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Structural Studies of Metalloporphyrins. 10.† Complexes of Water-Soluble Cobalt(III) Porphyrins with Amino Acids: NMR Study of the Conformation of the Complexes with Cobalt(III) Tetrakis[4-(*N*-methylpyridiniumyl)]porphine (CoTMPyP) and Cobalt(III) Tetrakis(4-carboxylatophenyl)porphine (CoTCPP)‡

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Cobalt(III) porphyrins of general formula Co(P)$(L)_2$, where P = tetrakis[4-(*N*-methylpyridiniumyl)]porphine (TMPyP) or tetrakis(4-carboxylatophenyl)porphine (TCPP) and L = amino acid, have been studied in water solution by ^{1}H NMR spectroscopy. Complexes with the stoichiometry Co(P)$(L)_2$ are the predominant species above pH 7, whereas at lower pH values Co(P)(L)(H_2O) species are also present. In the general case, the two amino acid ligands were bound to the cobalt atom through the NH_2 group. In the case of histidine, three different complexes with CoTMPyP, two symmetrical and one unsymmetrical, were detected in proportions that varied with the pH of the solution: at pH 7, histidine was bound to cobalt exclusively through imidazole N-3, and at higher pH (pH > 10), it was bound only through the NH_2 group. Similar behavior was found for two other amino acids containing two potential ligands: methionine and lysine. The predominant species at pH 10 were the NH_2-bound complexes, in both cases. The conformational analysis of these complexes has been performed by using two sets of NMR data, the vicinal interproton coupling constants, J_{NH-H_α}, $J_{H_\alpha-H_\beta}$, and $J_{H_\beta-H_\alpha}$, and the induced shifts, $\Delta\delta$. All amino acids studied adopt a largely predominant geometry characterized by a nearly eclipsed conformation around the N-C_α bond and conformation I around C_α-C_β. This geometry allows the side chain of the amino acid to interact with the porphyrin macrocycle by either stacking (aromatic amino acids), hydrophobic (e.g. leucine), or electrostatic (e.g. aspartic acid) interactions. Existence of the last interactions were confirmed by the conformational analysis of the complexes of the same amino acids with the anionic porphyrin CoTCPP, which revealed that only the polar amino acids aspartic acid, serine, and asparagine had significantly different geometries in the two types of complexes. Comparison of the constant K for the "open form" ↔ "closed form" equilibrium in the free and complexed amino acids has shown that the gain of stability $-\Delta\Delta G°$ of the closed form upon complexation increases in the order His < $TyrO^-$ < Phe < TyrOH < Trp. Temperature dependence of $\Delta\Delta G°$ values indicates that the enthalpy change contributes to the stabilization due to the stacking interaction in the case of the aromatic amino acids.

Introduction

Iron protoporphyrin IX, the prosthetic group of hemoproteins, is involved in many different biological processes: oxygen transfer, oxidation of organic or inorganic substrates by dioxygen or peroxidic derivatives, electron transfer, etc. These significantly different biological functions are controlled not only by the number and structure of the axial ligands of the metal but also by the local environment of the macrocycle.[1]

The characteristics of the metal found in hemoproteins, e.g. coordination number and geometry, redox properties, and magnetic properties, are all greatly affected by subtle structural modifications involving amino acid residues in the vicinity of the heme. This includes not only those residues that behave as ligands for the metal but also others that are in close contact with the porphyrin ring but not directly bound to the metal. It has often been assumed that these noncovalent interactions between the side chains of aromatic (tryptophan, tyrosine, phenylalanine) or nonpolar (valine, leucine, isoleucine) amino acids probably play an important role in the function performed by the hemoprotein, either by controlling the size of the cavity available to the substrate molecule when it binds to the heme or by modifying the local dielectric constant or polar character of this cavity.

In an attempt to obtain evidence for the existence of noncovalent interactions between amino acid side chains and the porphyrin ligand, we have prepared a series of ternary complexes of amino acids with water-soluble cobalt(III) porphyrins. The choice of cobalt(III) as the central metal atom was dictated by several criteria. Cobalt(III) porphyrins form stable hexacoordinated diamagnetic complexes with nitrogenous ligands, and these complexes are amenable to study by NMR spectroscopy due to the strong shielding of the ligand protons (up to 8 ppm) induced by the ring-current effect of the porphyrin ring. We previously reported the synthesis and stereochemical study of complexes of various amino esters with two cobalt(III) porphyrins in chloroform solutions: ClCoTPP[2] and ClCoDPDME.[2] We now extend this study to complexes of the usual amino acids with two water-soluble cobalt(III) porphyrins, CoTMPyP (**1**) and CoTCPP (**2**), whose structures are shown in Figure 1. An NMR study was carried out in aqueous solution in order to obtain information on the nature and extent of the various noncovalent interactions between the amino acid side chains and the porphyrin ligand. In particular the possibility of carrying this study on porphyrins bearing charges of opposite signs proved very useful to assess the importance of electrostatic interactions. The NMR analysis allows a complete structural study of these complexes. In particular, for those amino acids that possess two nitrogen atoms susceptible to metal coordination (e.g. histidine and lysine), it was possible to identify the various species present in solutions at different pH. A complete conformational analysis of these complexes is also reported, using two NMR parameters: the vicinal interproton coupling constants ^3J and the induced shielding $\Delta\delta$ of the various protons. This study clearly shows that the conformation of the complexed and uncomplexed amino acids are markedly different and that these geometrical modifications can be best rationalized by the existence of ligand-ligand interactions. Similar studies have been previously carried out by various authors[3-7] on ternary complexes of copper(II), palladium(II), nickel(II), cobalt(II), and zinc(II) in order to obtain evidence for ligand-ligand interactions. Both the stability constants of the complexes and their conformation were shown

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‡ Abbreviations used in this paper: TPP = tetraphenylporphine; DPME = deuteroporphine dimethyl ester; TMPyP = tetrakis[4-(*N*-methylpyridiniumyl)]porphine; TCPP = tetrakis(4-carboxylatophenyl)porphine.

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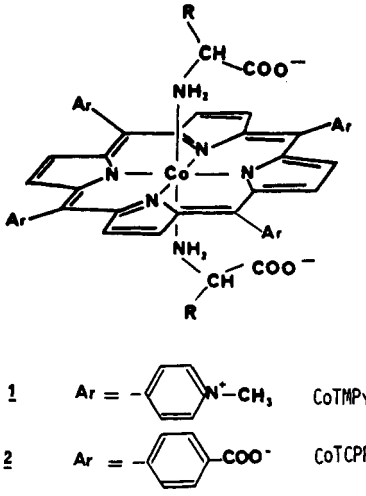

1 Ar = CoTMPyP

2 Ar = CoTCPP

Figure 1. Structures of $(L)_2$CoTMPyP and $(L)_2$CoTCPP with L = amino acid.

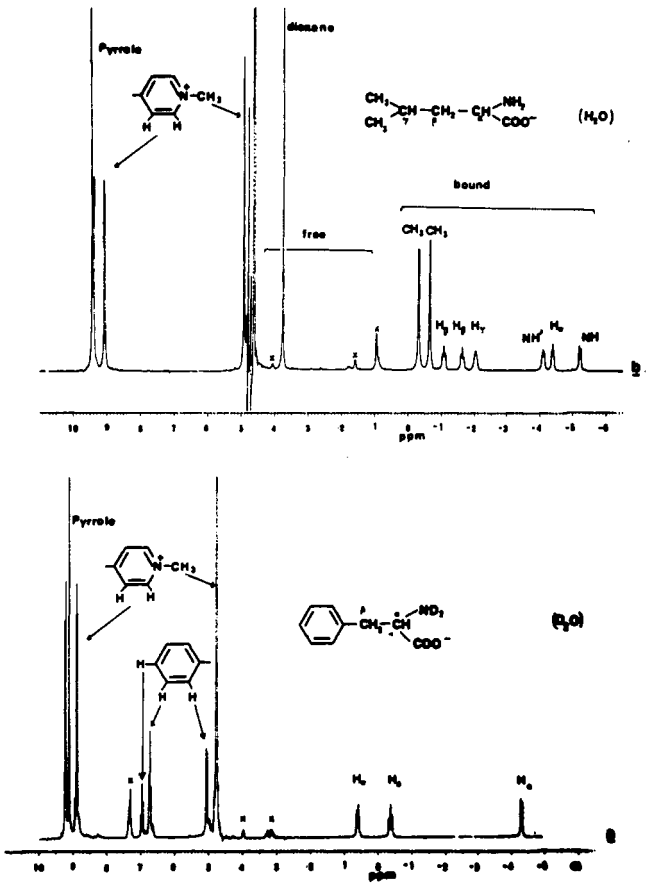

Figure 2. ^{1}H NMR spectra of the complexes CoTMPyP$(L)_2$: (a) L = Phe (D_2O); (b) L = Leu (H_2O).

to reflect such interactions, which appeared to be maximized in complexes containing an aromatic ligand, e.g. 2,2′-bipyridyl or *o*-phenanthroline and an aromatic amino acid or carboxylic acid.

