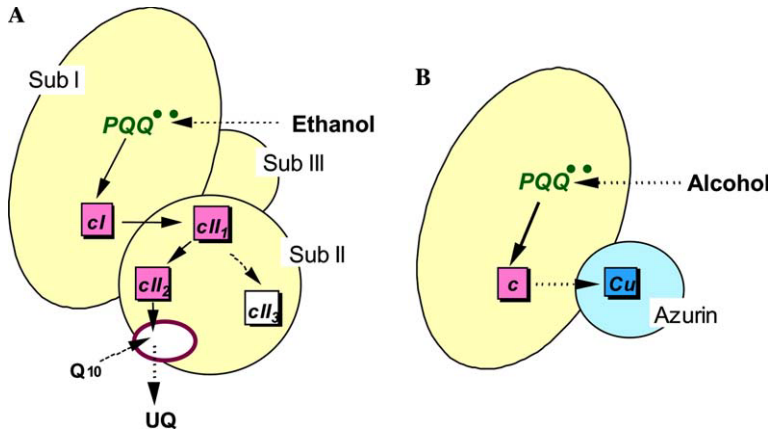

Fig. 7. Hypothetical mode for intra- and intermolecular electron transfer in types II and III ADHs. Estimated intramolecular electron transfer routes (solid arrows) and intermolecular electron transfer routes (dashed arrows) are shown in type III ADH (A) and type II ADH (B). In type III ADH, *c*I, cII_1, cII_2, and cII_3 represent the 4 heme *c* sites in subunit I (I) and subunit II (cII_1, cII_2, and cII_3) in ADH; Q_{10} may be present as bound quinone in vivo. In both cases, without any intermolecular electron acceptor (bound quinone, Q_{10} or bulk UQ in type III ADH, and a blue copper protein in type II ADH), PQQ is present in the semiquinone form (PQQ•), and hemes *c* (*c*I, cII_1, and cII_2 in type III ADH, and *c* in type II ADH) are present in the reduced form.

distance between PQQ and heme *c* is a little different in the three ADH crystals, these crystals may depict different transient states of the heme *c* domain moving from one conformation to another.

In type III ADH, intramolecular electron transfer is more complicated because there is an additional subunit containing three heme *c* prosthetic groups involved in the reaction [2,25]. The first step could involve the same kind of electron transfer from PQQ to heme *c* in the first quinohemoprotein subunit, as in the case of type II ADH. In a second step, the electrons could be transferred via the heme *c* site in the first subunit to one of the three heme *c* sites in the second cytochrome subunit; these latter heme *c* moieties may then be involved in electron transfer to and subsequent reduction of UQ. Kinetic studies of native ADH and of a reconstituted form of ADH from *G. suboxydans* were able to distinguish four heme *c* sites having different ferricyanide reduction properties, and at least one of the heme *c* sites in the subunit II (cII_2 or cII_3 site in Fig. 7A) was shown not to be involved in the UQ reduction [25]. Furthermore, in a redox titration study of *Acetobacter* (now *Acidomonas*) *methanolicus* ADH and of a hybrid ADH, it has been shown that the redox potentials of the 4 hemes *c* groups are substantially altered by dissociation of ADH into its separate subunits but are then restored by reconstitution of a hybrid ADH from the subunit I/III of *G. suboxydans* ADH and the subunit II of *A. methanolicus* ADH [45]. Although the potential of one of the heme *c* sites (cII_2 or cII_3, Fig. 7A) is considerably different from that in the native enzyme, the hybrid enzyme recovers normal UQ reduction activity. Thus, it has been suggested that one site, probably site cII_3, may not to be involved in intramolecular electron transfer from PQQ to UQ, and thus an electron from heme *c*I (+24 mV at pH 4.5) is transferred to heme cII_1 (+187 mV at pH 4.5), then to heme cII_2 (+190 mV), and then finally to UQ (+220 mV at pH 4.5), as shown in Fig. 7A.

The above notion is consistent with a recent finding that ADH of *G. suboxydans*, which is purified with Triton X-100 and has no bound UQ (see below), has an intense PQQ-semiquinone radical, whose *g* value is similar to that of ADH IIB in X-band EPR and also in W-band EPR, exhibiting a rhombic spectrum with g value of 2.00408 (manuscript in preparation). The results suggest that other prosthetic groups present downstream of the electron transfer pathway can accept a single electron or three electrons (after one or two cycles of electron donation, respectively) from $PQQH_2$; the latter "three electrons" scheme is consistent with the above notion that three heme *c* sites (*c*I, cII_1, and cII_2) are able to accept a total of three electrons after two rounds of $PQQH_2$ reduction, and thus PQQ would remain in the semiquinone form (PQQ·) (Fig. 7A). Furthermore, our recent finding shows that ADH isolated with mild detergent, dodecylmaltoside, has a tightly bound UQ (Q_{10}) in a high-affinity site, probably near the cII_2 site in the subunit II, and the enzyme having the bound Q_{10} exhibits almost the same UQ reduction activity as the enzyme having no bound UQ. In addition, the high-affinity site of UQ-free enzyme can be labeled with azido-Q_2 to produce a covalently cross-linked Q_2 without losing any UQ reduction activity (manuscript in preparation). Thus, a tightly bound Q_{10} or a covalently cross-linked Q_2 in the high-affinity site may serve as an electron mediator between a one-electron redox center (probably the heme cII_2 site) and a two-electron acceptor (bulk UQ) in the low-affinity site. The UQ thus reduced by ADH is further oxidized by a ubiquinol oxidase, *bo*- or *ba*-type, which transfers electrons to oxygen, thus making up an ethanol oxidase respiratory chain in acetic acid bacteria [2].

