The photo-oxidation of a methene carbon of a magnesium porphyrin is dependent on the electron distribution on the methene bridge carbon. The corresponding hydroxyoxophlorins were obtained by irradiating magnesium deuteroporphyrin and magnesium mesoporphyrin esters. Magnesium monoformyldeuteroporphyrin, magnesium porphyrin *a* esters and magnesium chlorins were not photo-oxidized. An additional factor is the stability of the metal porphyrin bond. In a series of magnesium porphyrins photo-irradiated in benzene, the stability of the metal porphyrin bond paralleled the thermodynamic stability[6] of these complexes. From magnesium-deuteroporphyrin only a trace of free porphyrin was found, but significant amounts of protoporphyrin were obtained from magnesium protoporphyrin, and magnesium dissociated even more rapidly from magnesium monoformyldeuteroporphyrin, magnesium porphyrin *a*, magnesium monovinyl- and magnesium monovinylmonocarbonyl-chlorin.

A strongly electron donating ligand completely suppresses the formation of hydroxyoxophlorins. Magnesium deuteroporphyrin and magnesium mesoporphyrin dipyridino-complexes in benzene could not be photo-oxidized. A similar effect was obtained with β-picoline, but not with α-picoline or lutidine. With the dipyridino-complexes of magnesium protoporphyrin and of magnesium monovinylmonohydroxyethyl deuteroporphyrin, however, photo-oxidation occurred, not at the methene bridge but on one of the pyrroles at the vinyl side chain to give magnesium dioxyprotoporphyrin and magnesium dioxymonovinylmonohydroxyethyldeuteroporphyrin. Free dioxyprotoporphyrin (IV) (ref. 1) is obtained on photo-oxidation of protoporphyrin in either benzene or pyridine. Complexing of the magnesium porphyrins with pyridine or picoline had the further effect of stabilizing the magnesium–porphyrin bond. The pyridine complex of magnesium diformyldeuteroporphyrin and magnesium porphyrin *a* could be photo-irradiated for long periods without dissociation of magnesium.

Only hydrophobic solvents permit the formation of hydroxyoxophlorins. None could be obtained when magnesium protoporphyrin was irradiated in alcohols, ketones or were dispersed in 'Emasol' phosphate buffer at *p*H 6·8. In ether neither photo-oxidation of the porphyrins nor dissociation of the magnesium occurred.

The photo-oxidation of magnesium porphyrins, to give specifically biliviolins on splitting of the ring, contrasts with the biliverdin obtained, which has three methene bridges, as the product of the opening of the porphyrin ring on chemical oxidation of pyridine haemochromes. The transformation of oxophlorin to biliviolin requires not only the addition of oxygen, because the methene carbon probably leaves as CO, or CHO, but also a reduction. Moreover, the photo-oxidation of the disodium complex of aetioporphyrin in pyridine[7] gave a mixture of verdins and violinoid pigments.

The specific formation of protobiliviolin from magnesium protoporphyrin raises the possibility that magnesium protoporphyrin is a precursor of the chromophores of *c*-phycocyanin and *allo*-phycocyanin, and possibly the chromophore of phytochrome. The absorption spectrum of magnesium protoporphyrin is very close to the photo-action spectra of the formation of phycocyanin[8].

Magnesium protoporphyrin has been implicated as a precursor[9] of chlorophyll, and the cell membrane could provide a hydrophobic environment, required for the formation of the oxophlorin.

I have now found[10] that at least one vinyl group appears to be present in the native chromophore attached to the protein, and the other vinyl present in the protobiliviolin could be involved in a covalent linkage to the protein.

The spectral shift observed (725–665 mμ) for phytochrome on going from the enzymatically active forms, Pfr, to the inactive form, Pr, closely mimics that found for hydroxyoxophlorins on going from neutral to alkaline solvents. This strongly suggests that the chromophore is not of the biliverdin type[11], but still has a bridge carbon present.

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MOLECULAR STRUCTURE

Nucleotide Sequence of 5*S*-ribosomal RNA from *Escherichia coli*

In 1964 Rosset, Monier and Julien[1] described and characterized a low molecular weight ribonucleic acid (*5S* RNA) that is present in the ribosomes of *Escherichia coli* in addition to the two larger components, the *16S* and *23S* RNA. It contains 120 nucleotide residues and in contrast to transfer RNA contains no "minor" bases. Using a two-dimensional fractionation procedure for the separation of nucleotides labelled with phosphorus-32 (ref. 2), we have determined the sequences of all the oligonucleotides obtained by complete digestion of ^{32}P-labelled *5S* RNA with ribonuclease *T1* and ribonuclease *A* (pancreatic ribonuclease)[3]. In order to arrange these nucleotides in the unique sequence of the *5S* RNA, we

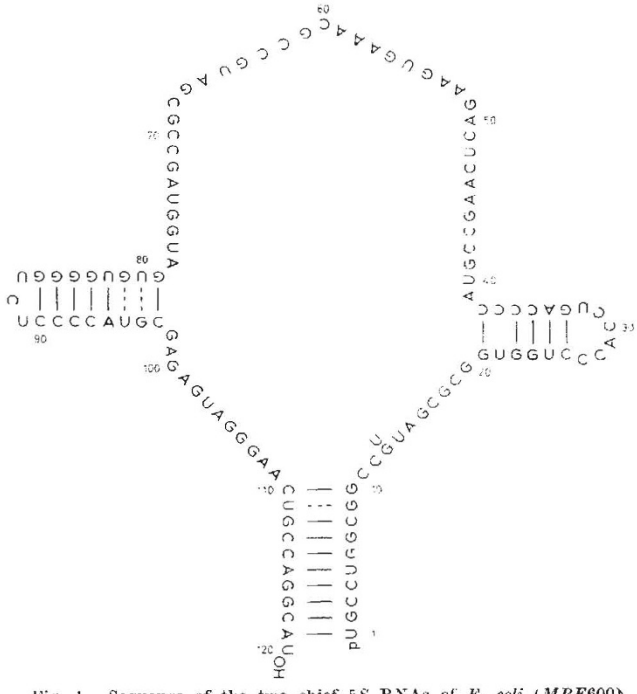

Fig. 1. Sequence of the two chief *5S* RNAs of *E. coli* (*MRE*600) showing regions involved in base-pairing. A solid line indicates a "standard" (G—C or A—U) pair and a dashed line a G—U pair, for example, between residues 81 and 95.

