

¹H-NMR STUDY OF STEREOSPECIFIC DIMERISATION OF DICYANODEUTEROHEMIN IN WATER. COMPLETE SPECTRAL ASSIGNMENT OF DEUTEROHEMIN

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1. Introduction

Ironporphyrins are an essential component of the active site of heme proteins. It can be assumed that the interaction with the polypeptide chain induces a specific electron density distribution in the porphyrin system. The spin density distribution, that is, the delocalisation of the unpaired electron of the iron in the paramagnetic iron-(III)-porphyrin complexes (hemins) can be detected by the ¹H-NMR spectroscopy. In order to unequivocally interpret this electron spin density distribution, selective assignment of the proton signals in the NMR-spectrum is necessary. In spite of many efforts however, only groupwise assignment has been achieved [1–3] except for the methyl substituents of dicyano-protohemin [4,5].

Previously we have investigated the complex formation of iron-(III)-deuteroporphyrin IX (deuterohemim) (see fig.3) with cyanide in water [6,7], and observed a dimerisation at high concentration [7].

We present here a detailed model of self-association of the dicyano-deuterohemim which allows specific assignment of all proton resonances.

2. Materials and methods

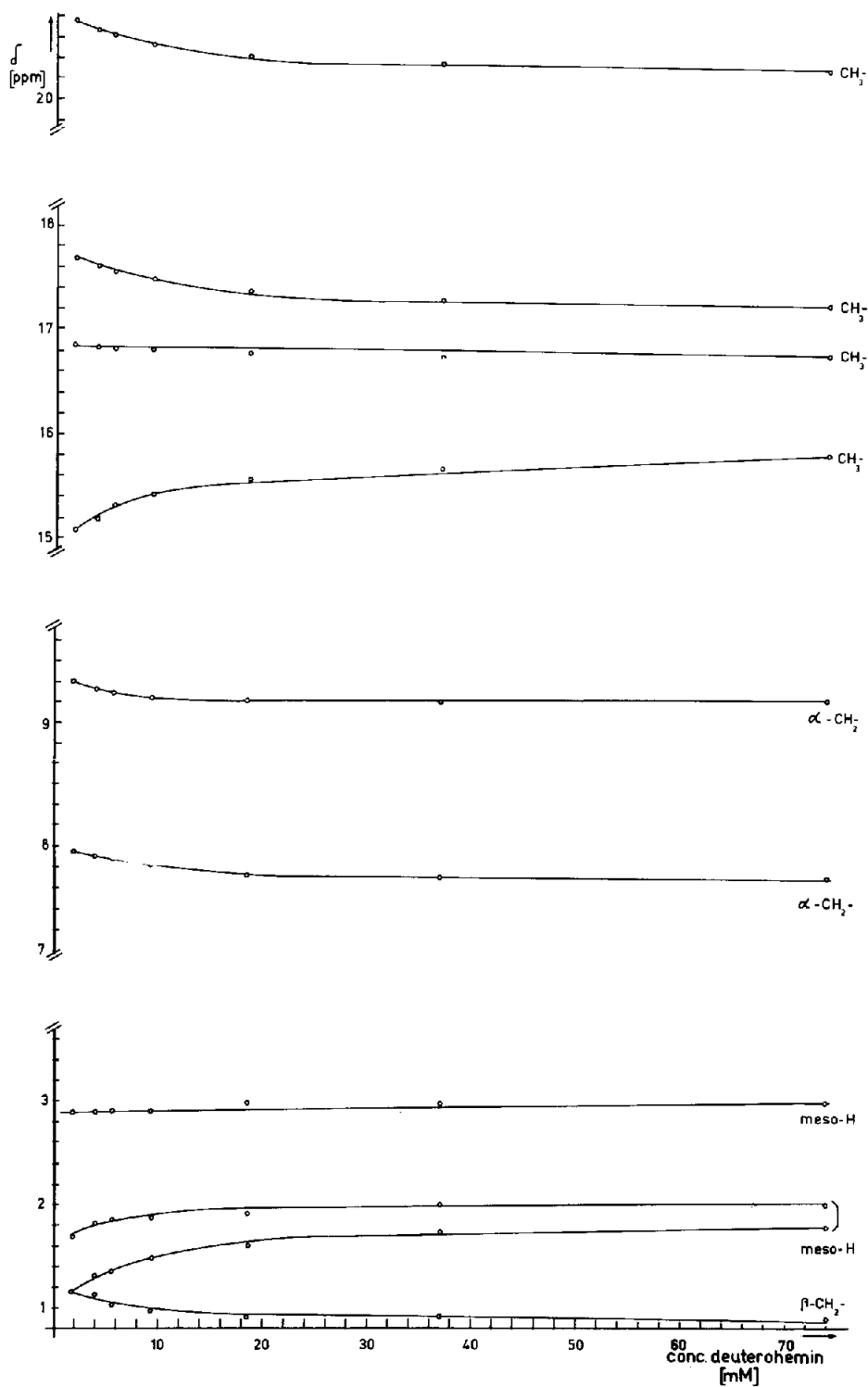
Deuterohemim was prepared and purified by the method of Gaughey et al. [8]. Solutions of the complexes were obtained by dissolution of deuterohemim in D₂O/NaOD and addition of a ten-fold molar excess