Materials and Methods

Materials. CoTMPyP and CoTCPP were prepared by methods described elsewhere[8,9] and were used as the Cl^- and Na^+ forms, respectively. All amino acids were purchased from Sigma and used without further purification. Dideuteriated (2*R*,3*R*)(2*S*,3*S*)-phenylalanine was prepared according to Kirby et al.[10]

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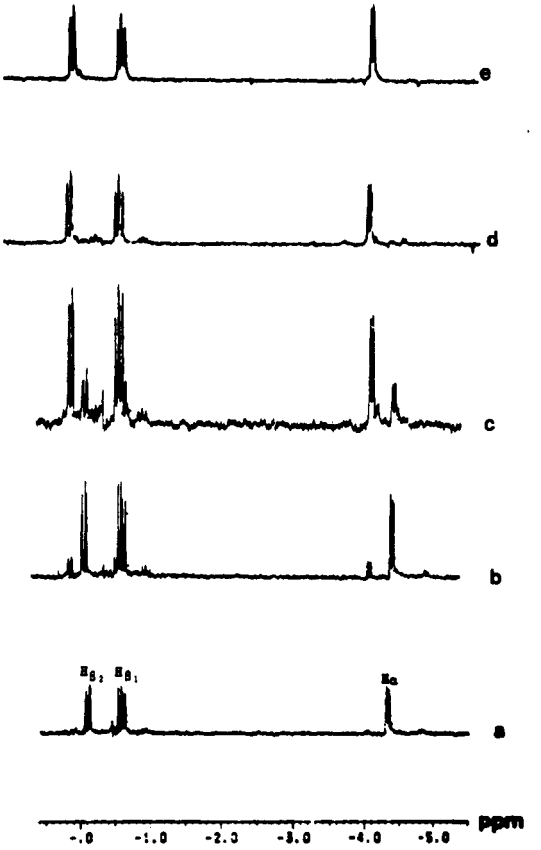

Figure 3. ^{1}H NMR spectra of a 2:1 mixture of Asn and CoTMPyP at various pH values: (a) pH = 2; (b) pH = 2.5; (c) pH = 3.3; (d) pH = 6.9; (e) pH = 10.2.

^{1}H NMR Spectroscopy. ^{1}H NMR spectra were measured with a Bruker AM spectrometer at 250 MHz with 0.05 M solutions of 1:2 or 1:3 CoTMPyP (or CoTCPP)/amino acid in aqueous solutions (D_2O or H_2O) and dioxane as internal standard (δ = 3.70 ppm vs TMS). We checked that the chemical shift of dioxane itself was not affected by the presence of porphyrin. Spectra in H_2O were obtained by irradiating the H_2O signal. The samples were prepared in deuteriated borax solutions (0.025 M), and the pH was adjusted to pH 10.5 (unless otherwise specified) by adding small amounts of NaOH (6 N). The digital resolution of the spectra was 0.1–0.3 Hz for the real part of the Fourier transform.

Results

^{1}H NMR Spectra and Structure of the Amino Acid–CoTMPyP Complexes. (1) General NMR Characteristics. The spectra of the amino acid–CoTMPyP complexes show three important general features.

(a) Protons of the coordinated amino acid are strongly shielded due to the ring-current effect of the porphyrin ring, with shieldings decreasing from H_α to H_β and to protons further remote on the side chain. This is best exemplified by the spectrum of the phenylalanine complex in D_2O (Figure 2a).

(b) The exchange between the bound and the free amino acid is slow on the NMR time scale, which is expected for cobalt(III) complexes. This allows the direct measurement of the chemical shifts and of the coupling constants. In most cases, a first-order analysis of the spectra was possible due to the large chemical shift differences, the proposed assignments being confirmed by double-resonance experiments.

(c) In the spectra measured in H_2O, e.g. the leucine complex (Figure 2b), two extra signals are observed at high field (ca. −4 to −6 ppm) that must be assigned to the diastereotopic NH_2 protons.[2] The magnetic nonequivalence of these protons indicates a slow exchange with the solvent protons and confirms that the amino acid is bound to the cobalt atom through the amino nitrogen atom.

(d) Measurement of the spectra at various pH values, e.g. the asparagine complex (Figure 3), further confirms the N-coordi-

Table I. Proton Chemical Shifts δ (ppm) in Complexes $(L)_2CoTMPyP$ (L = Amino Acid)

	NH			H_β			
	1	2	H_α	1	2	H_γ	
Gly			-3.18				
Ala	-4.71		-4.53	-1.72			
		-4.08					
Val	-5.60		-4.90	-1.62			
		-4.27			-1.55		
Leu	-5.28		-4.42	-1.71		-2.03	CH_3 -0.35
		-4.1			-1.12		
Ile	-5.66		-4.72		-1.57	-1.52	-0.40
		-4.2				-1.31	
						CH_3 -1.17	
Ser	-4.76		-4.38	0.71			
		-4.23			1.00		
Thr			-4.72		-1.02	0.89	
Asp	-3.59		-4.10	-0.57			
		-4.59			-0.40		
Asn	-4.37		-4.27	-0.72			
					-0.03		
Glu	-3.9			-1.32		-1.00	
		-4.22	-4.73		-0.92	-0.14	
Gln	-4.12			-1.27		-0.40	
		-4.24	-4.65		-1.03	0.14	
Arg	-4.96		-4.76	-1.55		-1.85	H_δ 1.65
		-4.14			-0.93	-1.45	
Phe	-6.2		-4.26	-0.45			H_o, 4.95, H_p 6.96
		-4.4			0.45		H_m 6.74
Tyr	-6.12		-4.5	-0.75			H_o 5.95
		-4.42			0.32		H_m 4.79
Trp	-5.95		-4.35	-0.41			H_2 5.04, H_6 7.1
		-4.3					H_4 5.97, H_7 7.23
					0.64		H_5 6.69

nation mode and gives evidence for the existence of two types of complexes depending on the pH. At low pH (<2), no high-field signals were observed, indicating that, in this pH range, no complexation occurs. At 2 < pH < 4, two sets of signals were observed at high field, indicative of two complexes: $CoTMPyP(L)(H_2O)$ and $CoTMPyP(L)_2$. As the pH was raised further, one set of signals disappeared, the other one becoming more intense until the intensities of the protons of the bound amino acid relative to those of the porphyrin protons reached a maximum corresponding to the stoichiometry $CoTMPyP(L)_2$ with L = amino acid. From pH 5 to pH 10.5, the position of the signals remained constant. These results clearly show that the amino acid is bound through its NH_2 group in its anionic form $R\text{-}CH(NH_2)COO^-$ and that the two aquo (or hydroxo) ligands that are initially bound to cobalt are progressively replaced as the pH is raised. This is in complete agreement with the reaction pathway first proposed by Pasternack et al.[8] for the substitution reactions of water-soluble cobalt(III) porphyrins (Scheme I). At pH ≥10.5, the only species present is $CoTMPyP(L)_2$.

Scheme I

$$CoTMPyP(H_2O)_2 + L \overset{K_1}{\rightleftharpoons} CoTMPyP(L)(H_2O) + H_2O$$

$$CoTMPyP(L)H_2O + L \overset{K_2}{\rightleftharpoons} CoTMPyP(L)_2 + H_2O$$

(2) Induced Chemical Shifts. In order to express the shieldings experienced by the various protons of the complexed amino acids, the induced shift

$$\Delta\delta = \delta_{free} - \delta_{bound}$$

was calculated for all protons, except NH_2, where δ_{free} and δ_{bound} are the proton chemical shifts for the free amino acid anion and the complexed amino acid, respectively.[11] The values of δ_{bound} were found to be pH and concentration independent. The values of δ_{bound} and $\Delta\delta$ for the $CoTMPyP(L)_2$ complexes are reported in Tables I and II. The values of the coupling constants are given in Tables V–VII. From these data, the following general observations can be drawn:

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Table II. Induced Chemical Shifts $\Delta\delta$ (ppm) in Complexes $(L)_2CoTMPyP$ (L = Amino Acid)

