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^{1}H NMR SPECTROSCOPIC STUDY OF HYDROGEN BONDS AND MOBILE NH PROTONS IN AQUEOUS SOLUTIONS OF PORPHYRIN IONS

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The concentration and temperature dependence of the chemical shifts of aromatic and β-protons of tetra-meso substituted porphyrin ions in aqueous solution have been investigated. A considerable increase in the localization temperatures of interior NH protons of porphyrins dissolved in water compared to solutions of porphyrins in organic solvents was observed. Types of association of the porphyrins studied and the possibility of intra- and intermolecular hydrogen bonds are discussed.

A characteristic behavior of free porphyrin bases is NH tautomerism, connected with the movement of mobile protons from one of a pair of nitrogen atoms to the other in the center of the macrocycle. The migration of these protons in symmetrical porphyrins in the basic state was revealed by ^{1}H NMR spectroscopy [1].

Electronic absorption and fluorescence spectra of individual tautomers of symmetrical porphyrins are identical [2]. Tautomers of porphyrins with unsymmetrical substitution have different life times and may be recorded by both optical and ^{1}H NMR methods [3, 4]. In most ^{1}H NMR spectroscopic studies the temperature dependence of the chemical shifts and the widths of the β-proton signals and also the carbon and nitrogen signals in ^{13}C and ^{15}N NMR spectra were investigated for characteristics of this process [5-7]. At low temperatures (170-200 K) different signals were observed for atoms of pyrroles with pyridine and pyrrole nitrogen atoms.

Signals of NH protons participating directly in the motion have been recorded in the ^{1}H NMR spectra of β-substituted tetraphenylporphyrins [8-10]. Values of the free energy and enthalpy of motion for the NH protons are negligibly different for porphyrins in the solid state and in $CDCl_3$ solution [11]. It was therefore concluded that proton transfer is intra- not intermolecular in these cases. Intermolecular contacts occur predominantly between the peripheral atoms of the porphyrin skeleton and do not affect the kinetics of the reaction occurring at the center of the macrocycle.

The porphyrins most widely used in medicine are water-soluble porphyrins, such as salts of hematoporphyrin derivatives, some derivatives of mesoporphyrin-IX, and porphyrin ions. The spectra of ionic porphyrins have been well studied [12-16]. Their interactions with DNA have been investigated.

The types of association and the mechanisms of NH-proton migration in aqueous solutions of ionic porphyrins may have characteristics connected with the dissociation of water molecules. We have therefore studied the concentration and temperature dependence of the chemical shifts of the signals of the β-protons of the porphyrin skeleton and the protons of the aromatic rings of substituents for sulfate and perchlorate salts of meso-tetra(N-methylpyridinyl)porphyrin (T-MePyP) and the sodium salt of meso-tetra(*p*-sulfonatophenyl)porphyrin (TFPS). The temperature variation of the ^{13}C chemical shifts were studied for the sodium salt of meso-tetra(*p*-carboxyphenyl)porphyrin in aqueous solution.

It is known from spectroscopic results [17] that the anions of the porphyrins TFPS and TFPK are characterized by association even at very low concentrations ($25 \cdot 10^{-6}$ mol/liter) and the equilibrium concentration for dimerization is quite large, 10^5 liter/mol [17]. The concentration dependence of the proton chemical shifts for TFPS in D_2O are shown in Fig. 1. The main characteristic of the spectra (for concentrations greater than $3 \cdot 10^{-4}$ mol/liter and T = 293 K) is the presence of two signals for the β-protons which indicates the localization of the NH(D) protons under these conditions. The decreasing distance

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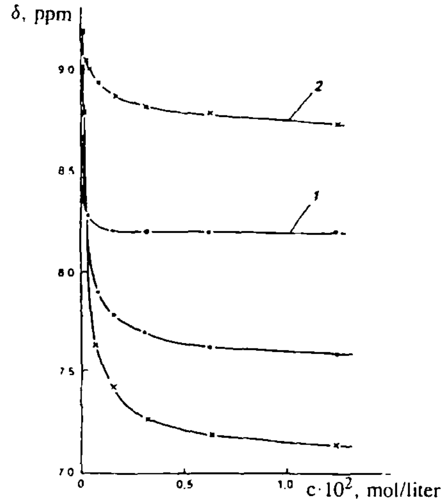

