BBA 45622

ELECTRON DONOR–ACCEPTOR PROPERTIES OF HAEMATOPORPHYRIN

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(Received August 9th, 1967)

SUMMARY

1. The interaction of haematoporphyrin with a wide range of electron donors has been studied spectrophotometrically in phosphate buffer solution at pH 9 where the spectrum is insensitive to small changes of pH.

2. It has been shown that haematoporphyrin forms 1:1 complexes, the difference spectra being temperature reversible and containing well defined isosbestic points.

3. Using the Benesi–Hildebrand equation and the variation of K_c with temperature, values of K_c and ΔH° have been derived for different electron donors. The values for the complexes with amino acids *e.g.* tryptophan and arginine are about 5 kcal/mole.

4. Haematoporphyrin forms similar 1:1 complexes with electron acceptors, *e.g.* trinitrobenzene and chloranil. The spectra of mixtures of haematoporphyrin and trinitrobenzene in both 50% aqueous ethanol and in a mixture of ethanol (20%) and toluene (80%) are similar to those obtained by GOUTERMAN AND STEVENSON for other trinitrobenzene complexes; the ΔH° values are also similar.

INTRODUCTION

The porphyrins are an important group of compounds in animal and plant metabolism. Theoretical studies[1] on the electronic structure of the porphyrins predict that they should be able to function both as good electron donors and acceptors. The β-carbon atoms of the pyrrole rings should be good electron donors because they carry an excess of π-electrons. The carbon atoms in the methene bridges, however, are electron-deficient and, consequently, should be good electron acceptors. CALVIN[2] has suggested that electron donor–acceptor (charge-transfer) complexes involving porphyrins play an important role in photosynthesis. GOUTERMAN AND STEVENSON[3] have shown that coproporphyrin and etioporphyrin form 1:1 electron donor–acceptor complexes with 1,3,5-trinitrobenzene. Some correlation has been noted between the number of electron-donating groups on the pyrrole rings and donor abilities of the porphyrins. MAUZERALL[4] has investigated porphyrin complexes with large organic cations such as 1-carbamidomethyl, 3-carbamyl pyridinium chloride and with planar heterocyclic molecules such as caffeine.

In this present study the electron donor and acceptor properties of haemato-

porphyrin have both been investigated spectroscopically in solution by studying the changes in its optical spectrum on the addition of various electron donors and electron acceptors.

MATERIALS AND METHODS

Chloranil from Hopkin and Williams was recrystallised 4 times from benzene.

Alanine, alloxan, triethylamine, cysteine (hydrochloride), cytosine, lysine, methyl cyanide, proline, pyridine, tyrosine, caffeine, indole, dimethyl sulphoxide, urea, asparagine, diethylamine, L-glutamic acid hydrochloride, glutaric acid, 1,3,5-trinitrobenzene, L-valine, glycine, L-proline, 6-aminohexoic acid were obtained from British Drug Houses Ltd.; haematoporphyrin hydrochloride, arginine, histidine, tetracyanoethylene were obtained from Koch-Light Laboratories. Serine was obtained from the National Biochemical Corp.

All spectra were recorded on a Unicam SP700 automatic recording spectrophotometer. Stoppered, fused silica cells (1 cm) were used and the cell compartment of the spectrophotometer was maintained at constant temperature to within $\pm 0.5°$.

In general, solutions were made up by adding the appropriate electron donor or acceptor to a solution of haematoporphyrin (about 10 μM) in the appropriate solvent to a concentration of 0.1 to 10 mM. Differential spectra of haematoporphyrin against haematoporphyrin *plus* added compound were recorded at known temperatures.

RESULTS AND DISCUSSION

Spectra obtained using unbuffered solutions

The difference spectra of haematoporphyrin *plus* additive *vs.* haematoporphyrin in 50% aqueous ethanol can be divided into 3 groups. The first consists of *n*-electron donors, *e.g.* diethylamine, triethylamine, valine, proline, and glycine[5,6]. The difference spectrum gives positive peaks at approx. 20200, 18800 and 16000 cm^{-1} and negative peaks at approx. 24000, 19200 and 17600 cm^{-1} as illustrated in Fig. 1.