		H_β			
	H_α	1	2	H_γ	
Gly	6.4				
Ala	7.75	2.94			
Val	7.95	2.85		2.47	
				2.48	
Leu	7.66	3.11		3.77	CH_3 1.59
			2.59		1.24
Ile	7.81	3.21		2.44	1.29
				2.55	
				CH_3 2.42	
Ser	7.73	3.04			
			2.71		
Thr	7.80	2.93		2.11	
Asp	7.66	2.86			
			3.04		
Asn	7.86	3.16			
			2.70		
Glu	7.95	3.15		3.19	
			2.66	2.30	
Gln	7.92	3.21		2.71	
			2.89	2.16	
Arg	7.89	3.16		3.46	H_δ 1.55
			2.54	3.06	
Phe	7.75	3.30			H_o 2.31, H_m 0.58
			2.47		H_p 0.34
Tyr	7.83	3.46			H_o 1.98
			2.55		H_m 1.08
Trp	7.94	3.43			H_2 2.07, H_6 0.18
			2.55		H_4 1.68, H_7 0.17
					H_5 0.59

Table III. Proton Chemical Shifts δ (ppm) in Complexes of CoTMPyP with Methionine, *N*-acetylmethionine, and Lysin (pH = 10.3) and Histidine

	NH			H_β			
	1	2	H_α	1	2	H_γ	
N_ϵ acet Lys			-4.67	-1.53	-1.11	-1.66	H_δ 0.3
						-1.43	H_ϵ 2.1
N_α acet Lys			2.86		0.03		H_δ -1.77
							H_ϵ -4.11
Met	-4.71	-4.15	-4.37	-1.30	-0.75	-0.14	SCH_3 1.27
						0.10	
N_α acet Met						-0.51	SCH_3 -2.96
His[a]	-5.95	-4.31	-4.42	-0.46	0.47		H_4 5.35
							H_2 6.93
His[b]			2.69	1.58			H_2 1.31
							H_4 0.37

[a] pH = 11.4. [b] pH = 7.

(a) The H_α protons are the more shielded and are characterized by values of the induced shift $\Delta\delta$, which vary very little; $\Delta\delta = 7.8 \pm 0.2$ ppm.

(b) When the amino acid contains two protons on C_β, these protons exhibit different though fairly constant $\Delta\delta$ values. The more shielded $H_{\beta 1}$ ($\Delta\delta = 3.2 \pm 0.2$ ppm) is also more strongly coupled to H_α, whereas the less shielded $H_{\beta 2}$ ($\Delta\delta = 2.6 \pm 0.2$ ppm) is only weakly coupled to H_α, which suggests that the bound amino acids must adopt highly specific conformations around $N\text{–}C_\alpha$ and $C_\alpha\text{–}C_\beta$ (see below).

(c) The NH_2 protons deserve a special comment. In all but the glycine complex, two distinct signals are observed, and since their coupling with H_α can be observed, they give rise to an ABX spectrum with two markedly different coupling constants. These coupling constants were accurately determined by spectrum simulation using the LAOCOON program. Furthermore, the proton NH_1, which exhibits the larger coupling constant with H_α ($J_{NH_1H_\alpha}$ = 5.2–9.7 Hz) has a fairly constant chemical shift ($\delta = 4.5 \pm 0.3$ ppm), whereas the other proton, NH_2, which has a much smaller

Table IV. Proton Chemical Shifts δ (ppm) and Induced Chemical Shifts $\Delta\delta$ (ppm) in Complexes $(L)_2CoTCPP$ (L = Amino Acid) (D_2O)

	δ, ppm				$\Delta\delta$, ppm			
	H_α	H_β	H_γ	other protons	H_α	H_β	H_γ	other protons
Ala	−4.52	−2.00			7.74	3.22		
Val	−4.64	−1.03	CH_3 −1.69		7.69	2.95	CH_3 2.55	
			−1.45				2.31	
Leu	−4.29	−1.88	−2.05	CH_3 −0.66	7.51	3.30	3.73	CH_3 1.56
		−1.22				2.68		
				−0.37				1.29
Ile	−4.59	−1.95	−1.60	−0.37	7.69	3.59	2.53	
			CH_3 −1.20				CH_3 2.44	
			−1.05				2.29	
Ser	−4.55	0.35			7.9	3.4		
		0.65				3.1		
Thr	−4.69		−1.36		7.79		2.56	
Asp	−4.2	1.50			7.76	3.79		
		0.30				2.94		
Asn	−4.13	−0.75			7.79	3.29		
		−0.55				3.12		
Glu	−4.66	−1.4	−1.08		7.89	3.24	3.29	
		−0.97	−0.37			2.81	2.58	
Gln	−4.58	−1.41	−0.51		7.85	3.27	2.81	
		−1.14	0.01			3.00	2.31	
Arg	−4.82	−1.78	−2.01	H_δ 1.56	8.01	3.39	3.62	H_δ 1.63
		−1.35	−1.49			2.96	3.10	
Phe	−4.10	−0.26		H_o 4.48	7.61	3.12		H_o 2.54
		0.12		H_m 6.45		2.88		H_m 0.88
				H_p 6.58				H_p 0.75
Tyr	−4.2	0.45		H_m 4.82	7.64	3.17		H_m 1.85
		0.19		H_o 6.1		2.69		H_o 0.94
Trp	−4.16	−0.25		H_2 4.93	7.75	3.27		H_2 2.19
		0.48		H_4 5.8		2.72		H_4 1.72
				H_5 6.63				H_5 0.45
				H_6 6.89				H_6 0.20
				H_7 7.05				H_7 0.27

Table V. Values of $^2J_{NH_1,NH_2}$, $^3J_{NH_1,H_\alpha}$, $^3J_{NH_2,H_\alpha}$ (Hz) and Φ_2 (deg) in Complexes $(L)_2CoTMPyP$ (L = Amino Acid) at T = 293 K

	J_{NH_1,NH_2}	J_{NH_1,H_α}	J_{NH_2,H_α}	ϕ^2
Ala	11.3	1.0	5.2	135
Val	11.5	1.6	8.0	150
Leu	11.5	2.0	8.0	150
Ile	12.0	1.5	7.0	145
Ser	11.5	2.0	7.0	140
Asp	11.0	1.7	7.7	145
Glu	12.0	1.7	8.0	150
Met	12.0	1.7	7.2	140
Arg	12.0	1.7	7.2	140
Phe	11.0	1.2	8.7	153
Tyr	12.1	1.2	9.7	160
His	11.0	1.2	9.0	158

Table VI. Coupling Constants $^2J_{\beta1\beta2}$, $^3J_{\alpha\beta1}$, and $^3J_{\alpha\beta2}$ (Hz) and Populations of the Various Conformers I–III in Complexes $(L)_2CoTMPyP$ (L = Amino Acid)

	$J_{\alpha\beta2}$	$J_{\alpha\beta1}$	$J_{\beta1\beta2}$	p_I	p_{II}	p_{III}
Val	4.50					
Leu	3.35	10.08	13.7	74	3	23
Ile	4.03					
Ser	3.90	8.10	12.2	64	4	32
Thr	6.50					
Asp	3.35	10.80	15.5	83	3	14
Asn	2.53	10.03	13.0	85	0	15
Glu	3.00	10.01		74	0	26
Gln	4.50	7.70	15.3	49	14	37
Met	3.65	9.40		67	4	29
Arg	3.35	9.45	14.5	68	3	29
Phe	2.90	11.60	13.0	92	0	8
Tyr	3.00	11.80	13.0	93	0	7
Trp	2.83	11.88	14.0	94	0	6
His	3.00	11.50	15.0	90	0	10

coupling constant with H_α (J_{NH_2,H_α} = 1.5 ± 0.4 Hz), has a much more variable chemical shift (δ = −3.5 to −6.2 ppm). The implications of these differences in terms of conformational analysis will be outlined below.

Table VII. Coupling Constants $^3J_{\beta\gamma}$ and $^2J_{\gamma\gamma}$ (Hz) in Complexes $(L)_2CoTMPyP$ (L = Amino Acid)

	$J_{\beta1\gamma1}$	$J_{\beta1\gamma2}$	$J_{\beta2\gamma1}$	$J_{\beta2\gamma2}$	$J_{\beta1\beta2}$	$J_{\gamma1\gamma2}$
Glu	7.2	6	6	7	15.3	16.0
Glu	6.4	4.5	5.3	6.22		15.4
Arg	3.9	11.5	11.5	3.8	14.5	15
Met	4.7	6.1	6.5	4		13.8
Leu	3.45		9.5			
Ile	3.6	8.3				

(3) Structures of the Complexes $CoTMPyP(L)_2$ with L = Histidine, Lysine, and Methionine. For most amino acids, metal complexation involves the NH_2 group, which is the most basic functional group of the molecule. In the case of histidine and lysine, there are two nitrogenous functions that are likely to bind to the cobalt atom. To some extent, this is true also for methionine, which contains a thioether moiety in addition to the NH_2 group. The presence of an additional potential donor group within the molecule suggests that three different complexes can in principle be formed: two that are symmetrical and one that is unsymmetrical, depending on whether the groups binding to the metal are the same or not. Because in each of these amino acids the potential ligands have different pK_a values, the structure of the corresponding complexes should be strongly pH dependent, which proved to be the case.

