

84% of the residues in the E and F helices are identical. This result implies that the residues in the E and F helices are conserved between the tetramer and dimer chains for some important purpose such as contact formation.

Thus, we decided to use the dimer electron density from our tetramer map as a search molecule in molecular replacement. Structure factors obtained from the inverse Fourier transform of the isolated density were used in rotation¹³ and translation¹⁴ function calculations against the dimer data. Unlike our previous molecular replacement calculations, these gave good solutions.

We tested the validity of this molecular replacement solution using various criteria. First, the *R*-value of 42.8% compares favourably with other successful solutions^{15,16}. Second, dimer placement in the crystal showed no interpenetration between neighbouring molecules even though packing considerations were not included in the translation function (see Fig. 3). In addition, standard difference and 'haem-less'¹⁵ difference Fourier maps were calculated, examined and found to support this solution. Finally, an anomalous difference Patterson¹⁷ for native dimer crystals contained peaks at all positions for the expected iron-iron vectors.

The 'cooperative dimer' hypothesis of Antonini⁷ and colleagues in the late 1960s has been shown to be inapplicable to vertebrate haemoglobin⁸, in which the uncooperative $\alpha_1\beta_1$ dimer appears to move as an essentially rigid body. However, dimeric clam haemoglobin is cooperative, and a probable structural basis for that cooperativity has been revealed in the crystal structure. Because the same mode of subunit assembly occurs in the tetramer, it is likely that Antonini's cooperative dimer is

the underlying cause of cooperativity also in tetrameric clam haemoglobin.

To ascertain how these structures change with the release of ligands and why the dimers do not form tetramers in solution, we intend to determine the structures of liganded and deoxy dimeric and tetrameric clam haemoglobins at high resolution.

We thank Drs Emilia Chiancone and Eraldo Antonini for drawing our attention to these interesting proteins and for providing us with purified dimeric and tetrameric haemoglobins; and Drs Janet Smith and Wayne Hendrickson for the symmetry-averaging programs and many helpful discussions. This work was supported by NIH grant AM 02528. W.E.R. was supported in part by NIH training grant GM 07231.

Received 14 February; accepted 3 May 1985.

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MATTERS ARISING

A complex of copper (II)-montmorillonite with a modified cyclodextrin

IN the article by Kijima *et al.*¹, structural organization of intercalated copper mono-(6- β -aminoethylamino-6-deoxy)- β -cyclodextrin (CDen) has been deduced from the interpretation of electron paramagnetic resonance (EPR) spectra of oriented film samples (CDen-Cu(II)-montmorillonite).

However, the invariance of the EPR spectrum towards the variation of the angle between the film plane and magnetic field is indicative of a random orientation of the adsorbed Cu(II) complexes rather than a spatial organization. For both models proposed in Fig. 3a and b, there should be a pronounced angular dependence of the spectrum with a maximum change at 45° in 'structure a' and a vanishing component at 90° in 'structure b'.

Since the statements in this article are inferred from an erroneous spectrum interpretation, the conclusions about the lipid bilayer orientation remain questionable.

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KIJIMA *ET AL.* REPLY—We admit that we have misinterpreted the electron spin resonance (ESR) spectra given in Fig. 2 of our paper. It is true that these spectra are indicative of a random orientation of the adsorbed Cu(II) complexes rather than a spatial organization, as pointed out by Motschi. This indication, however, appears impossible in view of the stereochemistry of hydrated Cu(II) ions in Cu(II)-montmorillonite¹. By using X-ray diffraction and scanning electron microscopy, we have re-examined the orientation of silicate layers in the film of CDen-Cu-montmorillonite used for ESR measurements. This re-examination leads us to conclude that the ESR spectra in Fig. 2 should be for a randomly oriented sample, not for an oriented film.

In considering the arrangement of intercalated CDen, ESR observations were used to justify two assumptions: (1) that the interlayer Cu(II) ion forms a coordination group [Cu(II) (*en*) (H₂O)₂]²⁺ with a square configuration and (2) that this square plane is perpendicular to the silicate layers. The correct data support assumption (1), but are inefficient in proving the other. However, it does not mean that the structure model proposed is invalidated. This is because it seems reasonable to adopt the second assumption, and because the structure model was fundamentally deduced from the interpretation of data for the interlayer spacing

and chemical composition of CDen-Cu-montmorillonite. Thus, our conclusions about the lipid bilayer orientation remain unchanged.

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