The dissociable electron acceptors glutamic acid hydrochloride, glutaric acid and tetracyanoethylene form another group whose difference spectrum has positive peaks at 23200, 19000, 18200 and 16600 cm^{-1} and negative peaks at 20200, 18000 17600 and 16000 cm^{-1}. This is illustrated in Fig. 2.

The non-dissociable electron acceptors chloranil and 1,3,5-trinitrobenzene possess difference spectra which have peaks in approximately the same position as those of glutaric acid but of different appearance (Fig. 3).

All spectra possess well marked isosbestic points and are furthermore temperature reversible as shown in Fig. 4.

Usually the presence of isosbestic points and temperature reversibility implies the formation of 1:1 reversible complexes[3]. From the variation of absorbance with concentration for a given wavelength, the equilibrium constant K_c of this supposed interaction can be evaluated by the Benesi–Hildebrand method[7] which is that the relationship

$$\frac{1}{\log I_0/I} = \frac{1}{K_c \cdot \varepsilon_c [a] \cdot [b]} + \frac{1}{\varepsilon_c [b]} \quad (1)$$

is valid provided $[a] \gg [b]$, where ε_c is the absorbance coefficient of the complex and $[a]$ and $[b]$ are the molar concentrations of the components.
A plot of

$$\frac{1}{\log I_0/I} \text{ vs. } \frac{1}{[a]}$$

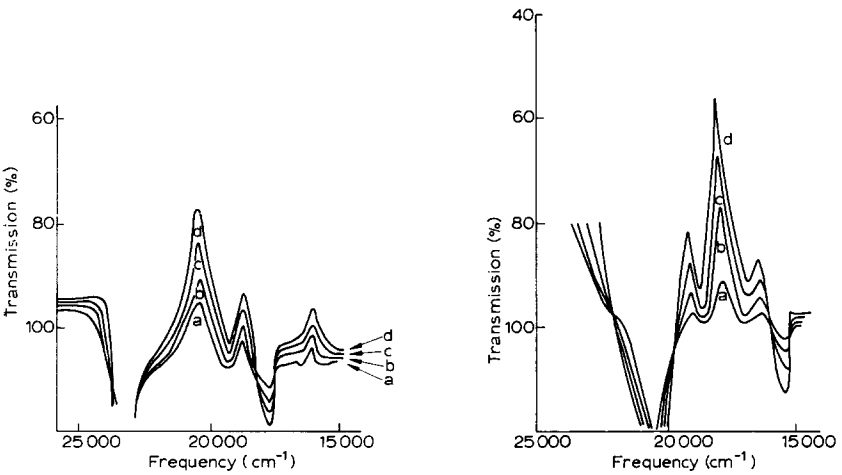


Fig. 1. Difference spectra. (a) 17.39 mM proline in 74.5 μM haematoporphyrin; (b) 34.78 mM proline in 74.5 μM haematoporphyrin; (c) 86.95 mM proline in 74.5 μM haematoporphyrin; (d) 34.5 mM proline in 74.5 μM haematoporphyrin *vs.* 74.5 μM haematoporphyrin in 50% aq. ethanol.

Fig. 2. Difference spectra. (a) 15.1 mM glutaric acid in 74.5 μM haematoporphyrin; (b) 30.3 mM glutaric acid in 74.5 μM haematoporphyrin; (c) 123 mM glutaric acid in 74.5 μM haematoporphyrin; (d) 245 mM glutaric acid in 74.5 μM haematoporphyrin *vs.* 74.5 μM haematoporphyrin in 50% aqueous ethanol.