have now studied the products of various partial ribonuclease digestions. This has involved the development of a number of new techniques which will be described in detail later. From the large number of partial digestion products obtained, we deduced the unique sequence shown in Figs. 1 and 2.

Fig. 1 is drawn to show the residues which are believed to be involved in base-pairing. These base-paired regions were identified as four sequences which were particularly resistant to digestion by ribonucleases. The longest double-stranded region is believed to be formed by base-pairing between the two ends of the molecule, and there are also two smaller "loops". Base-pairing between the two ends of the molecule is also found in transfer RNA, but otherwise there is less base-pairing in 5*S* RNA than in transfer RNA.

It will be noted that two residues are shown as occupying position 13. It appears that there are two 5*S* RNAs, presumably controlled by separate genes, one having a G in position 13 and the other a U. This was found in one strain of *E. coli* (*MRE*600, obtained from Dr H. E. Wade of the MRE Experimental Station, Porton, Wiltshire), while in another strain (*CA*265, obtained from Dr S. Brenner of this laboratory) a difference has been found in another position. It is probable that there are also other minor heterogeneities and therefore Fig. 1 illustrates the structure only of the two principal components of 5*S* RNA in *E. coli*, *MRE*600.

There are two sequences of ten and eight residues, respectively, that are repeated twice in the molecule. In Fig. 2 the structure is written so that the common sequences are aligned. There is considerable homology between the two parts of the chain, indicated by the boxed regions. This observation suggests that the 5*S* RNA may have evolved from a smaller RNA by a duplication of a part of the DNA sequence within the gene. There also appears to be some homology between the two ends of the molecule as shown by the underlining in Fig. 2. This could be explained by a separate duplication.

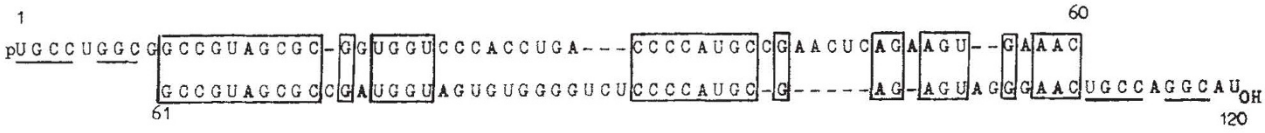

Fig. 2. Homologies between the two halves of the sequence of 5*S* RNA. The residues are numbered as in Fig. 1. Homologies are shown by the boxed areas. Dashes are where gaps have to be left in the sequence in order to maximize these homologies. The underlining shows similarities between the two ends of the molecule.

Previous work on RNA sequences has been confined to transfer RNAs and a number of complete sequences have been reported[4–7]. The 5*S* RNA is 120 residues long compared with 75–85 residues in the transfer RNAs, and the absence of "minor" bases makes interpretation somewhat more difficult. This work shows, however, that it is possible, using the small-scale techniques which we have developed, to determine the nucleotide sequence of an RNA labelled with phosphorus-32.

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BIOPHYSICS

Electron Spin Resonance in Biological Tissues

Several points arise from the recent communication by Dettmer, Driscoll, Wallace and Neaves[1].

First, the simple factors affecting electron spin resonance signals in tissues, which they set out to describe, seem to be only the rapid decay of free radical signals, as measured extensively by Commoner and Ternberg[2], Kerkut *et al.*[3] and mentioned briefly as a source of error by Mallard and Kent[4]. Far from not having appeared in print, as stated in their first paragraph, it is seen that many workers have noted this effect.

Second, the techniques of lyophilization used by Dettmer *et al.* seem to us to be unnecessary and, in certain respects, dangerous. It was shown by Truby *et al.*[5] that lyophilization can create unwanted free radicals in organic materials, and Varian Associates, Inc., even show an example of this in their literature. We think that it is unnecessary to use this technique because adequate techniques now exist for the study of whole tissues at both room temperature and low temperatures[2–4].

That lyophilization has some effect can be shown from the spectra of lyophilized samples which Dettmer *et al.* show in their communication. These spectra, when observed in the light of the methods of analysis of Searle *et al.*[6] and Lebedev[7], are seen to represent asymmetric lines, possibly arising from *g*-value anisotropy. The lines have a Lorentzian shape and the anisotropic splitting in terms of individual line width is about 3·0.

On the other hand, the lines observed by other workers, although still asymmetric, can be shown to have a value of anisotropic splitting approximately equal to 2·0, which applies both at room and low temperatures down to 77° K. In addition, the spectra under these conditions result from individual lines of Gaussian shape. Because these Gaussian spectra are in the presence of water (and therefore an environment containing many protons) the Gaussian shape may result from the broadening effect of this. The difference in the apparent anisotropic splitting between the Lorentzian and the Gaussian spectra may arise from a difference in individual line width between the two cases. Because no details of line widths are published by Dettmer *et al.*, however, it is not possible to follow this further.

Alternatively, it is possible that the differences described here arise merely because these authors are observing electron spin resonance signals generated by the lyophilization process, and this in itself represents a serious pitfall.

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