of carefully dried KCN. The pD was adjusted to 10, and the identity and purity of the complex were examined on a Unicam SP 700-spectrophotometer. The NMR-spectra were recorded at 100 MHz and 26°C using a KRH-100-spectrometer (Zentrum für wissenschaftlichen Gerätebau der AdW der DDR) and a JEOL JNM-PFT 100-spectrometer. Chemical shifts are given in parts per million (ppm) from internal sodium-2, 2,3, 3-tetradeutero-3-trimethylsilyl-propionate. Negative values indicate low field shifts.

3. Results and discussion

The resonances of ¹H-NMR-spectra of the dicyano-deuterohemim show a significant and characteristic concentration dependence in the range of 1 mM to 100 mM (fig.1a and 1b) typical of association effects. This shift dependence has been analysed quantitatively using the computer program reported by Winkler [9] which allowed estimation of parameters for an association model if the model functions depend on the parameters in a nonlinear fashion. Assuming a monomer-dimer equilibrium and fitting the model functions to the experimental shifts the parameters of dimerisation were computed as shown in table 1.

The curves resulting from the equilibrium functions coincide essentially with the values found experimentally (fig.1a and 1b), thus confirming the assumption of a monomer-dimer equilibrium as the first approximation. However, the occurrence of higher aggregates



(a)

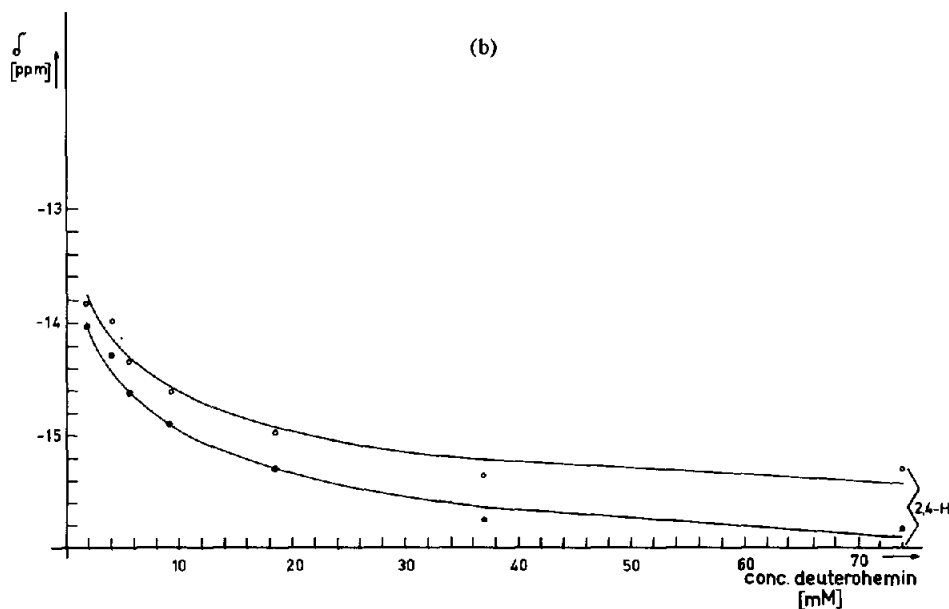


Fig. 1a and 1b: Dependence of chemical shifts of dicyano-deuteroheemin on the complex concentration. The points are experimental values, while the lines represent theoretical values of functions for a monomer-dimer equilibrium.