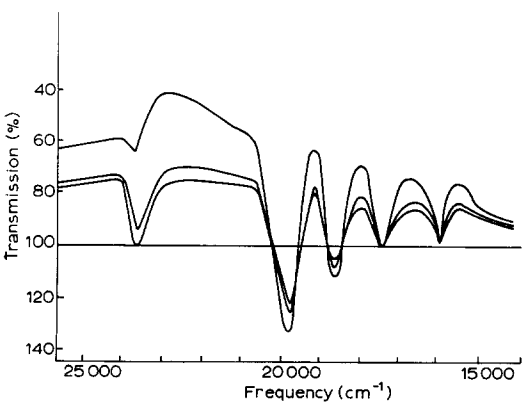
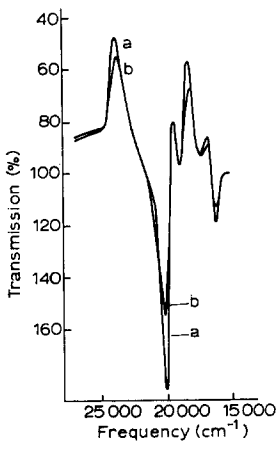


Fig. 3. Difference spectra. (a) 41.4 mM trinitrobenzene in 80.8 μM haematoporphyrin; (b) 12.4 mM trinitrobenzene in 80.8 μM haematoporphyrin; (c) 8.3 mM trinitrobenzene in 80.8 μM haematoporphyrin in ethanol–toluene (20:80, v/v).

for constant [*b*] will yield a straight line from which K_c and ε_c may be evaluated. All the donors and acceptors did in fact yield such straight lines. Some examples of Benesi–Hildebrand plots are shown in Fig. 5. The values of K_c and ε_c, which have been derived from such plots, are given in Table I.

In general K_c values mirror the donor and acceptor strengths of the compounds. The apparent extinction coefficients of the supposed complexes do not follow any



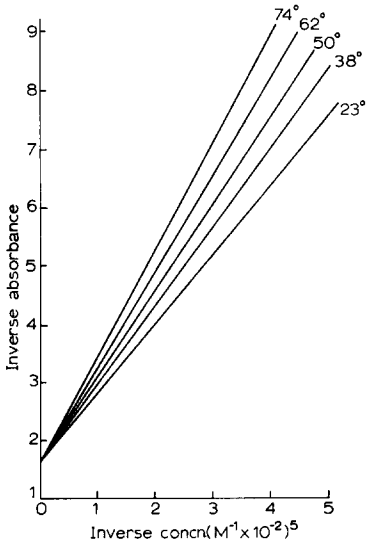


Fig. 4. Difference spectrum. 0.52 M glutaric acid in 74.5 μM haematoporphyrin. (a) at 28°; (b) at 49°; all in 50% aq. ethanol.

Fig. 5. Benesi–Hildebrand plots. Plot of inverse absorbance *vs.* inverse concentration of tetracyanoethylene in 74.5 μM haematoporphyrin in 50% aq. ethanol.

TABLE I

APPARENT HAEMATOPORPHYRIN COMPLEXES

	K_c (*l/mole*) at 23°	ε_c	*Additive concn. (mM)*	*Haemato-porphyrin concn. (μM)*	ΔH^0 (*kcal per mole*)	(*cm*$^{-1}$)
Donors						
Diethylamine	4420	1790	0.01 to 1.4	74.5	—16.0	20 40[illegible]
Triethylamine	7250	3018	0.1 to 0.3	74.5		20 40[illegible]
Glycine	110	2150	14 to 200	74.5		20 40[illegible]
Valine	36.3	2740	10 to 140	74.5		20 40[illegible]
Proline	10.2	2465	5.6 to 350	74.5		20 40[illegible]
Acceptors						
Glutamic acid hydrochloride	206	7950	5 to 65	74.5		18 30[illegible]
Glutaric acid	6.86	5500	15 to 520	74.5		18 30[illegible]
Tetracyanoethylene	126	9670	1 to 10	86.3	— 1.7	18 30[illegible]

* Frequency at which measurements taken.

regular pattern but this is a common feature of such complexes[9]. Triethylamine is known to be a particularly strong electron donor[8]. Diethylamine, containing only two strongly electron-releasing alkyl groups should be somewhat weaker. The amino acids which contain no strong electron-releasing groups other than the lone pair of electrons on the nitrogen atom should behave as moderate electron donors. Of the acceptors, tetracyanoethylene is one of the strongest known[9].