Fig. 1

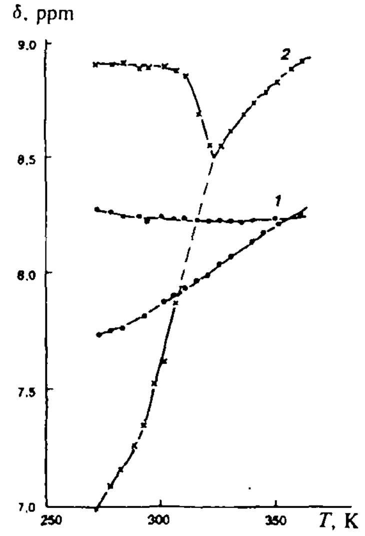

Fig. 2

Fig. 1. Concentration dependence of the chemical shifts of the protons of the Na salt TFPS in D_2O at 20°C: *1*) aromatic protons of the substituent; *2*) β-protons.

Fig. 2. Temperature dependence of the chemical shifts of the protons of the Na salt TFPS in D_2O. Concentration $3 \cdot 10^{-3}$ mol/liter: *1*) aromatic protons of the substituent; *2*) β-protons.

between these signals (and their broadening) for dilute solutions indicates the dependence of the migration of the internal protons of the porphyrin on the degree of association of the molecules and consequently that interaction of the NH protons is intermolecular. At a concentration of $2 \cdot 10^{-4}$ mol/liter at room temperature a single signal is observed for the β-protons (9.2 ppm). The signals of the aromatic protons of the meso-substituents coalesce at a concentration close to $3.6 \cdot 10^{-4}$ mol/liter; the broader signals of protons ^3H and ^5H appear at weaker field than the signals of protons ^2H and ^6H. The shift of the ^1H NMR signals of the β-protons and aromatic protons to weaker fields on dilution of the solution agrees with the proposed stacking interaction between TFPS molecules in the associate.

It has been shown that on dimerization porphyrins do not achieve the "centered stack" conformation with maximum symmetry; instead they take the "displaced stack" conformation with optimal electrostatic interaction between the π-systems [18]. It is possible that this same conformation occurs for TFPS, which may obtain additional stabilization by intermolecular hydrogen bonds $O_2SO^-\ldots H-N$, analogous to the $COO^-\ldots H-N$ interaction proposed by Blumenfel'd [19] for protoporphyrin dimers. The ^3H and ^5H protons of TFPS are then influenced by the π-current of the macrocyclic which decreases the values of their chemical shifts. The equilibrium constant for associate formation as a result of formation of the $O_2SO^-\ldots H-N$ bond, calculated by Lippert's method [20] from the concentration dependence of the chemical shifts of the *m*-protons, is $2 \cdot 10^2$ liter/mol at 295 K.

As the temperature is increased (see Fig. 2) there is a noticeable change in the position of the high field signal (^3H and ^5H) while the position of the low field signal for the ^2H and ^6H protons of the phenyl substituent is practically unchanged. At 348 K (concentration $3 \cdot 10^{-3}$ mol/liter) the lines coalesce. This is in agreement with the fact that the association constant based on formation of the hydrogen bond with the SO_2 group is smaller than the association constant based on stacking interactions. The *o*-protons of the phenyl substituent on the porphyrin have a greater influence on the formation and destruction of the "stack" than the formation of the hydrogen bond with the SO_2 does.