Table 1
Calculated parameters of self-association of dicyano-deuteroheemin; chemical shifts of the monomer (δ_M), of the dimer (δ_D) and the interaction chemical shifts ($\Delta\delta$).

Proton signals	δ_M [ppm]	δ_D [ppm]	$\Delta\delta$ [Hz]	Error $\Delta\delta$ [Hz]
1,3,5,8 - CH ₃	20.96	20.06	- 89.78	10.57
	17.93	16.97	- 95.48	10.59
	16.89	16.69	- 19.40	10.43
	14.80	16.01	- 120.60	10.69
- <u>CH₂</u> - CH ₂ - COOH	9.51	8.97	- 53.72	10.48
	8.08	7.53	- 55.27	10.48
- CH ₂ - <u>CH₂</u> - COOH	1.26	0.76	- 50.19	10.47
α -, β -, γ -, δ -H	2.81	3.05	24.61	10.44
	1.61	2.15	53.93	10.48
	0.88 ^a	2.06 ^a	118.00 ^a	13.99
2,4-H	- 13.08	- 16.13	- 305.00	12.00
	- 13.16	- 16.71	- 354.70	12.51

^aThe signal of the *meso*-H at 1,2 ppm was overlapped by the β -CH₂-signal, and was not observable.

in concentrated solutions cannot completely be ruled out.

The calculated association constant K_{ass} of $102 \pm 21 \text{ M}^{-1}$ indicates strong interactions to occur between the two molecules. Dicyanohemin complexes have so far been described as a monomer species also in aqueous solutions [10–12], but these investigations were carried out with optical methods in 10–100/ μM solutions where the dimer is completely dissociated (see fig.1a and 1b) and therefore, the dimerisation cannot be detected with optical methods. Only in alcoholic solution at temperatures below -80°C dimerisation of dicyano-protohemim has been observed by NMR-spectroscopy [13].

The concentration dependence or the interaction shifts (table 1) of the protons of dicyano-deuterohemim enable us to determine the geometry of the dimer, because these shifts reflect the strength of the interaction mechanisms, and thus are a function of distances.

The interaction chemical shifts of protons of diamagnetic aromatic molecules were shown to be caused mainly by ring current shifts [14]. Now, if the transfer of spin density through the space and, consequently, the contact term is negligible, the theoretical interaction chemical shift for hemim proton is given by

$$\Delta\delta = \Delta\delta_{\text{RC}} + \Delta\delta_{\text{PC}}^{\text{M}} + \Delta\delta_{\text{PC}}^{\text{L}\sigma} + \Delta\delta_{\text{PC}}^{\text{L}\pi} \quad (1)$$

where $\Delta\delta_{\text{RC}}$ is the ring current term, $\Delta\delta_{\text{PC}}^{\text{M}}$ the pseudocontact term caused by dipole–dipole interaction of the electron at the iron with the corresponding proton [16–18], $\Delta\delta_{\text{PC}}^{\text{L}\sigma}$ and $\Delta\delta_{\text{PC}}^{\text{L}\pi}$ are the pseudocontact shifts from interactions with the electron spin delocalized to the ligands through σ - and π -bonds respectively [16–18]. In the case of low spin iron-(III)-porphyrins $\Delta\delta_{\text{PC}}^{\text{L}\sigma}$ is negligible.

With the aid of another computer program using equation 1 the theoretical chemical shifts of the two dicyano-hemins were fitted to the experimentally based interaction shifts by continuous variation both of the distance of the two heme planes and of their position relative to each other. The details of the optimisation calculation will be reported elsewhere [19]. The main features of the model of dimers are the following. The heme planes show a parallel stacking at a distance of 4.0–4.5 Å, where the hemim planes are turned at an angle of 180° to each other so that

the propionic acid side chains point in opposite directions. The plane centers are displaced by 2.3 Å in parallel and 0.35 Å anti-parallel to the axes in acid side chain direction. The model derived on this basis for the dimer is illustrated in fig.2.

Low spin iron-(III)-complexes show significant π -electron donor-acceptor properties [20], this should be the main reason for dimer formation. In addition the cyanide ligands of the two hemes with a spacing of 2.5 Å may have a stabilising effect on the dimeric structure via dipole–dipole interaction.