(a) Complexes with Histidine. The NMR spectra of 2:1 mixtures of histidine/CoTMPyP were measured at various pH values between pH 5 and pH 11. It was anticipated that because of the differing pK_a values of the imidazole and NH_2 groups, the predominent species at lower pH values should be complex A (Figure 4) involving imidazole groups as ligands, whereas, at high pH values, complex C with the more basic NH_2 group as ligand should be favored. At intermediate pH, a mixture of the symmetrical complexes A and C with the unsymmetrical one B should be observed. The experimental results are in complete agreement with these predictions. At pH 5 (Figure 5a), one predominant set of signals is observed for the histidine protons, the signals at highest fields being singlets (1 H) at δ = 0.37 and 1.31 ppm, which are assigned to H_2 and H_4 of the imidazole ring. The strong

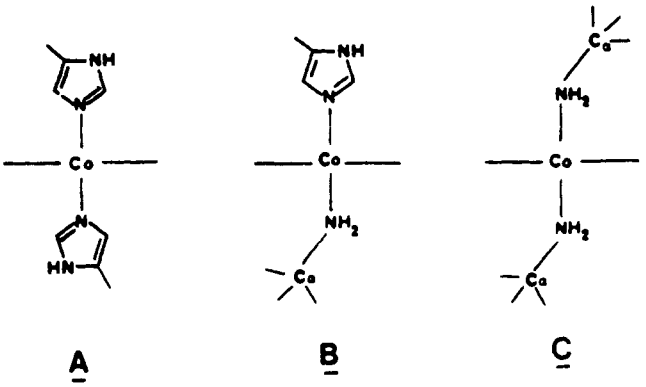

Figure 4. Possible structures of the complexes of histidine with CoTMPyP.

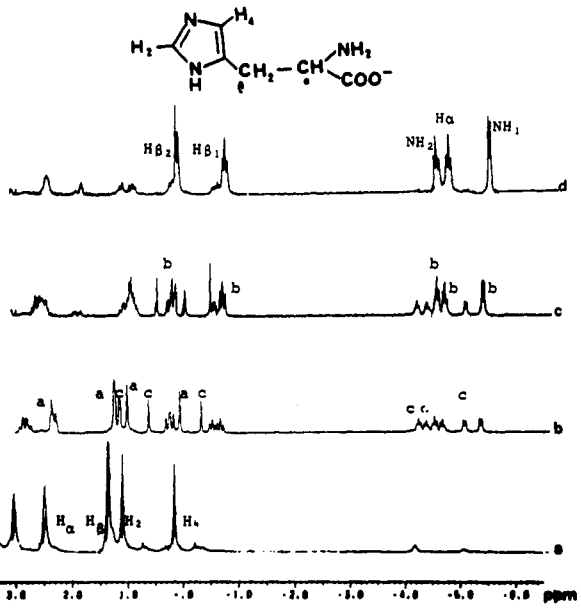

Figure 5. ^{1}H NMR spectra (high-field region) of a 2:1 mixture of His with CoTMPyP at various pH values: (a) pH = 5; (b) pH = 7.5; (c) pH = 10.2; (d) pH = 11.4.

shielding experienced by these protons clearly reflects the binding of the amino acid through the imidazole N_3 atom (Table III).

At pH 11 again only one set of signals is observed (Figure 5d), which is very similar, except for the aromatic protons, to that observed in the case of the phenylalanine complex (Figure 2a). The characteristic high-field signals for the NH_2 and H_β protons clearly indicate that, at this pH, complex C is the predominant species (Table III).

At intermediate pH values (Figure 5b,c), a third group of signals in addition to those of complexes A and C can be observed. The relative intensities of these various signals are observed to vary with pH. This third group of signals consists of three quartets at high field, which can be assigned to H_α and NH_2 protons, and two singlets at δ = 0.9 and −0.1 ppm, corresponding to the imidazole H_2 and H_4 protons. The fact that not only the NH_2 and H_α protons but also the imidazole protons undergo strong shieldings can be accommodated only by the unsymmetrical structure B.

(b) Complexes with Methionine. Mixtures of methionine and CoTMPyP (3:1) exhibit NMR spectra that vary greatly with pH (Figure 6). At pH 9.8, a "normal" spectrum is observed with a highly shielded H_α proton, which is assigned to the complex with NH_2–cobalt coordination. At low pH (3.9), two singlets are observed at δ = −3.0 and −3.2 ppm, corresponding to CH_3 groups and indicating coordination of methionine by the thioether sulfur atom. This was confirmed by measuring the spectrum of the *N*-acetylmethionine–CoTMPyP complex, which showed a 3 H singlet at δ = −2.96 ppm. In this case the only available ligand

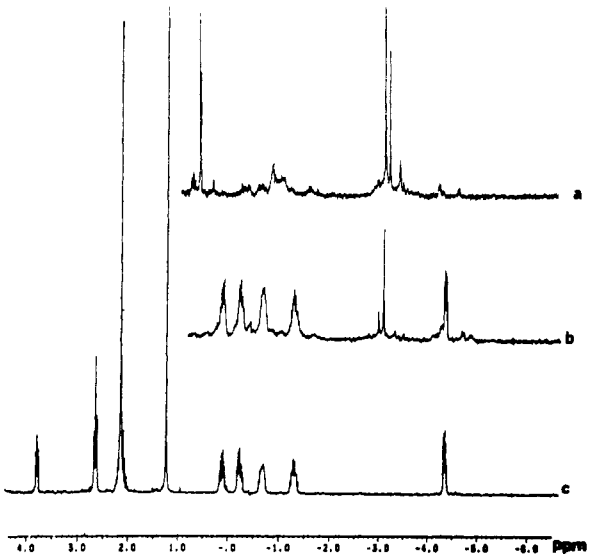

Figure 6. ^{1}H NMR spectra (high-field region) of a 3:1 mixture of Met with CoTMPyP at various pH values: (a) pH = 3.9; (b) pH = 5.5; (c) pH = 9.8.

for cobalt is the $-S-CH_3$ group (Table III). The appearance of two singlets in the spectrum of methionine at pH = 3.9 can be rationalized by the presence of trivalent asymmetric sulfur atoms in the complex resulting in magnetically nonequivalent diastereotopic $S-CH_3$ groups.

(c) Complexes with Lysine. The spectra of a 3:1 mixtures of lysine, N_ϵ-acetyllysine, and N_α-acetyllysine and CoTMPyP were measured at pH = 10.3. The complexity of the lysine complex spectrum in the high-field region and a comparison of this spectrum with those of the *N*-acetyllysine complexes (Table III) strongly suggest that, at this pH value, the lysine complex is a mixture of the N_α- and N_ϵ-bound species. The multiplet at δ = −4.61 ppm has a chemical shift that is characteristic of H_α protons in NH_2-bound amino acid complexes, whereas the multiplet at −4.11 ppm is typical of a CH_2 group adjacent to a coordinated NH_2 group.[2] In the N_α-acetyllysine complex, these two protons appear in the vicinity of δ = −4.2 ppm but as two multiplets instead of one. The nonequivalence observed for these two diastereotopic protons is quite remarkable considering that they are five bonds away from the asymmetric C_α atom.

Variations of the pH induce modifications in the relative intensities of the signals corresponding to the N_α- and N_ϵ-bound complexes, but the predominance of the N_α-bound species at all pH values is observed and may well reflect a stabilization of this species due to a favorable electrostatic interaction between the $-COO^-$ group of the amino acid and the positively charged porphyrin ligand.

(4) Complexes of Amino Acids with CoTCPP. Complexation of the amino acids with CoTCPP occurs at the same conditions as with CoTMPyP, the optimal pH for the formation of the 2:1 complex being 10–10.5. The NMR spectra of the CoTCPP complexes are qualitatively similar to those with CoTMPyP. The δ and $\Delta\delta$ values are very similar for the two types of complexes (Table IV), except for those complexes with aspartic acid, asparagine, and serine, which show very different patterns of signals for the H_α, $H_{\beta 1}$, and $H_{\beta 2}$ protons. This suggests a different geometry for the two types of complexes involving these three amino acids.

Conformational Analysis. Two sets of parameters have been used to study the conformation of the bound amino acid: $^3J_{HH}$ and $\Delta\delta$.

