A UV Study of the Magnesium Haematoporphyrin Complex

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The interaction between magnesium and haematoporphyrin has been studied spectrophotometricaly as a function of concentration, pH and temperature and the existence of a strong 1 : 1 complex has been established. The Benesi-Hildebrand equation was used to determine K_c's at various wavelengths and temperatures, at pH's of 7.4, 8.2, and 9.0. The enthalpy of complex formation is seen to change from +9.78 kcal/mol to −9.50 kcal/mol as the pH changes from 8.2 to 9.0. This change may be a reflection of the change in the various ligands which coordinate to the magnesium ion in addition to the porphyrin ring.

The porphyrins are a group of compounds that play an extremely important role in the metabolism of plants and animals. A large number of porphyrin derivatives have been isolated, all of which contain the basic prophyrin nucleus.[1-6] One of the first porphyrins to be isolated was haematoporphyrin, about which this report is concerned.

In the present study, addition of magnesium acetate to haematoporphyrin produced marked changes in the difference spectra and these changes were analyzed as a function of pH, concentration and temperature. Equilibrium constants and ΔH's for a 1 : 1 complex have been determined at the pH's of 7.4, 8.2, and 9.0 for the following equilibrium:

$$PH_2 + Mg^{2+} \rightleftharpoons \{MgPH_2\}^{2+} \quad (1)$$

As is indicated in the text, the above equilibrium is far stronger than a displacement equilibrium of the type studied by Brisbin and Balahura.[7] They have measured the equilibrium constants for copper(II) and zinc(II) complexes with haematoporphyrin which result in the following proton displacements:

$$Zn^{2+} + PH_2 \rightleftharpoons ZnP + 2H^+ \quad (2)$$

$$Cu^{2+} + PH_4^{2+} \rightleftharpoons CuP + 4H^+ \quad (3)$$

In their analysis, the 500—600 mμ region was used, and K_C for Eq. (2) was 1.3×10^{-7} at a pH of 7.0.

Experimental

Reagent grade chemicals were used throughout the investigation. Haematoporphyrin dihydrochloride was obtained initially from Calbiochem and finally in recrystallized form from Mann Laboratories. Tris buffer solutions at 0.05 M were used at all pH's.

It should be emphasized that unless the commercial haematoporphyrin is recrystallized, it will be found to contain numerous impurities. However, it can be easily separated from its impurities by the use of Sephadex G-25 in a borate buffer of pH 8.6. This separation has been extensively studied by Momenteau, Ropars and Rougee[8] and Rimington and Belcher.[9]

Survey spectra were recorded with a Beckman DK-2 and a Beckman DU was used to record the actual absorbances. Each spectrophotometer was thermostatted to within 0.1 °C of the desired temperature. The wavelengths used for the analysis were 425, 430, 435, 440, and 445 mμ. The solutions were made up such that the metal concentration was at least 100 times greater than the heamatoporphyrin concentration over a ten-fold range. At least ten points were used at each of the five wavelengths, and the resulting formation constants are an average of the results at each of these wavelengths. As is required in the analysis, Beer's law was obeyed by al of the data used.

Results and Discussion

Some typical difference spectra are given in Fig. 1. Here magnesium acetate was added to haematoporphyrin in a tris buffer of pH 9.0. The haematoporphyrin absorbance was subtracted out and yielded

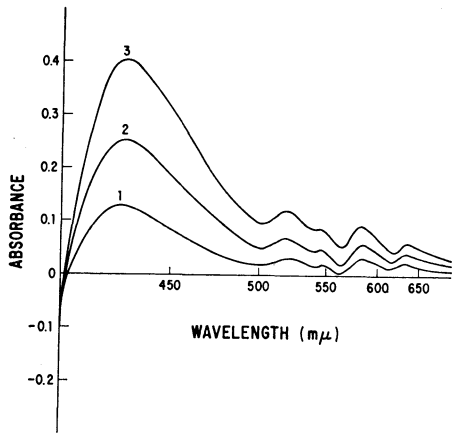

Fig. 1. Recorded difference spectra of the complex as a function of concentration at 25 °C in a tris buffer, pH 9.0. In all cases the haematoporphyrin concentration is 10^{-4} M. The concentrations of magnesium acetate are 10^{-2} M in 1, 4×10^{-2} M in 2 and 0.1 M in 3.