The K_c values of the two non-dissociable acceptors, chloranil and trinitrobenzene do not fall into this regular pattern. This will be explained further but it is interesting to note that the value of 6.06 l/mole at 23° which was obtained for the K_c value of the haematoporphyrin–trinitrobenzene complex compares well with the values of 12 l/mole for the etioporphyrin–trinitrobenzene complex at 26° and of 5.2 l/mole for the tetraphenylporphin–trinitrobenzene complex at 27° which were obtained by GOUTERMAN AND STEVENSON[3]. Etioporphyrin contains 8 electron-releasing alkyl groups at the pyrrole rings positions and is thus a strong donor. It would be reasonable to suppose that the presence of electron-attracting carbonyl groups at the pyrrole rings in haematoporphyrin should cause a reduction in the donor strength of haematoporphyrin as compared to etioporphyrin.

The enthalpy of dissociation $\Delta H°$ of molecular complexes can be obtained from the variation of K_c with the absolute temperature T using the thermodynamic relationship[9]

$$\frac{d}{dT} \ln K_c = \frac{\Delta H°}{RT^2} \tag{2}$$

Values of K_c at different temperatures have been obtained for two of the compounds. Tetracyanoethylene and diethylamine yield $\Delta H°$ values of −1.7 kcal/mole and −16.0 kcal/mole respectively. The results are tabulated in Table II.

TABLE II

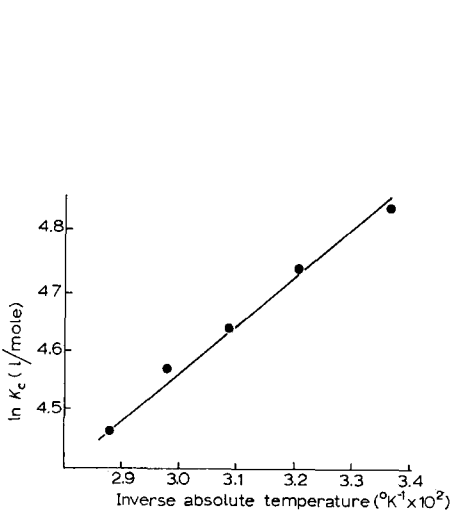
THERMODYNAMIC DATA OF APPARENT COMPLEXES OF HAEMATOPORPHYRIN

Complex with	K_c *(l/mole)*	*Temp.* (°K)	$\Delta H°$ *(kcal/mole)*
Tetracyanoethylene	126	296	
	114	311	
	103	323	− 1.7
	96.5	335	
	86	347	
Diethylamine	4420	296	
	2500	311	−16.0
	685	323	
	226	335	

The relationship 2 can be integrated to give

$$\ln K_c = \frac{-\Delta H°}{R(T_1 - T_2)}$$

hence a graph of ln K_c *vs.* $1/T$ should yield a straight line. A typical plot is illustrated in Fig. 6.



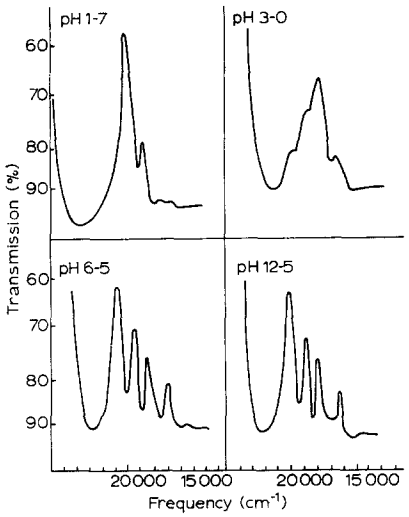


Fig. 6. Plot of ln K_c *vs.* inverse absolute temperature for the tetracyanoethylene–haematoporphyrin system in 50% aq. ethanol.

Fig. 7. Absorption spectra of approx. 10 μM haematoporphyrin at various values of pH.