$^3J_{HH}$ coupling constants between H_α and H_β protons have been used repeatedly to study the conformation around the C_α–C_β bond of amino acids and peptides in solution. In the present study, the shielding induced by the porphyrin ring current spreads the resonance signals of the amino acid side-chain protons over a large range of chemical shifts, which makes the measurement of nearly

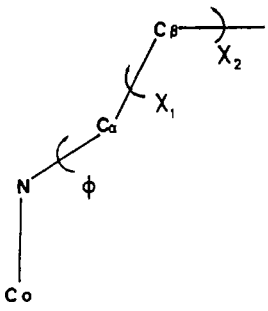

Figure 7. Dihedral angles ϕ, χ_1, and χ_2 in amino acids.

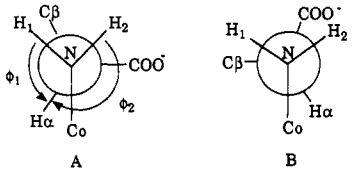

Figure 8. Conformations A and B around the N–C_α bond.

all vicinal coupling constants possible. Moreover, by using H_2O as solvent, it was possible to observe the signals of the diastereotopic NH_2 protons and measure the J_{NH,H_α} coupling constants.

The $\Delta\delta$ values give an estimate of the shielding induced by the macrocycle for any proton. In order to relate the $\Delta\delta$ values to the position of the protons with respect to the porphyrin ring, we adapted the ring-current model we previously developed for the CoDPDME–amino ester complexes to the CoTMPyP–amino acid case.[12] A detailed account of the results obtained with this model in the conformational analysis of various flexible molecules (amines, amino acids) will be published separately.[13] We will only mention here some results that confirm or complement the conclusions drawn from the coupling-constant-based analysis.

The methodology that was used to achieve our conformational analysis consists of determining the conformational state of the bound amino acid around each bond, starting from the N–C_α bond and progressing along the side-chain C–C bonds as far as the vicinal coupling constants are available (Figure 7).

(1) Conformation around the N–C_α Bond. The conformational analysis around the N–C_α bond made use of the $^3J_{NH,H_\alpha}$ coupling constants (Table V). As mentioned before, the two coupling constants have significantly different values in all complexes. The smaller value (^3J = 1.5 ± 0.5 Hz) is associated with the NH proton, which has a fairly constant chemical shift (δ = −4.5 ± 0.3 ppm), whereas the larger value (^3J = 7 ± 2 Hz) concerns the NH proton with a much more variable chemical shift (δ = −3.5 to −6.2 ppm). The large difference observed between these two coupling constants strongly suggests that the molecule adopts a predominant, if not unique, conformation around the N–C_α bond. In order to define more precisely this geometry, a Karplus-type equation relating the $^3J_{NH,H_\alpha}$ values to the dihedral angles between the N–H and C_α–H_α bonds was used. Several equations of the general form

$$^3J_{NH,H_\alpha} = A + B\cos\phi + C\cos^2\phi$$

have been proposed, which differ by the values of the coefficients A–C.[14] We have arbitrarily used the coefficients proposed by Bystrov (A = 0.4, B = −1.1, C = 9.4),[14a] which appear to be of more general use in the literature than the other sets of coefficients.

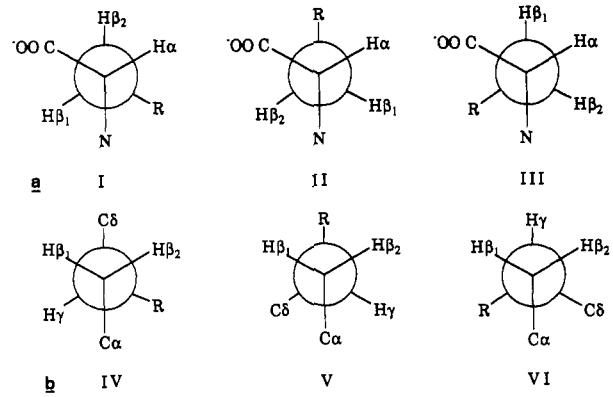

Figure 9. Conformations I–III around C_α–C_β (a) and IV–VI around C_β–C_γ (b).

The results of our calculations are shown in Table V. They suggest that for all complexes, a single conformation around N–C_α is present, which is characterized by one of the dihedral angles φ_1 close to 100° (±10°), with the other angle φ_2 ranging from 130° to 160°. Two possible conformations A and B characterized by these dihedral angle values can be envisaged (Figure 8). The choice between these two geometries implies an identification of the two NH protons. This can be done by careful examination of their chemical shift variations throughout the series of complexes. In conformation A, proton NH_1 is located closer to the amino acid side chain than to the carboxylate group, whereas the reverse is true for proton NH_2. Consequently, if conformation A is the correct one, NH_1 should have a small $^3J_{NH_1,H_\alpha}$ coupling constant and at the same time have a chemical shift that should be largely dependent on the amino acid structure, while proton NH_2 should show a larger $^3J_{NH,H_\alpha}$ constant and a much more constant δ value. This is exactly what is observed experimentally. Moreover, if the amino acid adopted conformation B, this would bring the side chain in very close contact with the porphyrin ring, which would result in very unfavorable ligand–ligand interactions. In conformation A, the carboxylate group is brought closer to the macrocycle, but because it is much less bulky than most side chains, the interaction is much less destabilizing. Conformation A is in fact closer to an eclipsed than to a staggered conformation. The same type of geometry around N–C_α was previously proposed for CoDPDME complexes of amino esters[12] and indeed observed in the X-ray structure of the CoTPP((1-phenylethyl)amine)$^{2+}$ complex.[15]

The small variations observed for the dihedral angles ϕ_1 and ϕ_2 throughout the series can also be assigned to steric factors. The smaller ϕ_2 angle (ϕ_2 = 130°) is observed for alanine, which has the least bulky side chain of all the amino acids. As the size of the side chain increases, ϕ_2 values increase reaching a maximum with aromatic amino acids (ϕ_2 = 160°). This variation corresponds in fact to a decrease of the eclipsed interaction between the side-chain atoms and N–H_1. It thus appears that in conformation A, unfavorable ligand–ligand interactions are minimized at the expense of increased eclipsed repulsions.

(2) Conformation around the C_α–C_β Bond. The conformational states around the C_α–C_β bond were deduced from the ^3J coupling constants by using a Karplus-type equation. It has been assumed by many authors[16] that amino acids and peptides predominantly adopt the staggered conformations I–III around the C_α–C_β bond, (Figure 9a). The real conformational state in most cases is a mixture of these conformations in relative amounts that can be

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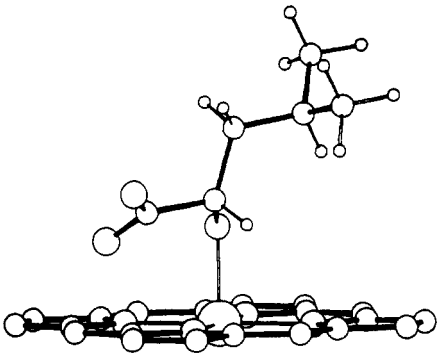

Figure 10. Preferred geometry of leucine in the complex $(Leu)_2CoTMPy$. (Only one of the amino acid ligand is shown; the *N*-methylpyridyl groups are omitted.)

deduced from the experimental $^3J_{\alpha\beta1}$ and $^3J_{\alpha\beta2}$ coupling constants by using the following relationships:

$$J_{\alpha\beta1} = p_I J_{\alpha\beta1}(g-) + p_{II} J_{\alpha\beta1}(t) + p_{III} J_{\alpha\beta1}(g+)$$

$$J_{\alpha\beta2} = p_I J_{\alpha\beta2}(g-) + p_{II} J_{\alpha\beta2}(t) + p_{III} J_{\alpha\beta2}(g+)$$

$$p_I + p_{II} + p_{III} = 1$$

where p_I, p_{II}, and p_{III} are the molar fractions of the various conformations and $J_{\alpha\beta1}(g-)$, $J_{\alpha\beta1}(t)$, etc. are the values of the vicinal coupling constants in the individual conformations I–III.