When aqueous solutions of T-MePyP sulfate were diluted from 0.05 to $2 \cdot 10^{-4}$ mol/liter and solutions of T-MePyP perchlorate were diluted from $6 \cdot 10^{-3}$ to $2 \cdot 10^{-4}$ mol/liter the proton signals of these compounds underwent a notable shift to strong field. We note that ^1H NMR spectroscopic studies of solutions with porphyrin concentrations greater than $2 \cdot 10^{-3}$ mol/liter and, according to [12], dilution of the solutions had practically no effect on the signals of the protons of the pyridine ring, while the methyl proton signals were shifted somewhat to weaker field.

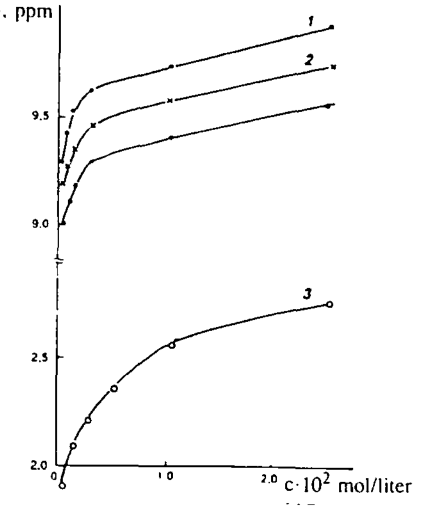

Fig. 3

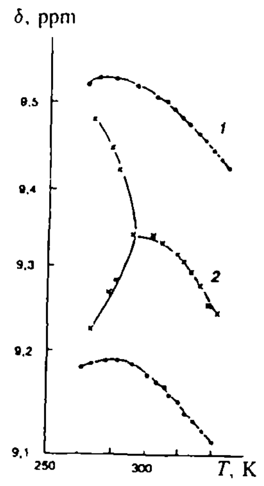

Fig. 4

Fig. 3. Concentration dependence of the chemical shifts of the protons of T-MePyP sulfate in D_2O at 20°C: *1*) phenyl protons; *2*) β-protons; *3*) methyl protons.

Fig. 4. Temperature dependence of the chemical shifts of the protons of T-MePyP sulfate in D_2O. Concentration $3 \cdot 10^{-3}$ mol/liter: *1*) phenyl protons; *2*) β-protons.

The concentration dependence of the chemical shifts of T-MePyP sulfate is shown in Fig. 3. The direction of the shift of the β-proton signals appears greater in the planar associate of T-MePy+P (network) via mono- or doubly charged anions than in the formation of stacks which is the most widespread form of association in porphyrins [18]. If it is assumed that the substitutions in the meso-position are coplanar with the porphyrin ring in the associated state, then when the associate breaks down it is possible that they rotate and the conformation of the substituted porphyrin would be close to the propeller conformation as in tetraphenylporphyrin [21]. In this case the shift of the ^1H NMR shift of the aromatic proton signals to high field is understandable because the protons are under the influence of the shielding magnetic field arising from the π-current of the porphyrin ring.

On the other hand it should be noted that when the temperature is decreased to close to the coalescence temperature and below (Fig. 4), the shift of the ^1H NMR signals of the aromatic protons to high field slows and even changes sign, which suggests the existence of two types of porphyrin associates: planar (network) and the development of stacks at low temperature when the migration of the NH protons is slowed down. In this case we are inclined to conclude that the energy of association of "stacked" porphyrins is less (they begin to dissociate at a lower temperature) than the planar association via anions. These results confirm the suggestion [12] that anionic porphyrins have a greater tendency to stacking interactions than cationic porphyrins.

We now examine the temperature behavior of the β-proton signals for TFPS dissolved in D_2O at a concentration of $3 \cdot 10^{-3}$ mol/liter. Heating the solution from 270 to 305 K caused a strong shift of the strong field signal to weaker field (by 450 Hz) without affecting the position of the low field signal (see Fig. 2). The high field signal is broader than the low field signal which may arise from spin–spin interaction of the NH protons. Above 350 K this signal becomes so broad as to be practically unobservable.

