BINDING OF IMIDAZOLE AND 2-METHYLIMIDAZOLE BY HEMES IN ORGANIC SOLVENTS. EVIDENCE FOR FIVE-COORDINATION.

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SUMMARY : By means of absorption spectroscopy we show that in benzene solutions, only one molecule of 2-methylimidazole is bound with a great affinity by deuteroheme ($K = 1.25\ 10^4\ M^{-1}$) and mesotetraphenylheme ($K = 2.4\ 10^4\ M^{-1}$). Besides, two overlapping steps may be distinguished when hemes bind imidazole molecules. The equilibrium constants are $K_1 = 4.5\ 10^3\ M^{-1}$ and $8.8\ 10^3\ M^{-1}$, $K_2 = 6.8\ 10^4\ M^{-1}$ and $7.9\ 10^4\ M^{-1}$ for deuteroheme and mesotetraphenylheme respectively.

INTRODUCTION : Thermodynamical studies of ligand binding by iron porphyrins are of interest with regard to the structure-function relationship of hemoproteins. They may give material for an answer to the question : how does hemoprotein tertiary structure has an effect upon the physico-chemical properties of iron porphyrins and conversely. As pointed out in our previous paper (1), studies in non-aqueous solutions may be more fruitful. We reported the preparation (1) and the spectral properties (2) of ferrous porphyrins (hereafter called hemes) dissolved in various organic solvents. They were unambigously proved as being ligand - free - monomers is benzene (2). It is well known that hemes bind simultaneously two nitrogenous ligands such as pyridine, imidazole, histidine... in aqueous solutions (3). On the other hand, Collman and Reed (4) have pointed out that only one 2-methylimidazole molecule (2-MeIm) may bind hemes leading to five-coordinated iron. This paper emphasizes the 2-MeIm affinity for hemes dissolved in benzene. Moreover, we show that two overlapping steps can be distinguished when hemes bind two imidazole molecules. The equilibrium constants are given for each step. Two hemes have been studied : ferrous

deuteroporphyrin dimethyl ester ($DeutFe^{II}$) and ferrous mesotetraphenylporphin ($TPPFe^{II}$).

MATERIALS AND METHODS : Preparation and reduction of iron III porphyrins have been described elsewhere (1). Solvents and chemicals were of the purest avalaible grade. The coordination studies, using absorption spectroscopy were carried out as follows : a benzene solution of heme was contained in optical cells (1 mm and 1 cm) sealed to a glass pipe stopped by a teflon cap. The solution was bubbled with nitrogen. The deoxygenated ligand solution was added to the heme solution by means of an air-protected Hamilton micro-syringe. Spectra were recorded using a Bausch and Lomb Spectronic 505 spectrophotometer. Temperature was regulated to 25 $\pm$ 0,1° C.

RESULTS : 1) 2-methylimidazole

Addition of 2-MeIm to a heme solution induces a spectral evolution. Well - defined isobestic points indicating the presence of only two absorbing species are obtained. The reaction is very rapid and fully reversible. It may be represented by :

$$H + n.L \rightleftharpoons H(L)_n \qquad [1]$$

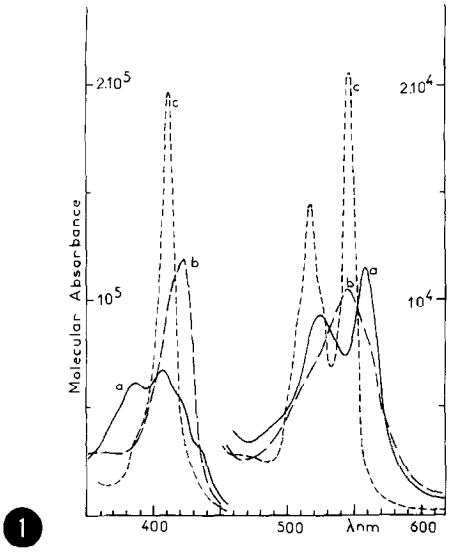
and analysed by means of the standard equation :

$$\log \frac{A - A_o}{A_\infty - A} = \log K + n.\log [L] \qquad [2]$$

where A_o, A_∞ and A are the absorbances of the initial, final and mixed species respectively. The mathematical analysis demonstrates that $DeuFe^{II}$ and $TPPFe^{II}$ bind only one 2-MeIm molecule (n=1) with affinity constants : $K = (1.25 \pm 0.15)\ 10^4\ M^{-1}$ and $K = (2.4 \pm 0.3)\ 10^4\ M^{-1}$ at 25° C, respectively. The bare heme and monocoordinated heme spectra are depicted on figures 1 (a,b) and 2 (a,b).

