Interactions of Water-soluble Zinc Porphyrin with Amino Acids

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Abstract

Equilibrium constants for replacement in aqueous solution of the axial ligand of tetrakis(4-*N*-methylpyridyl)porphinezinc(II) by a variety of amino acids at pH 9 have been determincd spectrophotometrically. In addition, the equilibrium constant was determined for glycine as a function of pH. From the pH studies, it appears that the substitution of glycine for OH^- at the Zn center is thermodynamically less favorable than for replacement of H_2O. Stabilization due to intramolecular non-covalent bonding between side-chains of several L-amino acids and the porphyrin macrocycle has also been observed.

Introduction

Metalloporphyrin complexes occupy an important position in biology because of the diversity of their essential functions. This function often depends on secondary interactions (hydrophobic or electrostatic) between the porphyrin ring and its environment [1, 2]. We are interested in evaluating these secondary interactions and how they influence the metalloporphyrin ligation reactions. As part of our studies we now report on the reactions of tetrakis(4-*N*-methylpyridyl)porphinezinc(II) (ZnTMPyP) with a variety of amino acids.

Experimental

ZnTMPyP was prepared by a method described elsewhere [3]. All other chemicals were purchased from Kodak and Sigma Chemical Co. and used without further purification. Visible spectra were determined on a Varian 2200 spectrophotometer thermostated at 25 °C. Porphyrin solutions were freshly prepared and protected from direct sunlight and fluorescent light. All the equilibrium experiments were carried out at an ionic strength of 0.1 M with NaCl. The spectrophotometric data were analysed by application of the SIMPLEX minimization fitting routine on a Prime computer [4].

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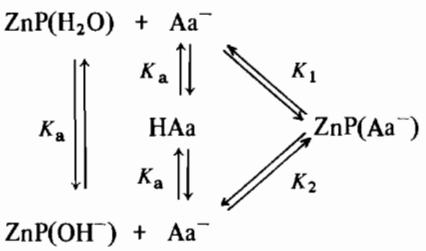
Results and Discussion

ZnTMPyP (ZnP) was shown to obey Beer's law over a concentration range from 2×10^{-6} M to 2×10^{-5} M. The acid dissociation constant for ZnTMPyP-(H_2O) was determined by spectrophotometric titrations with sodium hydroxide. A value of $pK_a = 11.95$ was obtained.

Spectral experiments for the substitution of the axial ligand by glycine (Gly) were conducted in the pH range 8.9–11.8. From these spectral data we obtain the K_{app} for the different pH values, where

$$K_{app} = \frac{[ZnP(Aa^-)]}{[ZnP][Aa^-]} \quad (1)$$

We have found that the thermodynamic scheme which accounts for all our spectral data is:



The two acid/base forms of ZnTMPyP in aqueous solution, ZnP(H_2O) and ZnP(OH^-), can be considered from the spectrophotometric titration data through eqn. (2)

$$\frac{1}{K_{app}} = \frac{1}{K_1} + \frac{1}{K_2}[OH^-] \quad (2)$$

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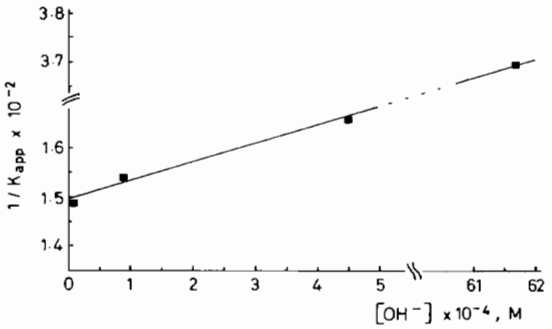

Fig. 1. Plot of $1/K_{app}$ *vs.* $[OH^-]$ for the coordination of Gly to ZnTMPyP.

where

$$K_1 = \frac{[ZnP(Aa^-)]}{[ZnP(H_2O)]\,[Aa^-]} \quad (3)$$

and

$$K_2 = \frac{[ZnP(Aa^-)]\,[OH^-]}{[ZnP(OH^-)]\,[Aa^-]} \quad (4)$$

A plot of $1/K_{app}$ *versus* $[(OH^-)]$ (Fig. 1) gives a value of $K_1 = 66.8\ M^{-1}$ and $K_2 = 0.3\ M^{-1}$. Using:

$$\frac{K_1}{K_2} = \frac{K_a}{K_w} \quad (5)$$

derived from eqns. (3) and (4), a calculated value of $pK_a = 11.65$ is obtained. This is in good agreement with the pK_a determined from the pH titration of ZnTMPyP.

Similar experiments with different amino acids were conducted at pH 9. The K_1 and K_2 values for these reactions were determined by recasting eqn (2):

$$K_{app} = \frac{K_1}{1 + (K_a/[H^+])} \quad (6)$$

The results of these titrations are given in Table I.

From the values of Table I it is apparent that the stability constants vary markedly with the structure of the amino acid and these variations probably reflect the intervention of secondary interactions between the macrocycle and the amino acid side-chain, either electrostatic or hydrophobic, which increase or decrease the binding energy.

TABLE I. Equilibrium Constants and Thermodynamic Parameters for the Complexes of Amino Acids to ZnTMPyP

Aa	K_{app}	pH	K_1 (M^{-1})	K_2 (M^{-1})	$\Delta G°$ (kJ/mol)	$\Delta\Delta G°$ (kJ/mol)
Gly	65	9.00	66.8	0.3	−10.30	
Leu	197.6	9.42	198	1.8	−13.00	−2.67
Glu	172.3	9.02	173	1.6	−12.63	−2.33
Arg	22.3	9.02	22.3	0.2	−7.60	+2.7
Phe	308.5	9.02	309	2.80	−14.05	−3.75
Trp	332.2	9.02	1330	12.2	−17.63	−7.33

The order of stability:

$$ZnP(Arg) < ZnP(Gly) < ZnP(Glu)$$

can be best rationalized by assuming favorable or unfavorable interactions between the cationic porphyrin ligand and the charges present on the amino acid side-chain: negative for glutamic acid, positive for arginine.

Hydrophobic interactions between the amino acid side-chains and the porphyrin ring can explain the order:

$$ZnP(Trp) > ZnP(Phe) > ZnP(Leu) > ZnP(Gly).$$

Similar results were obtained by Sigel *et al.* [5, 6] and Yamauchi *et al.* [7] in a study of ternary complexes involving Cu or Zn, aromatic amines and various amino acids. In order to quantify the increase or decrease in stability due to these secondary interactions, we have calculated the free energy $\Delta\Delta G_{Aa}$ of the following reaction:

$$ZnP(Gly) + Aa \rightleftharpoons ZnP(Aa) + Gly$$

from the values of ΔG_{Aa} and ΔG_{Gly} (Table I) which are the free energies of the substitution of water by the amino acid Gly, respectively.

It is interesting to note that the increase in stability is slightly greater for Phe than for Glu and Leu, and is dramatically larger for Trp than for Phe. The second trend shows that the hydrophobic interaction is enhanced when the π system is more extended.

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