LETTERS TO THE EDITORS

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Thermodynamic Data for Myoglobin, Hæmoglobin and Cytochrome-c Reactions, and the Position of the Hæm Groups

CONANT[1] first put forward the idea that the hæms in hæmoglobin are held by two bonds of unequal strength between the iron and groups in the protein on both sides of the hæm disk, the weaker bond breaking when combination with oxygen, carbon monoxide, etc., occurs. This crevice configuration has been elaborated to explain various features of its reactions by Coryell and Pauling, Wyman, and St. George and Pauling; but Keilin has laid stress on other aspects, particularly the combination with large molecules such as nitrosobenzene and 4-methyl imidazole, which suggest that the hæm iron is not embedded in a crevice but is readily accessible[2].

Unfortunately, few of the experimental techniques which give direct quantitative evidence on molecular configuration are applicable to hæmoproteins. From a study of unit-cell dimensions in crystals of metmyoglobin and its 4-methyl imidazole complex, Kendrew and Parrish[3] conclude that the hæm group is more likely to be located on the surface than in a deep crevice. No similar data are available yet for methæmoglobin. But information relating to the crystalline state, valuable as it is, does not necessarily give a true picture of the configuration of such large molecules in aqueous solution, the medium in which their reactions have almost exclusively been studied, and one which is more strictly comparable to their environment *in vivo*. On the other hand, a comparison of thermodynamic data for corresponding hæmoprotein reactions, especially the entropy changes, does offer a basis for judging to what extent the changes in configuration which accompany the reactions are similar, and hence for judging to what extent the actual configurations of the hæm groups are also similar, when the molecules are in solution.

Fortunately, there is no doubt that the hæm in cytochrome-*c* is bound in a crevice. It reacts extremely slowly, if at all, with the usual ligands between *p*H 4 and 11, and its spectroscopic and magnetic properties show the hæm to be bonded as in a hæmochromogen, where the fifth and sixth co-ordination positions of the iron, on either side of the hæm, are occupied by nitrogenous substances[4]. The thermodynamic data for its reactions can therefore be taken as characteristic of hæm in a crevice configuration. There is a very great contrast between the data for cyanide-complex formation by ferricytochrome-*c* and ferrimyoglobin, as shown in Table 1 [5,6]. Although the affinities (free-energy changes) are quite similar, the ferrimyoglobin reaction is greatly favoured by the change in heat content, yet the entropy change is very disadvantageous; whereas the ferricytochrome-*c* reaction is even slightly endothermic and its complex forms entirely by virtue of the very favourable entropy change. This kind of contrast is just what would be expected if the hæm were in a crevice in cytochrome-*c* and relatively exposed on the surface in myoglobin. In the former case, energy would be required to break the bond to the more weakly held amino-acid residue, thus diminishing the net exothermicity of complex formation; but the entropy change would be favourable because a considerable part of the protein would gain far more freedom of movement. The great magnitude of the differences, 20 kcal./mole and 55 e.u., suggests that in myoglobin the sixth co-ordination position is far more exposed to ligand attack, and if a crevice configuration is present then it is in no way comparable to that in cytochrome-*c*.

Table 1. THERMODYNAMIC DATA FOR THE FORMATION OF CYANIDE COMPLEXES BY REACTION WITH THE CYANIDE ION, AT 25° C.

	ΔG^0 (kcal./mole)	ΔH^0 (kcal./mole)	ΔS^0 (e.u.)
Ferrimyoglobin	− 11·4	− 18·6	− 24
Ferricytochrome-*c*	− 8·3	+ 1·1	+ 31·3
Differences:	− 3·1	− 19·7	− 55·3

Table 2. THERMODYNAMIC DATA FOR FERRIMYOGLOBIN (METMB) AND FERRIHÆMOGLOBIN (METHB) REACTIONS, AT 25° C.

Reaction		ΔG^0 (kcal./mole)	ΔH^0 (kcal./mole)	ΔS^0 (e.u.)
Complex formation with OH^-	MetMb	− 6·9	− 7·7	− 2·6
	MetHb	− 7·1	− 9·5	− 7·9
Complex formation with F^-	MetMb	− 2·0	− 1·5	+ 1·8
	MetHb	− 2·4	− 2·5	− 0·6
Reduction by H_2 (standard cell reaction)	MetMb	− 2·7	− 13·7	− 37·0
	MetHb	− 3·6	− 15·2	− 39·0

Results for the cyanide reaction of ferrihæmoglobin are not yet available, but a comparison with ferrimyoglobin is possible for three other reactions as shown in Table 2 [6–8]. These results, like those in Table 1, refer to highly purified hæmoprotein preparations; with ferrihæmoglobin, hæm–hæm interaction was completely absent since hyperbolic and not sigmoid dissociation and titration curves were obtained. The great similarity between the results, which differ at most by only 1·8 kcal./mole in ΔH^0 and 5·3 e.u. in ΔS^0, suggests very strongly that the hæm is very similarly situated in both hæmoproteins in this purified condition. If anything, for all three reactions the entropy changes are less favourable for ferrihæmoglobin and the change in heat content more favourable; so, in view of the above comparison with ferricytochrome-*c*, ferrihæmoglobin shows to an even less extent than ferrimyoglobin the characteristic behaviour of hæm situated in a crevice.

A discussion of hæm–hæm interaction in relation to the entropy changes in hæmoprotein reactions and those with inorganic co-ordination complexes is being published elsewhere[9].

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