Using the dimer model of dicyano-deuterohemim, the optimisation calculation [19] has afforded selective assignment of the experimentally found chemical shifts to the various protons. Thus it was possible to make the first complete assignment of the NMR-spectrum of dicyano-deuterohemim as shown in fig.3.

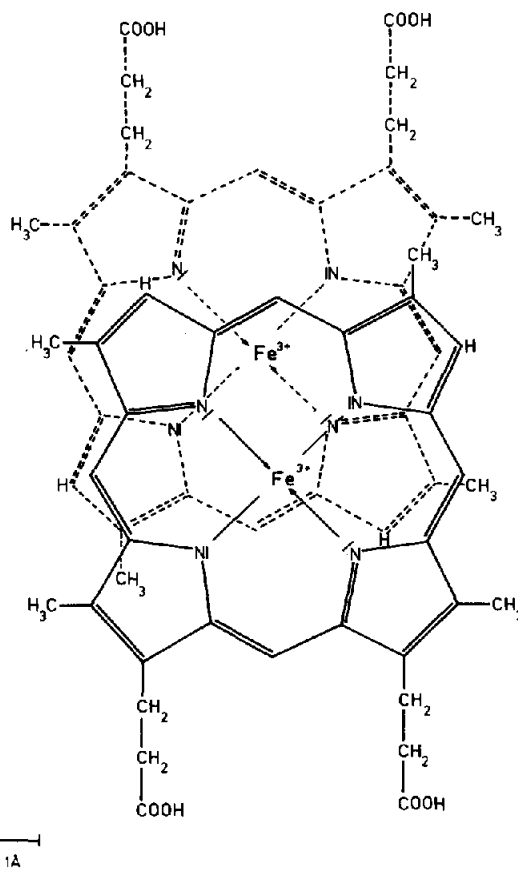


Fig.2. Model of the dicyano-deuterohemim in water.

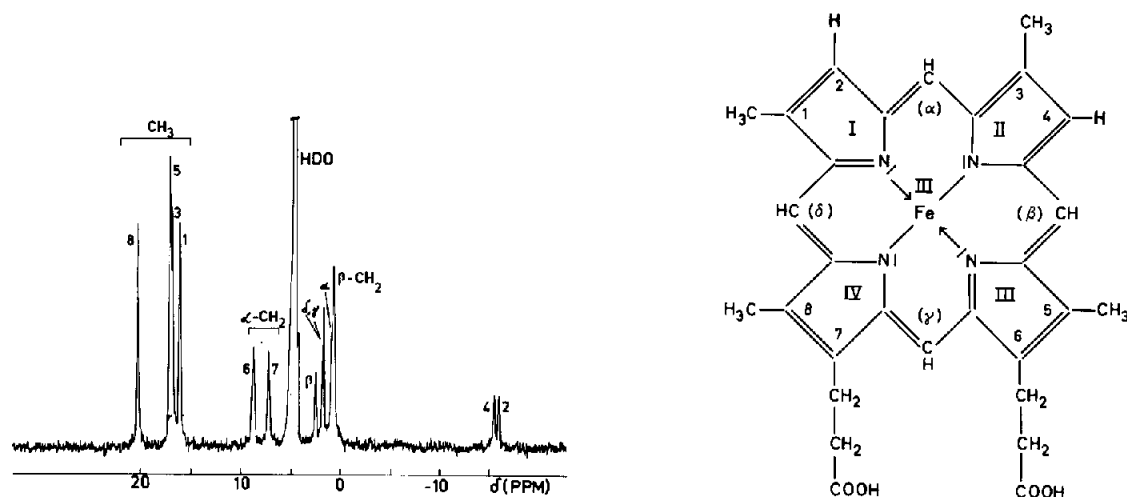


Fig.3. Assignment of the proton signals of dicyano-deuteroheemin (the formula of deuteroheemin is given at the top right) in water (pD = 10, T = 26°C, conc. = 0.08 M, conc. CN⁻ = 1M).

The partial assignment of methyl groups reported by us for dicyano-deuteroheemin [7] and by Cavaleiro et al. for dicyano-protioheemin [4] was introduced in the computer program for simplification and control.

Knowing the chemical shift of each proton in the NMR-spectrum of deuteroheemin it is now possible to characterise more precisely the influence of ligands on the spin density distribution of deuteroheemin and the location of nonaxially bound effectors [21].

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