Effect of pH on the spectra

The effect of change of pH on the spectrum of approx. 10 μM haematoporphyrin has been investigated. Fig. 7 shows the absorption spectra at different values of pH. It is seen that, between pH 1.7 and 6.5, there is a very marked change of spectrum from a 2-peaked form to a 4-peaked form. The pH of a 10 μM solution of haematoporphyrin in 50% ethanol is about pH 4, *i.e.* in the region of maximum change. Consequently the difference spectra of haematoporphyrin *vs.* haematoporphyrin *plus* acid or base are identical with those of haematoporphyrin *plus* dissociative electron acceptor or electron donor. Furthermore the absorbance of a 10 μM solution of haematoporphyrin in 50% ethanol at a given wavelength is purely a function of pH and is independent of the actual donor or acceptor. This is illustrated in Fig. 8. This implies that firstly, the spectra we have hitherto assumed to be due to molecular complexing are merely due to protonation or deprotonation of haematoporphyrin. Secondly the spectra are independent of the ionic strength of the solutions as no difference is detectable between an aliphatic amine or an amino acid at the same pH although the ionic strength is clearly different. The results given in Tables I and II although obviously not due to the type of molecular complexing found by the earlier workers with porphyrins[3,4] are still of interest. ILMET AND KRASIJ[10] have noted that plots of dissociation constants of aza-naphthalenes in water *vs.* the K_c values of the complexes formed with iodine in non-polar solvents are linear. Hence the values obtained for apparent K_c values are nevertheless a measure of the basicity or alkalinity of the substances and hence, indirectly, a measure of the electron-donating, or accepting, ability. Indeed as can be seen from Table I, these apparent K_c values do correlate with the electron-donating, or accepting, ability.

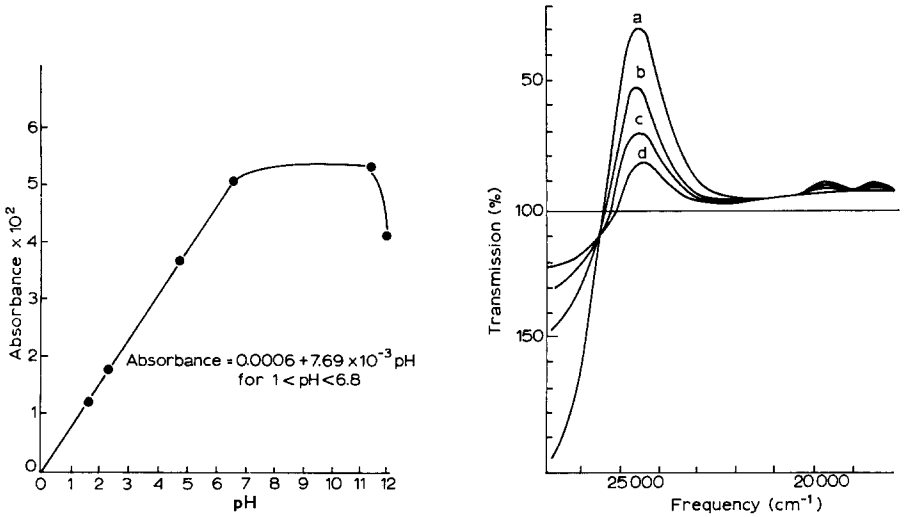


Fig. 8. Relation between absorbance at 20400 cm^{-1} and pH for 10 μM haematoporphyrin in 50% ethanol.

Fig. 9. Difference spectra. (a) 65.7 mM tryptophan in 20.9 μM haematoporphyrin; (b) 32.9 mM tryptophan in 20.9 μM haematoporphyrin; (c) 19.7 mM tryptophan in 20.9 μM haematoporphyrin; (d) 13.1 mM tryptophan in 20.9 μM haematoporphyrin in pH 9 buffer.