In order to make use of these relationships, one must unequivocally identify the signals of the two protons $H_{\beta1}$ and $H_{\beta2}$. For that purpose, the complex of CoTMPyP with ($^2H_2$-2*S*3*S*,2*R*3*R*)-phenylalanine[10] was synthesized. Comparison of the spectrum of this complex with that of the undeuteriated phenylalanine complex revealed that the pro-R $H_{\beta1}$ proton was more shielded and more strongly coupled to H_α than the pro-S $H_{\beta2}$ proton. As was noted above, for complexes of amino acids that have two H_β protons, these protons exhibit remarkably constant $\Delta\delta$ and J_{H_α,H_β} values. Just as in the phenylalanine complex, the more shielded $H_{\beta1}$ proton is always more strongly coupled to H_α, and we assume that it has the pro-R configuration, the less shielded $H_{\beta2}$ having the pro-S configuration.[17]

Several Karplus-type equations $^3J = f(\theta)$ have been proposed in the literature.[14-16] They differ by the values of the various $J_{\alpha\beta1}(g-)$, etc. coefficients, which take into account the influence of substituent electronegativity and orientation on the ^3J values. We used the sets of parameters proposed by Kopple et al.[16c]

$$J_{\alpha\beta1}(g-) = J_{\alpha\beta1}(g+) = J_{\alpha\beta2}(g-) = J_{\alpha\beta2}(g+) = 3.25 \text{ Hz}$$

$$J_{\alpha\beta1}(t) = J_{\alpha\beta2}(t) = 12.4 \text{ Hz}$$

to calculate p_I, p_{II}, and p_{III} for both the free and the complexed amino acids. The results of these calculations are reported in Table VI.

Examination of Table VI reveals that when amino acids are complexed to CoTMPyP, they adopt very similar conformations, not only around the N–C_α bond but also around the C_α–C_β bond. The large values of $J_{\alpha\beta1}$, in apolar amino acids, imply a single predominant conformation, namely conformation I (Figure 9a). In this geometry, which for example accounts for 74% the total conformations of leucine and more than 90% of those of the aromatic amino acids, the C_α–C_β bond lies approximately parallel to the porphyrin ring. This places the substituent on C_β (isopropyl, phenyl, hydroxyphenyl, indole, or imidazole) at a distance that is adequate for hydrophobic or stacking interactions (≈3.5 Å).

(17) We could also confirm that in the cases of the proline complex the more shielded H_β proton has the pro R configuration by measuring the coupling constants between the COO^- carbon atom and $H_{\beta1}$ or $H_{\beta2}$.[16c] The values of the coupling constants $J_{COO,H_{\beta1}}$ = 3 Hz and $J_{COO,H_{\beta2}}$ = 3 Hz indicate that conformation I is the predominant conformation and therefore that H_β, which shows the greatest coupling constant with H_α, has the pro R configuration.

Table VIII. Values of $\Delta\delta_{obs}$ and $\Delta\delta_{calc}$ in Complexes of Leucine and Phenylalanine with CoTMPyP

	$\Delta\delta_{obs}$	$\Delta\delta_{calc}$[a]	$\Delta\delta_{obs}$	$\Delta\delta_{calc}$[a]
H_α	8.09	7.69	7.80	7.84
$H_{\beta1}$	3.05	3.15	3.32	2.95
$H_{\beta2}$	2.55	2.61	2.34	2.39
H_γ	4.21	3.79		
CH_3	1.30	1.28		
CH_3	2.06	1.61		
H_o			2.27	2.32
H_m			0.54	0.67
H_p			0.33	0.30

[a] $\Delta\delta_{calc}$ obtained with d_{Co-N} = 1.983 Å and Co–N–C_α = 116°.

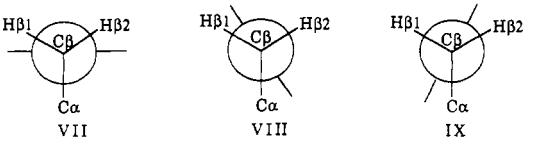

Figure 11. Conformations VII–IX around C_β–C_γ in aromatic amino acids.

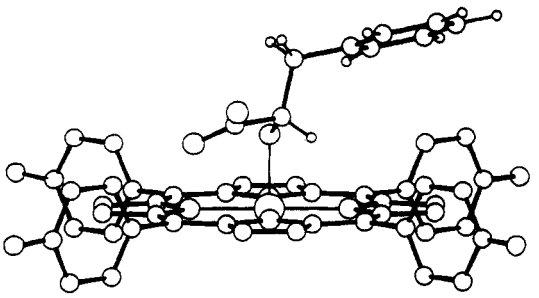

Figure 12. Preferred geometry of phenylalanine in the complex $(Phe)_2CoTMPyP$. (Only one of the amino acid ligands is shown.)

In the case of complexed polar amino acids the predominance of conformation I is not as pronounced as with the apolar amino acids though a significant increase in the relative amount of conformer I is observed when going from the free to the bound amino acid. More detailed comments on this general phenomenon will be given below.

(3) Conformations around the C_β–C_γ Bond. The values of the $^3J_{\beta\gamma}$ coupling constants are reported in Table VII. As in the case of the conformation around the C_α–C_β bond, we suppose that only the staggered conformations IV–VI can contribute significantly (Figure 9b).

In the case of the leucine complex, the strongly differing $J_{\beta\gamma}$ coupling constants suggest a single predominant conformation. On the basis of the previous identification of the two β protons, we propose conformation IV (R = CH_3) as the preferred rotamer. If we consider now the overall conformation of leucine in the complex, we come to the conclusion that the geometry represented in Figure 10 is the preferred one in solution. This is confirmed by the calculations of the induced shifts $\Delta\delta$ performed on the various protons of the amino acid.[13] Inspection of the results (Table VIII) reveals reasonably good agreement between calculated and experimental values. In particular, the relatively strong shielding of the H_γ proton is in accord with the fact that the C_γ–H_γ bond is pointing toward the porphyrin ring.

With the aromatic amino acids phenylalanine, tyrosine, and tryptophane, the conformation around the C_β–C_γ bond cannot be deduced from the coupling constant values, as the C_γ carbon atom bears no proton. Examination of the molecular models of the complexes in their preferred geometry around C_β–C_γ strongly suggests that the aromatic rings should adopt the more stable conformation VII rather than the less stable ones VIII or IX (Figure 11). In the latter geometries, close-contact interactions occur between the porphyrin ring and the aromatic ring protons that are absent in conformation VII. In this geometry, the aromatic ring of the amino acid lies approximately parallel to the

Table IX. Values of $^3J_{\alpha\beta1}$, $^3J_{\alpha\beta2}$, p_l, K, $\Delta G°$, and $\Delta\Delta G°$ (kJ/mol) (See Text) at T = 293 K

	free amino acid					complexed amino acid					
	$J_{\alpha\beta1}$, Hz	$J_{\alpha\beta2}$, Hz	p_l, %	K_l	$\Delta G°$, kJ/mol	$J_{\alpha\beta1}$, Hz	$J_{\alpha\beta2}$, Hz	p_l, %	K_c	$\Delta G_c°$, kJ/mol	$\Delta\Delta G°$, kJ/mol
Leu	7.98	5.40	52	1.1	−0.2	10.08	3.35	74	3.0	−2.70	−2.50
Ser	5.01	4.25	19	0.2	3.6	9.10	3.90	64	1.8	−1.44	−4.04
Asp	8.79	3.88	61	1.5	−1.04	10.91	3.35	84	5.1	−4.02	−2.97
Asn	9.07	4.65	64	1.8	−1.41	11.03	2.53	85	5.7	−4.26	2.85
Glu	6.7	5.55	38	0.6	1.25	10.00	3.00	74	2.8	−2.52	−3.42
Gln	7.0	6.5	34	0.5	1.7	7.70	4.50	49	1.0	0.04	1.70
N_ϵ-acetLys	8.6	4.73	58	1.4	−0.8	9.54	2.60	69	2.2	−1.93	−1.13
Arg	6.05	5.37	30	0.5	1.7	9.45	3.35	68	2.1	−1.82	−3.57
Met	6.5	5.5	35	0.5	1.7	9.40	3.65	67	2.0	−1.70	−3.40
Phe	7.49	5.25	46	0.8	0.44	11.68	2.90	92	11.5	−6.00	−6.40
TyrOH	7.42	5.16	46	0.9	0.4	11.80	3	93	14.4	−6.54	−6.94
$TyrO^-$	7.30	5.38	44	0.8	0.57	11.63	3	92	10.9	−5.86	−6.26
Trp	7.30	5.02	44	0.8	0.57	11.88	2.83	94	15.7	−6.75	−7.10
His	7.70	5.09	49	1.0	0.13	11.50	3	90	9.0	−5.39	−5.40

porphyrin plane, as shown in Figure 12 in the case of the phenylalanine complex. Calculation of $\Delta\delta$ using the ring-current model[13] brings support to this assumption. The $\Delta\delta$ values for the various protons of phenylalanine were calculated by assuming that the conformational states around the N–C_α and C_α–C_β bonds were those determined by using the ^3J coupling constants (Table VIII). The Co–N bond length and Co–N–C bond angle are identical with those used for calculating $\Delta\delta$ values for leucine. The results shown in Table VIII indicate a reasonably good agreement between calculated and experimental values, lending strong support for the predominance of conformation VII around C_β–C_γ. Detailed $\Delta\delta$ calculations have not been performed for the complexes with tyrosine and tryptophane, but the comparison of the $\Delta\delta$ values of the aromatic protons of these two amino acids with those of phenylalanine strongly suggests that the preferred geometry for tryptophane and tyrosine is similar to that of phenylalanine. In particular, the $\Delta\delta$ values of H_o in tyrosine ($\Delta\delta$ − 1.98 ppm) and H_2 in tryptophane ($\Delta\delta$ = 2.07 ppm) are close to the $\Delta\delta$ value of H_o in phenylalanine ($\Delta\delta$ = 2.31 ppm).