Two factors affect the position of the β-proton signals: dissociation of the stacking associate (shift to weak field) and activation of the movement of the NH protons (shift to strong field for β-protons of pyrrole rings with a pyridine nitrogen atom and a shift to weak field for β-protons of pyrrole rings with a pyrrole nitrogen atom). The two factors effectively compensate each other for the low field signal (up to 305 K) but are cumulative for the higher field signal. Above 305 K the second factor prevails over the effect of associate dissociation for the low field signal which moves to stronger field. The coalescence temperature is 320 K, above which the single signal for the β-protons shifts to low field under the influence of dissociation of the associate. The coalescence temperature for the β-proton signals in this case is almost 70° higher than in chloroform solution.

An estimate of the energy of the transfer of ND protons by an approximate solution of the Eyring equation [22] gave $\Delta G^{\neq} = 14.4$ kcal/mol at a concentration of $3 \cdot 10^{-3}$ mol/liter. The energy barrier was observed to depend on the porphyrin anion concentration in water solution: increasing the concentration to $6 \cdot 10^{-3}$ mol/liter increased the free energy for movement of the ND protons to 15.6 kcal/mol, whereas decreasing the concentration to $4 \cdot 10^{-4}$ mol/liter gave $\Delta G^{\neq} = 13.1$ kcal/mol. This confirmed the conclusion reached above that NH(D) shift is not only an intramolecular process but also an intermolecular process and is limited by the energy of the NH(D)...N(second porphyrin molecule) interaction.

Since all of the experiments described were carried out in D_2O, presence of intermolecular exchange with the solvent should change the interior NH protons of the porphyrin into ND. We therefore studied the ^1H NMR spectra of TFPS in H_2O with a concentration of $6 \cdot 10^{-3}$ mol/liter at various temperatures between 280 and 320 K. It was established that the chemical shifts of the β-protons were not changed on exchanging the interior protons for deuterons since the distance between the β-protons and the interior protons is about 5 Å and these atoms do not affect one another. However the coalescence temperature for the β-proton signals decreased by about 20°C, because the rate of movement of protons is about 10 times as great as that of deuterons. The free energy for motion for NH-protons consequently decreased to 13.9 kcal/mol (15.6 kcal/mol for the same TFPS concentration in D_2O).

The high temperature averaging of the NH(D) protons, brought about by a complex mixture of intra- and intermolecular processes of proton exchange, is also characteristic of the other porphyrin anion we have investigated, the Na salt of TFPK. The ^{13}C chemical shifts for aqueous solutions of TFPK with a concentration of 0.05 mol/liter at various temperatures are shown in Table 1. The NH(D) protons are localized above 290 K. On lowering the temperature of a $3 \cdot 10^{-3}$ mol/liter solution of the sulfate T-MePyP from 340 K to room temperature shifted the ^1H NMR signals to weaker field which confirms our ideas on the type of associate in porphyrin cations. Cooling the solution of this porphyrin below 285 K causes splitting of the β-proton signal into two signals of equal intensity (Fig. 4) which indicates slowing of the NH(D) interchange and their localization. At 268 K the difference in chemical shifts of the β-proton signals was 37.5 Hz with a width of 15 Hz.

The upper limit for the barrier to NH proton exchange, estimated by approximate solution of the Eyring equation [22], is $\Delta G^{\neq} = 14.1$ kcal/mol. This experiment was carried out in heavy water so the free energy refers to deuteron transfer, not proton transfer. The ratio $K^{HH}/K^{HD} \sim 10$, therefore $\Delta G^{\neq}{}_{HH} \sim 13$ kcal/mol. Comparison with results from meso(tetralkyl)-porphyrin powders and for organic solvents ($\Delta G^{\neq} = 11.4$-12.7 kcal/mol at 298 K [23]) shows some increase in the size of the energy barrier for NH proton exchange in aqueous solutions of porphyrins and also a considerable increase in the coalescence temperatures (285-320 and 240-250 K) for the porphyrin β-proton signals. Consequently it is necessary to take into account intermolecular proton exchange between NH protons and water protons for both anionic and cationic porphyrins, i.e, the proton transfer process NH(D)...N will be limited not only by the energy of the interaction NH(D)...N with neighboring molecules but also by the energy of the interaction N...H(D)−O where OH is the hydroxyl group of water (the solvent).