Spectra obtained in buffered solution

From Fig. 8 it can be seen that the spectrum of haematoporphyrin is fairly constant in the region of pH 7 to 11. The interaction of various electron donors with haematoporphyrin in pH 9 aqueous phosphate buffer has been studied. The difference spectra obtained are quite different from those obtained in unbuffered solutions and are illustrated in Fig. 9. Again the spectra are found to be temperature reversible and contain well defined isosbestic points. The spectrum of haematoporphyrin is insensitive to small changes of pH at pH 9 and no change of pH greater than 0.1 unit was detected on the addition of any of the electron donors listed in Table III. Using the Benesi–Hildebrand equation and the variation of K_c with temperature, various values of K_c and ΔH° for different electron donors have been derived and are given in Table III. The results are very interesting, the ΔH° values being a thermodynamic quantity are a better indication of complexing capacity than K_c values and they show similar values for complexes of amino acids of about 5 kcal/mole. The value for caffeine is much higher, being 10.8 kcal/mole, while that for 6-methylaminopurine is 3.5 kcal/mole. Another interesting point is that there is no apparent interaction between the aliphatic α-amino acids in pH 9 buffer. A wide variety of n-electron donors has also been shown to interact with haematoporphyrin from their difference spectra. Another point of interest is that whereas tryptophan forms a complex with haematoporphyrin having ΔH° -5.2 kcal/mole (a similar value to that of aliphatic amino acids), indole does not react. This suggests that the tryptophan is interacting *via* its amino group, specifically by the lone-pair electrons on the nitrogen, rather than *via* a π-electron from the indole ring.

TABLE III

INTERACTIONS OF HAEMATOPORPHYRIN

	ΔH^0 (*kcal/mole*)	K_c (*l/mole*) *			
(a) Amino acids in pH 9 buffer					
Tryptophan	−5.2	7.18_{21}	6.33_{31}	4.45_{41}	2.84_{52}
6-Aminocaproic acid	−5.5	1.13_{21}	0.86_{29}	0.74_{40}	0.37_{49}
Arginine	−5.0	12.35_{24}	8.72_{85}	7.04_{45}	
Histidine	Reacts but too insoluble to measure				
Phenylalanine	Very weak interaction				
(b) Other materials in pH 9 buffer					
Caffeine	−10.8	2220_{28}	1288_{40}	497_{56}	
6-Methylaminopurine	− 3.5	150_{27}	85_{41}		
Pyridine	Very weak interaction				
Cytosine, diethylamine, methyl cyanide, dimethyl sulphoxide	Strong interaction				
(c) Other materials in mixture of ethanol–toluene (20:80, v/v)					
1,3,5-Trinitrobenzene	−5.2	102_{21}	78.6_{31}	73.3_{41}	
(d) Materials reacting in 50 % ethanol					
1,3,5-Trinitrobenzene		6.06_{23}			
Chloranil		588_{23}			

(e) Materials showing no interaction in pH 9 buffer

Glycine	Cysteine (hydrochloride)	Serine	Tyrosine
Alanine	Lysine (hydrochloride		Proline
Sodium glutamate	Asparagine	Citrulline	Orthinine (hydrobromide)
Alloxan	Ammonia	Indole	Allantoin
Glutaric acid			

* Subscripts give the temperature in °C at which K_c evaluated.

Spectra obtained using electron acceptors

The interaction of the two non-dissociable electron acceptors 1,3,5-trinitrobenzene and chloranil in unbuffered 50 % ethanol cannot be due to protonation or deprotonation effects but must be due to a molecular complex most likely of an electron donor–acceptor type. In addition an identical spectrum to that in 50 % ethanol is obtained with trinitrobenzene and haematoporphyrin in a mixture of 20 % ethanol and 80 % toluene (Fig. 3) and a ΔH° value of −5.2 kcal/mole is obtained. Furthermore the spectra and ΔH° values obtained are similar to those obtained by GOUTERMAN AND STEVENSON for other trinitrobenzene–porphyrin complexes[3].

CONCLUSIONS

It can thus be seen that, under conditions of controlled pH, haematoporphyrin forms 1:1 molecular complexes with electron donors which appear to be due to electron donor–acceptor forces. Furthermore haematophorphyrin can form similar complexes with electron acceptors but with haematoporphyrin now acting as the electron donor

not the electron acceptor. Several other biomolecules[5,6,11] have been studied previously, but almost invariably they have been shown to be either good electron donors or acceptors but not both. Various discussions on the role of charge transfer in biological systems have involved the use of simultaneous or alternate charge donor and acceptor interactions[12,13]. It has been shown here that interactions of both types can occur with one type of molecule, *viz.* haematoporphyrin.

ACKNOWLEDGEMENT

The spectrophotometer was made available by the Medical Research Council with a special grant to M.A.S.

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