In the case of the arginine complex, two of the $^3J_{\beta\gamma}$ coupling constants are small (Table VII), ca. 3.8 Hz, the two others being large, ca. 11.5 Hz. These values are strongly in favor of conformation IV (R = H), which places the positively charged guanidinium group far from the porphyrin ring.

By contrast, in the complexes with glutamic acid and methionine, the $^3J_{\beta\gamma}$ coupling constants all take values that are not very different from the ^3J value for an equimolar mixture of the three possible staggered conformations IV–VI (R = H), i.e. 7 Hz. We therefore conclude that for these complexes there is no preferred conformation around C_β–C_γ.

Discussion

Ligand–Ligand Interactions in $CoTMPyP(L)_2$ Complexes with L = Amino Acid. From the results of the preceding conformational analysis, we can speculate on the nature of the ligand–ligand interactions that control the geometry of the complexed amino acid molecules. Studies of the conformation of the free amino acids in solution have shown that a large number of factors influence the geometry adopted by the molecule:[18] ionization state of the amino acid, interaction between the various groups present in the molecules (steric, electrostatic, hydrophobic, hydrogen bonding), and to a lesser extent solute–solvent and solute–solute interactions.

The most general conclusion which can be drawn from this conformational study is that complexation of the amino acids to CoTMPyP compels the former to adopt a predominant conformational state which is a combination of the nearly eclipsed conformation A around the N–C_α bond and the staggered conformation I around C_α–C_β bond. In order to quantify the amount of extra stabilization observed for conformation I in the various complexes, we will consider the intramolecular equilibrium that

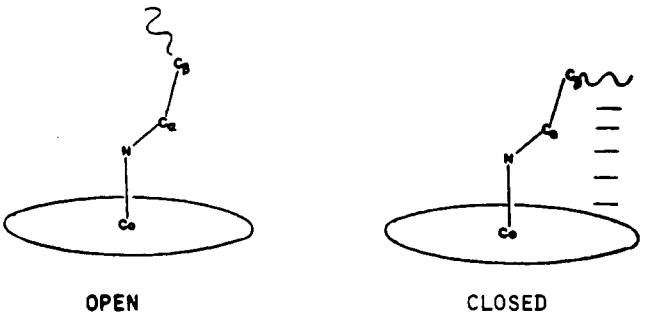

Figure 13. "Open" and "closed" forms of the amino acid ligands.

exists between the "closed" conformation I and the "open" conformations II and III (Figure 13):

"open" forms (II, III) ⇌ "closed" form (I)

Following Sigel's assumption,[5] in the closed form I, the amino acid side chain is located closer to the porphyrin ring than in the open forms II and III. If, on complexation, the percentage of form I increases, this must be attributed to stabilizing ligand–ligand interactions.

The constant for the above equilibrium is

$$K = \frac{[\mathrm{I}]}{[\mathrm{II}] + [\mathrm{III}]} = \frac{p_\mathrm{I}}{p_\mathrm{II} + p_\mathrm{III}}$$

If K_L and K_C are the values of this constant for the free and complexed amino acid, respectively, then the stability increase $\Delta\Delta G°$ due to ligand–ligand interactions can be estimated:

$$\Delta\Delta G° = \Delta G°_\mathrm{C} - \Delta G°_\mathrm{L} = -RT \ln (K_\mathrm{C}/K_\mathrm{L})$$

In Table IX the values for K and $\Delta G°$ for the free and bound amino acid and $\Delta\Delta G°$ values for the various amino acids are listed. Inspection of this table reveals that, except glutamine for which the closed form appears to be destabilized on complexation, all amino acids are characterized by negative $\Delta\Delta G°$ values, reflecting the stability increase of the closed conformation.

The aromatic amino acids exhibit the largest negative $\Delta\Delta G°$ values with the following order of decreasing stability of form I: Trp > TyrOH > Phe > $TyrO^-$ > His. This order is quite comparable to the variation in stability observed by Yamauchi et al. for the case of ternary Cu(II) complexes of the general formula Cu(DA)(AA), where DA is an aromatic diamine.[7c] Interestingly, these authors also noticed the decrease in stability that accompanies the dissociation of the tyrosine phenol OH group. These results further suggest that the increased stability of the closed form results, at least in part, from intramolecular hydrophobic interactions between the aromatic rings of the amino acid and the porphyrin ring. This stacking of aromatic rings induces a greater stabilization of form I than the hydrophobic interaction between the *sec*-butyl chain of leucine and the porphyrin macrocycle, which is in agreement with the observations made by

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Table X. Coupling Constants $^2J_{\beta1\beta2}$, $^3J_{\alpha\beta}$, and $^3J_{\beta\gamma}$ (Hz) in Complexes $(L)_2$CoTCPP (L = Amino Acid)

	$J_{\alpha\beta1}$	$J_{\alpha\beta2}$	$J_{\beta1\beta2}$	$J_{\beta1\gamma1}$	$J_{\beta1\gamma2}$	$J_{\beta2\gamma1}$	$J_{\beta2\gamma2}$
Val	3.3						
Leu	9.4						
Ile	3.8			4.5	9.3		
Ser	4.1	7.5	11.5				
Thr	4.33						
Asp	4.00	7.25	15.8				
Asn	3.5	8.4	15.0				
Glu	8.2			4	9	9.5	3.5
Gln	7.5			7	6.5	7.5	5.5
Arg	9.0						
Trp	11.5		12.9				

Martin et al.[6] in the case of ternary dipeptide complexes of Pd(II).

The stabilization of the closed form observed in the case of the polar amino acid cannot be rationalized so easily. The $\Delta\Delta G°$ values for these amino acids are less negative than for the aromatic amino acids, which makes any tentative explanations less reliable. However, in the case of the complex of aspartic acid, it can be proposed that this stabilization is brought about by a favorable electrostatic interaction between the negatively charged β-carboxylate of aspartate and the positively charged pyridinium substituents. If this is true, one should observe a completely different conformation of the amino acid when it is complexed to the anionic cobalt(III) porphyrin CoTCPP. Indeed, the $\Delta\delta$ and ^3J values for the H_α and H_β protons are quite different in the CoTMPyP and CoTCPP complexes of aspartic acid (Tables IV and X). More precisely, in the latter complex, $H_{\beta1}$, which is weakly coupled to H_α ($J_{\alpha\beta1}$ = 4.0 Hz), is more shielded than $H_{\beta2}$, which is more strongly coupled ($J_{\alpha\beta2}$ = 7.25 Hz). This is the reverse of what was observed with the CoTMPyP complex. This observation strongly suggests that conformation II (Figure 9a) is the predominant conformation in the CoTCPP complex, instead of conformation I. This geometry places the β-carboxylate group of aspartate as far as possible from the negatively charged porphyrin ligand. Similar variations in the induced chemical shifts $\Delta\delta$ and ^3J coupling constants are observed with asparagine and serine when the TCPP ligand is substituted for the TMPyP ligand. This suggests that polar interactions also play a role in the stabilization of form I in the CoTMPyP complexes.

By contrast, the NMR data of the CoTMPyP and CoTCPP complexes of glutamine and arginine are quite similar, which suggests that a change in the structure of the porphyrin ligand does not result in a change of overall geometry for these amino acids. In the case of glutamic acid, the most important change concerns the values of the $J_{\beta\gamma}$ coupling constants. The values in the CoTCPP complex are in good agreement with a predominance of conformation II, which places the γ-COO^- group far from the porphyrin ring whereas, in the CoTMPyP complex, the $J_{\beta\gamma}$ values were not very different from one another, suggesting a mixture of the three conformations around C_β–C_γ. It is interesting to note that the ^3J and $\Delta\delta$ values are nearly identical for the two types of complexes, CoTCPP and CoTMPyP, in the cases of apolar or aromatic amino acids. This further confirms that with these amino acids, hydrophobic interactions are the main contributors to the stabilization of conformation I, whereas, with the polar amino acids, this stabilization is the result of favorable electrostatic interactions between the cationic TMPyP ligand and an electronegative substituent on the β-carbon atom of the side chain. With amino acids having longer side chains, these interactions probably become weaker and the dominating factor again appears to be the stabilizing interactions between the aliphatic part of the side chain and the porphyrin ligand.