2) Imidazole

Upon addition of imidazole to heme solutions the well-known hemochrome spectra are ultimately observed (fig. 1c and 2c), but the



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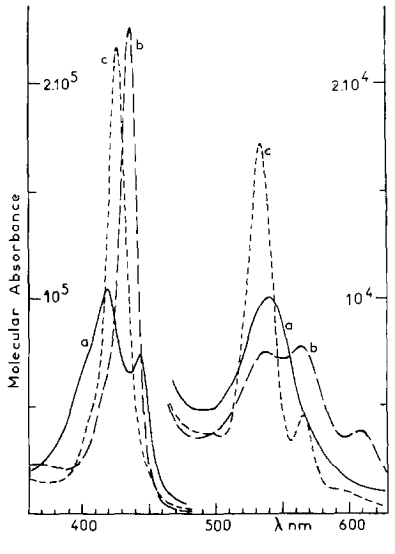

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Figure 1 : Spectra of iron II deuteroporphyrin dimethyl ester derivatives in benzene : a ———— : bare deuteroheme ; b — — — — : mono2-methylimidazoledeuteroheme ; c - - - - - - : bisimidazoledeuterohemochrome.

Figure 2 : Spectra of iron II mesotetraphenylporphin derivatives in benzene : a ———— : bare mesotetraphenylheme ; b — — — — : mono2-methylimidazolemesotetraphenylheme ; c - - - - - : bisimidazolemesotetraphenylhemochrome.

whole set of intermediate spectra does not show isobestic point (see fig.3a), and absorbance variations cannot be plotted according to equation (2) with n=2. Thus, an intermediate formation of monoimidazoleheme is suggested, according to the following equilibria :

$$H + L \underset{}{\overset{K_1}{\rightleftharpoons}} HL \qquad\qquad HL + L \overset{K_2}{\rightleftharpoons} HL_2 \qquad [3]$$

An analysis of these reactions may be carried out as follows :

obviously : $[P] = [H] + [HL] + [HL_2]$ (where $[P]$ is the total porphyrin concentration).

$$\text{It ensues that } [HL] = [P] \cdot \frac{K_1[L]}{1 + K_1[L] + K_1.K_2[L]^2} \qquad [4]$$

$$\text{reaches a maximum value for } [L_{max}] = \frac{1}{(K_1.K_2)^{1/2}} \qquad [5]$$

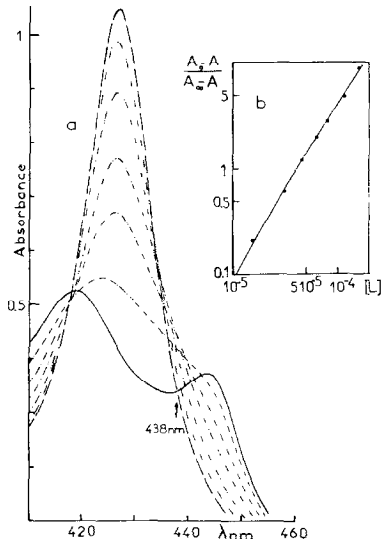

Figure 3 : Imidazole addition to a mesotetraphenylheme benzene solution
a/ Set of spectra showing the lack of isobestic points in the Soret region.
——— : mesotetraphenylheme ; — — —: bisimidazolemesotetraphenylhemochrome ; — .. — : intermediate spectra.
b/ Plot of $\log \frac{A - A_o}{A_\infty - A}$ versus $\log [L]$ (λ = 427 nm)
• : experimental points
——— : theoretical curve calculated with $K_1 = 8.8\ 10^3\ M^{-1}$, $K_2 = 7.9\ 10^4\ M^{-1}$ and $\varepsilon_{HL}/\varepsilon_H = 1.44$ (see text).

$$\text{and } \frac{[HL]_{max}}{[P]} = \frac{(K_1/K_2)^{1/2}}{2 + (K_1/K_2)^{1/2}} \qquad [6]$$