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the resulting curves. It was routinely possible to vary the absorbance reversibly with respect to temperature.

A Job's plot made at 430 mμ of a pH 9.0 solution is given in Fig. 2 and establishes the presence of a 1 : 1

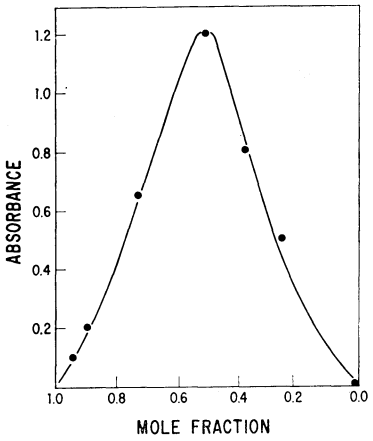

Fig. 2. Job's plot of magnesium-haematoporphyrin complex in a pH 9.0 buffer at 430 mμ at 28 °C.

magnesium-haematoporphyrin complex. Having defined the stoichiometry of the complex, it was possible to analyze the difference spectra of the complex, using the Benesi-Hildebrand (BH) method.[10] In this approach, the relationship

$$1/\log [I_0/I] = 1/K_c\varepsilon_c[M][H] + 1/\varepsilon_c[H] \quad (4)$$

is valid, providing that [M] ⋙ [H], where ε_C is the absorbance coefficient of the complex and [M] and [H] are the molar concentrations of the magnesium ion and haematoporphyrin, respectively.

A plot of $[H]/\log [I_0/I]$ *vs.* 1/[M] gives a slope of $1/K_C\varepsilon_C$, a y intercept of $1/\varepsilon_C$ and a x intercept of $-K_C$. It should be pointed out that if K_C is small, or if Beer's law is not obeyed, then the BH method can no longer be used.

Tables 1 and 2 contain the results of the UV analysis at the pH 's 7.4, 8.2 and 9.0. It should be emphasized that at a pH of 7.4, one is approaching the limits of solubility with respect to haematoporphyrin. As can be readily seen, the formation constants roughly decrease with increasing pH in the vicinity of 37 °C and the enthalpy of complex formation goes from endothermic to exothermic between a pH of 8.2 and 9.0.

In order to explain the drastic change in enthalpy with pH, one may invoke either of the following models or a combination thereof. The initial model (I) is concerned with the changing species that are coordinated to the magnesium ion in addition to the porphyrin nucleus. This effect may be due solely to

10) H. A. Benesi and J. H. Hildebrand, *J. Amer. Chem. Soc.*, **71**, 2703 (1949).

Table 1. Complex formation constants and extinction coefficients

T(°C)	K^c pH=7.4	ε_c
40	26.0±0.0	12000
35	25.0±1.2	12500
30	25.1±0.5	13200
25	22.2±1.8	12200
	pH=8.2	
40	32.5±1.2	13300
35	27.5±0.9	14000
30	18.6±0.3	15400
25	14.7±0.7	16000
	pH=9.0	
40	5.6±0.9	34500
35	7.5±0.3	29400
30	10.9±1.6	18200
25	12.5±1.1	17800

Table 2. Enthalpies of complex formation

pH	ΔH(kcal/mol)
7.4	+1.14
8.2	+9.78
9.0	−9.50

a more exothermic bond between the metal and hydroxyl ion than that between the metal ion and a water molecule or a tris anion. However, there is also the possibility of a polymerization effect where the ionized propionic sidechains from one porphyrin complex coordinate to the magnesium of another complex. This was suggested by Loach and Calvin for the manganese haematoporphyrin complex.[11] As porphyrins and metalloporphyrins are known to dimerize, this is not an unreasonable idea, particularly at high pH's.

Model (II) would concern itself with the effect of pH upon the pyrrole hydrogen atoms and the subsequent or simultaneous complex formation between the dianion and the magnesium ion. Unfortunately, the neutral species is stable even in concentrated sodium hydroxide[6] and it would appear that this event is unlikely.

In conclusion, it seems that the environmental changes around the magnesium ion drasticly affect its thermodynamic properties with respect to the complex. As a result, the function of the complex as an efficient electron acceptor may be considerably altered by a pH variation of less than one unit.

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