Hence the following conclusions may be drawn from the results presented.

1. From a comparison of the concentration and temperature dependence of the ^1H NMR proton shifts of cationic porphyrins (meso-tetra(*p*-methylpyridyl)porphyrin sulfate and perchlorate) and an anionic porphyrin (Na salt of meso-tetra(*p*-sulfonatophenyl)porphyrin) it follows that anionic and cationic porphyrins can have different types of association and the type of association of the latter can depend on the counterion. Anionic porphyrins have a greater tendency to stacking interaction than do cationic porphyrins. Therefore intermolecular NH...N interactions are less expressed in cationic porphyrins (while intermolecular NH...O−H interaction is maintained) which causes, firstly, the lower temperature of NH proton delocalization in comparison with anionic porphyrins (compare T-MePyP and TFPS) and, secondly, facilitates reactions with nucleosides [12, 13].

2. The coalescence temperature for the ^1H NMR signals of the β-protons caused by activation of NH-proton transfer between nitrogen atoms of the macrocycle is considerably higher (by 30-70°) for aqueous solutions of ionic porphyrins than for solutions of porphyrins in organic solvents or in the solid state. The energy for NH(D)-proton transfer is also increased correspondingly.

3. The process of NH-proton transfer in aqueous solutions of anionic porphyrins depends on their concentration, i.e., on the degree of association of the porphyrin.

4. The characteristics of NH(D) proton exchange in aqueous solutions of ionic porphyrins permits the conclusion that the process is both intra- and intermolecular and is limited by the energies of interaction with water molecules H−O−H...N and interaction with nitrogen atoms of associated porphyrin molecules.

TABLE 1. Chemical Shifts of ^{13}C NMR Signals for the Na-Salt of Tetra(*p*-carboxyphenyl)porphyrin in D_2O*

T, K	COO^-	Chemical shift (relative to TMS)						
		$C\alpha$	$C\beta$	meso-C	$C_{(1)}$	$C_{(2)}, C_{(6)}$	$C_{(3)}, C_{(5)}$	$C_{(4)}$
351	175,62	137,59	131,48	120,36	132,80	128,03	135,25	143,86
323	175,90	136,81	135,72	119,86		128,20	135,79	143,53
290	176,15	153,76 135,98	137,47 129,01	119,78	132,41	128,29	136,45	143,48
283	176,31 316,19	153,64 129,10	137,52	119,77	132,40	128,31	136,29	143,51

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*Internal standard — dioxane.

EXPERIMENTAL

^1H and ^{13}C NMR spectrum were measured with a Bruker WM-250 spectrometer. Concentrations of solution were varied from $5 \cdot 10^{-2}$ to $2 \cdot 10^{-4}$ mol/liter. The solvent was D_2O. The temperature range for measurements was 265-350 K. To study the effect of replacing D_2O by H_2O on NH(D) proton transfer the ^1H NMR spectrum of aqueous (H_2O) solutions of the Na salt of TFPS was recorded at several temperatures (285, 292, 315, and 325 K). A program for suppressing the H_2O signal was used in recording these spectra. The internal standard used was sodium dimethyl-2-silapentanesulfonate. The free energy for NH-proton transfer was carried out by approximate solution of the Eyring equation [22]:

$$\Delta G \neq\ = 19.14 \cdot T_{\mathrm{coal}}(10.32 - \log(\Delta \nu / T_{\mathrm{coal}}))$$

where T_{coal} is the temperature corresponding to coalescence of the β-proton signals on localization of the NH protons, $\Delta\nu$ is the difference in chemical shifts of the β-protons in the region of slow NH-proton exchange. Use of the approximate solution of the Eyring equation gave values of the free energy $\Delta G \neq$ which are independent of temperature.

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