On the other hand, we can write the following equations :

$$A_o = \varepsilon_H \cdot [P]; \quad A = \varepsilon_H [H] + \varepsilon_{HL}[HL] + \varepsilon_{HL_2}[HL_2]; \quad A_\infty = \varepsilon_{HL_2}[P]$$

from which it may be deduced : $A - A_o = (\varepsilon_{HL} - \varepsilon_H)[HL] + (\varepsilon_{HL_2} - \varepsilon_H)[HL_2]$ [8]

$$\text{and } \frac{A - A_o}{A_\infty - A} = \frac{(\frac{\varepsilon_{HL}}{\varepsilon_H} - 1)K_1[L] + (\frac{\varepsilon_{HL_2}}{\varepsilon_H} - 1)\ K_1K_2[L]^2}{(\frac{\varepsilon_{HL_2}}{\varepsilon_H} - 1) + (\frac{\varepsilon_{HL_2}}{\varepsilon_H} - \frac{\varepsilon_{HL}}{\varepsilon_H})\ K_1[L]} \qquad [9]$$

Equation [8] may be used if the second term on its right side cancels ($\varepsilon_{HL_2} = \varepsilon_H$), and if the first have some measurable value ($\varepsilon_{HL} \neq \varepsilon_H$).

These conditions are fulfilled in the Soret region (TPP : λ = 438 nm, see fig. 3a; Deut : λ = 420 nm). Then, [HL] may be determined provided that ε_{HL} is known. The divergences from isobestic points which are observed in the visible or the Soret regions, support the hypothesis that monoimidazoleheme and mono2-methylimidazoleheme spectra are similar. In a first approach we assume their identity. Thus, $[HL]_{max}$ and $[L_t]_{max}$ may be determined ($[L_t]$ is the total ligand concentration which is added to the heme solution). The free ligand is given by $[L] = [L_t] - \frac{K_1[L] + 2K_1K_2[L]^2}{1 + K_1[L] + K_1K_2[L]^2} \cdot [P]$ from which we deduce $[L]_{max} = [L_t]_{max} - [P]$. Then, equation [5] and [6] allow the determination of K_1 and K_2. Following values of K_1 and K_2 are obtained : $K_1 = 8.8.10^3$, $K_2 = 7.9.10^4$ and $K_1 = 4.5.10^3$, $K_2 = 6.8.10^4$ M^{-1} for mesotetraphenylheme and deuteroheme, respectively. Furthermore the experimental curves $\log \frac{A - A_o}{A_\infty - A}$ versus log [L] are plotted for some wavelengths (heme or hemochrome maxima). Generally, these curves do not greatly differ from straight lines, but their slopes are lower than 2. They are reasonably fitted by the theoretical curves calculated according to eq. [9] with the assumed molecular absorbances and the above mentioned affinity constants (see fig. 3b). As monoimidazoleheme spectra are not exactly known, values of K_1 and K_2 cannot be thought to be accurate. However, the deviations from theoretical curves which are observed by varying the assumed molecular absorbance of the intermediate species and the affinity constants suggest that affinity constants are given within 30% and gives support to our hypothesis on the monoimidazoleheme spectra.

DISCUSSION : As pointed out by Collman and Reed (4), repulsive interactions between the hydrogen atoms on the methyl group and the electrons of the porphyrin ring may account for the particular behavior of 2-methylimidazole. These interactions should be greater if iron came in the porphyrin plane which is a prerequisite for low spin hemochrome formation. Actual-

ly, the reaction of heme with 2-methylimidazole affords only a five-coordinated high spin species in which iron lies outside the porphyrin plane. Moreover, our results demonstrate that, in this compound, the interactions between the methyl group and the porphyrin ring are weak. Indeed, the affinities of hemes for 2-methylimidazole and for the first imidazole which cannot give rise to repulsive interactions, are quite similar. A base - strengthening effect of the methyl group which reinforces the iron nitrogen bond may account for the somewhat larger 2-MeIm-heme stabilities.

According to the high stabilities of these five-coordinated species, the bond between iron and only one nitrogenous ligand is expected to be strong. This fact must be taken into account in discussing the heme-globin linkage in deoxymyoglobin or deoxyhemoglobin.

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