Temperature Dependence of $\Delta\Delta G°$. To estimate the $\Delta\Delta H°$ and $\Delta\Delta S°$ values associated with the stability increase of conformation I, $\Delta\Delta G°$ values were measured for several amino acids by using the above methodology at several temperatures between 270 and 350 K (Figure 14). The results, which are reported in Table XI, clearly indicate that the amino acid complexes are all characterized by negative values of $\Delta\Delta H°$ and $\Delta\Delta S°$. Therefore, the stability increase largely depends on $\Delta\Delta H°$. It was previously

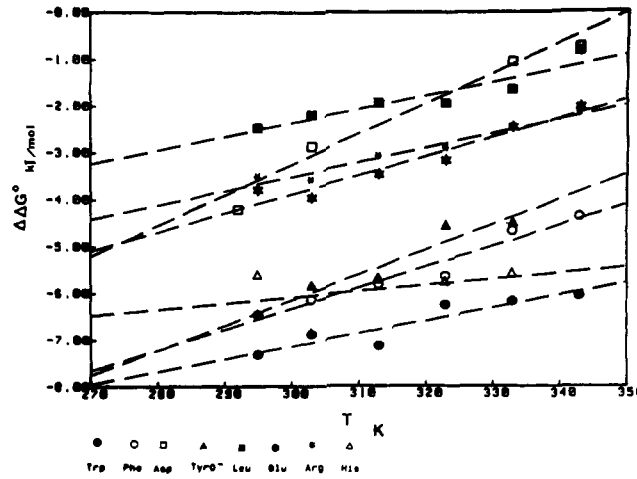

Figure 14. Plot of $\Delta\Delta G°$ (kJ·mol^{-1}) vs T (K).

Table XI. Thermodynamic Parameters $\Delta\Delta H°$ and $\Delta\Delta S°$ (See Text)

	$\Delta\Delta H°$, kJ/mol	$\Delta\Delta S°$, J/mol deg		$\Delta\Delta H°$, kJ/mol	$\Delta\Delta S°$, J/mol deg
Leu	−11.0	−28.9	Phe	−19.6	−44.2
Asp	−22.7	−65.0	$TyrO^-$	−22.2	−53.5
Glu	−15.9	−40.1	Trp	−15.3	−27.1
Arg	−12.6	−30.2	His	−9.9	−12.8

observed that this was also true for the self-stacking of nucleic bases[19] and for the stacking of aromatic residues[7c] in ternary palladium(II) complexes involving diamines and dipeptides with aromatic amino acid residues. The same tendency was also observed in the case of the complexes formed by nucleosides and nucleotides with the cationic free-base porphyrin $TMPyPH_2$.[20] It is therefore quite likely that the stacking of aromatic residues with the porphyrin ring is assisted by charge transfer from the former to the latter. This assumption is further substantiated by the order of decreasing $-\Delta\Delta G°$ values mentioned above.

Conclusion

A ^1H NMR study of aqueous solutions containing 2:1 or 3:1 mixtures of amino acid L and cobalt(III) water-soluble porphyrins CoTMPyP and CoTCPP indicates that, at basic conditions (9.5 < pH < 10.5), the complexes $CoTMPyP(L)_2$ or $CoTCPP(L)_2$ are the only species present with the NH_2 group bound to the metal. At lower pH values the 1:1 complex is also present in equilibrium with the 2:1 complex.

When the amino acid contains two groups susceptible to coordination to the cobalt atom, e.g. histidine, methionine, and lysine, ^1H NMR spectroscopy reveals the presence of several complexes in proportions that vary with pH. For histidine and methionine, at low pH values where the NH_2 group is protonated and not available for coordination, binding occurs through the imidazole or $-S-CH_3$ groups, respectively. At higher pH values, binding of the amino acid to the metal involves the more basic NH_2 group. In the case of lysine, the α-NH_2 group gives a stronger complex than the ϵ-NH_2 probably because of an extra stabilization due to electrostatic interaction between the COO^- group and the positively charged pyridinium groups.

The conformational states of the amino acid CoTMPyP complexes were fully characterized by using the interproton vicinal coupling constants for the H_α and H_β protons and also between the other protons present in the molecules: NH_2, H_γ, and H_δ. In a few cases, the induced shifts $\Delta\delta$ were used either to confirm the results obtained by using the coupling constants or to complement these results as for example in the case of the aromatic amino acids.

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Binding of the amino acids to the cobalt center of CoTMPyP or CoTCPP induces the amino acids to adopt a predominant conformational state that in most cases brings the side chain of the ligand in close proximity to the porphyrin ligands. This conformational state, which surprisingly appears to vary little among the series of amino acids, is quite different from that of the free amino acid, at least around the C_α–C_β bond and must be stabilized by ligand–ligand interactions. Among these, stacking and electrostatic interactions are major contributors. Quite recently we have extended this study to the complexes of amino acids with zinc(II) water-soluble porphyrins:[21] we were able to measure the complexation constants for different types of amino acids and found large variations among these, which again could be rationalized in term of ligand–ligand interactions. The stacking interactions between aromatic amino–acids and the free-base H_2TMPyP are of sufficient magnitude to allow the existence of molecular complexes between these amino acids and the water-soluble porphyrin.

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Modeling the Heteroleptic Equilibria of Organized Molecular Systems by Using a Double-Titration Technique: A Novel Determination of the Fundamental Equilibrium Constants in a Ternary System Involving a Macrocyclic Cobalt(II) Complex, Dioxygen, and Competing Axial Ligands

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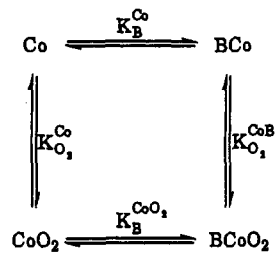
The equilibrium relationships in a ternary system containing (2,9,10,17,19,25,33,34-octamethyl-3,6,13,16,20,24,27,31-octaazapentacyclo[16.7.7.2^{8,11}.2^{3,6}.2^{13,16}]octatriaconta-1,8,10,17,19,24,26,31,33-nonaene-κ^4N)cobalt(II) chloride (abbreviated as [Co^{II}MeVD]Cl$_2$), 4-(hydroxymethyl)pyridine (4-HOCH$_2$py), and dioxygen in water have been examined. A double-titration method was devised by quantifying the concept of an *effective equilibrium constant* and resolving it into the underlying fundamental equilibrium constants. The simplicity and power of this method was shown by applying it to solve simultaneously all the equilibrium constants involved in this heteroleptic ternary system. The equilibrium constants are (oxygen binding to the solvated complex) $K_{O_2}^{Co}$ = 0.075 Torr^{-1}, (axial ligand binding to the deoxygenated complex) K_B^{Co} = 11.4 M^{-1}, (axial ligand binding to the oxygen complex) $K_B^{CoO_2}$ = 1071 M^{-1}, and (oxygen binding to the axially ligated complex) $K_{O_2}^{CoB}$ = 7 Torr^{-1}. The advantages, limitations, and broad applications of the method are discussed.

Introduction

Historically, the equilibria of greatest interest to coordination chemistry were homoleptic multiple equilibria,[1-5] in which molecules of the same ligand successively replace water molecules from the coordination sphere of a metal ion. Attention has, of course, been given to heteroleptic equilibria (involving unlike ligands) but as a rather less common phenomenon.[3,5-9] Recently, multiple heteroleptic equilibria have been increasingly important[10-31] because of the development of the modern fields of bioinorganic chemistry, bioorganic chemistry, inclusion chemistry, and those subjects collectively recognized as supramolecular chemistry. Biomimicry and homogeneous catalysis should be replete with examples of such phenomena in which several different species must be organized prior to some rate-determining process. Here we present a general approach to the problem with the example of the binding of an axial base and an oxygen molecule to a metal ion that is coordinated to a macrocyclic ligand (Scheme I (Co = [Co^{II}MeVD(H$_2$O)]Cl$_2$, VD refers to the 16-membered cyclidene structure with two piperazine molecules as bridgeheads and a durene as the bridge); see Figure 1). We are further interested[32-39] in the complex problem in which all of these species are at equilibrium along with a fifth equilibrant, a guest molecule, in a system that mimics the so-called *ternary complex* of the monooxygenase enzyme cytochrome P-450.[40-44] In the P-450 ternary complex, the enzyme complexes with both O_2 and the substrate molecule (our guest molecule). In reality our model system is a pentad: metal ion, macrocycle/host, axial ligand, O_2, and substrate/guest. Because of the large formation constants of macrocyclic ligands, one may assume that equilibrium to be complete and treat the system as a quaternary one. It is clear

Scheme I



and systematic to describe such a system by a cube of separate equilibria as shown, in projection, in Scheme II. Note that each

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