The intermolecular electron transfer in soluble type II ADH is different from that of the membrane-bound type III ADH. Both type II and III ADHs having heme *c* prosthetic group(s) can donate electrons in vitro to an artificial electron acceptor, ferricyanide, unlike MDH or type I ADH. For in vivo intermolecular electron transfer, type II ADH is likely to donate electrons to a soluble redox protein, as do the type I ADH and MDH enzymes (Fig. 7B). For example, a blue copper protein, azurin, isolated from *P. putida* HK5, has been shown to be an efficient intermolecular electron acceptor from ADH IIB, with a first order rate constant of approximately $50\,s^{-1}$ as determined by in vitro steady state kinetic analysis at 25 °C [37]. The apparent K_m for the reaction decreases with increasing ionic strength, indicating that the interaction of the two proteins is mediated by hydrophobic forces. In azurin, one of the histidine ligands to the buried copper atom is exposed to solvent and is surrounded by a surface patch of hydrophobic residues. In ADH IIB, the heme group is buried within the cytochrome domain except for the propionic acid-containing edge of the heme which is exposed to solvent [31]. This edge is partially surrounded by a charge-neutral surface formed by a polypeptide segment consisting of residues 605–631 that could form a putative azurin-binding site [31]. The interaction of azurin with ADH IIB mediated through these charge neutral regions would be consistent with the observed ionic strength dependence of the reaction. Thus, in a freely reversible bimolecular reaction, ADH IIB would transfer electrons from the heme *c* domain (+188 mV at pH 8.0) to azurin (+280 mV at pH 8.0). The reduced azurin then would transfer electrons to the membrane-bound cytochrome oxidase, producing a butanol oxidase respiratory chain in *P. putida* HK5. Azurin has also been shown to be an electron acceptor for another quinohemoprotein, amine dehydrogenase from *P. putida* [16].

Physiology of quinohemoprotein ADHs

As described above, there are several different type II ADHs capable of oxidizing different xenobiotic alcohols such as long-chain alcohols, heterocyclic alcohols, aromatic alcohols, and so on. The biogenesis of these quinohemoprotein ADHs could be controlled at the expression level on the basis of their growth substrate, and sometimes be replaced by type I ADH or NAD-dependent ADH. In *P. putida* HK5, quinohemoprotein ADH IIB or ADH IIG is produced in the presence of 1-butanol, or 1,2-propanediol in the culture medium, respectively, as the sole carbon and energy sources while ethanol induces quinoprotein ADH I as the principal ADH in these cells [11]. In *R. eutropha*, THFADH is induced with THFA, while NAD-dependent ADH is produced instead of quinohemoprotein ADH when ethanol is used as the growth substrate [46]. In contrast, in *C. testosteroni*, qhEDH is produced with ethanol as a growth substrate while NAD-dependent ADH is induced with 1-butanol (unpublished observation). When NAD-dependent ADH works on the oxidation of alcohol, in general the rate of cell growth of these strains usually increases while the oxidation rate of alcohol decreases. This is because NAD-dependent ADH is more efficient, functioning by coupling to the NADH oxidase respiratory chain which consists of three energy coupling sites, NADH dehydrogenase, the cytochrome bc_1 complex, and cytochrome oxidase. In the case of the type II ADH-dependent alcohol oxidase respiratory chain, cytochrome oxidase is the only energy coupling site. Thus, expression of these type II ADHs could be important for the rapid degradation of xenobiotic alcohols. This notion is supported by the finding in *P. butanovora* [13,14], where both BOH and BDH (type I and II ADHs, respectively) are produced in butane or 1-butanol grown cells, that the disruption of either or both of these enzymes causes severe growth defects at high concentrations of 1-butanol. This suggests that the type II ADH, BDH, is linked to the less-energy-efficient respiratory chain and is thus more efficient in the detoxification of 1-butanol owing to the high substrate oxidation rate. In addition, in the case of PVA-degrading *Pseudomonas* sp. VM15C, PVADH is constitutively synthesized but as the apo-enzyme, which is activated only when PQQ is supplied by a symbiont *P. putida* [26]. This situation is also observed in *C. testosteroni* qhEDH, where the enzyme is also produced as the apo-form [47]. Thus, degradation of xenobiotic alcohols in these strains seems to occur only when PQQ-producing microorganisms such as pseudomonads are present in their habitat.

On the other hand, type III ADH of acetic acid bacteria seems to behave differently from a type II ADH in terms of physiological function, since the type III ADH is well known to provide rapid oxidation of ethanol to produce a high concentration of acetic acid, which is very toxic against almost all microorganisms [2]. Although such a production of toxic acetic acid with type III ADH seems in striking contrast to the detoxification of alcohols by type II ADH, acetic acid bacteria seem to require this ability to accumulate high levels of acetic acid as part of their living strategy [48]. And also, similar to type II ADH, the ethanol oxidase respiratory chain with type III ADH seems to adapt for rapid oxidation. Actually, by the defect of type III ADH in *Acetobacter pasteurianus* SKU1108, the strain turns out to grow even better than the wild strain in ethanol-containing medium, where two NAD-dependent ADHs, present in only a small amount in the wild-type strain, are dramatically increased in the cytoplasm, concomitant to the increase of the key enzyme activities in TCA and glyoxylate cycles [49]. Thus, it is clearly shown in acetic acid bacteria that the type III ADH is extensively involved in acetic acid production, while NAD-dependent ADH is used only for ethanol assimilation through the TCA and glyoxylate cycles.

Quinohemoprotein ADHs seem to adapt to a rapid oxidation of a vast array of substrates for the production of useful materials and also for oxidation of molecules harmful to the cells. Thus, these quinohemoproteins are potentially very useful from a biotechnological viewpoint for bioconversion and bioremediation, together with high enantioselectivity of certain chiral substrates.

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