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Formation of Molecular Complexes of a Water-Soluble Porphyrin with Amino Acids: Importance of Hydrophobic Interaction

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Amino acid interacts with 5,10,15,20-tetrakis(4-sulfonatophenyl)-21*H*,23*H*-porphine (H_2TPPS_4) to form molecular complex. The formation constants (log K) defined as $HA+H_2TPPS_4 \rightleftharpoons HA\cdot H_2TPPS_4$ were found to be 2.00±0.05, 1.43±0.03, 0.83±0.02, −0.41±0.07, and −0.96±0.09 for L-tryptophan (Trp), L-tyrosine (Tyr), L-phenylalanine (Phe), L-leucine (Leu), and L-valine (Val) respectively, at 25 °C and an ionic strength of 0.1 mol dm^{-3} ($NaNO_3$), where HA denotes a neutral form of amino acid. The formation constants are linearly correlated to the hydrophobicity of the side-chain of amino acid: L-tryptophan exerts the largest interaction with porphyrin among them. Amino acids also interact with porphyrin adsorbed to an anion-exchange resin. Partition coefficient and retention volume were determined by batch and column methods using the porphyrin-loaded resin. The distribution coefficient and the retention volume of amino acid increase with the hydrophobicity of side-chain of amino acid. The formation of the molecular complex was also confirmed by ^1H NMR spectra for H_2TPPS_4/Trp and H_2TPPS_4/Leu.

Water-soluble porphyrin associates strongly with aromatic ligands such as pyridines, 1,10-phenathrolines,[1–3] and quinolines.[4] The interaction with porphyrin depends on the nature of associating ligands. The possible kinds of interaction with porphyrins are (1) aromatic-ring stacking or π–π complexation with 1,10-phenanthrolines,[1] (2) van der Waals interaction between anionic porphyrin and anionic aromatics,[4] (3) electrostatic interaction between positively charged metal–18-crown-6 complexes and porphyrin plane,[5] and (4) intercalation of porphyrin into DNA.[6] Aim of these studies is to elucidate factors which govern the interactions and to determine the extent to which the interactions occur.

We have recently reported two studies which show the importance of hydrophobicity of side-chains of amino acids involving both aromatic and aliphatic amino acids: one is catalytic effect of amino acid on the reaction of zinc(II) with 5,10,15,20-tetrakis(4-sulfonatophenyl)-21*H*,23*H*-porphine (H_2TPPS_4),[7] and other is enhanced stability constant of binary and ternary copper(II) complexes with amino acids.[8] In the kinetic study we have proposed the formation of molecular complexes as a reaction intermediate, where Zn(II)–amino acid complex sits on porphyrin plane. However, the formation constants were not directly determined because of short-life time of the reaction intermediate. To overcome this problem we decided to study the formation constant of the molecular complex of tetrakis(4-sulfonatophenyl)-21*H*,23*H*-porphine with amino acids in the absence of zinc(II). The use of amino acids (L-valine (Val), L-leucine (Leu), L-phenylalanine (Phe), L-tyrosine (Tyr), and L-tryptophan (Trp) allows a systematic variation of the hydrophobicity of the side-chain of amino acid.

We have determined the formation constant of the molecular complex spectrophotometrically. In addition, we found an interaction between amino acid and the porphyrin-loaded resin. Distribution coefficient and retention volume of amino acids were determined by batch and column methods using the porphyrin-loaded resin.

The formation constants of the molecular complexes, the distribution coefficients, and the retention volumes of amino acids are correlated to the hydrophobicity of side-chain of amino acids. Amino acid with higher hydrophobicity interacts more strongly with porphyrin. In addition, ^1H NMR spectra for a mixture of H_2TPPS_4 and L-tryptophan or L-leucine give upfield shifts due to the hydrophobic interaction between porphyrin plane and side-chain of amino acid.

Experimental

Reagents. 5,10,15,20-Tetrakis(4-sulfonatophenyl)-21*H*,23*H*-porphine (H_2TPPS_4) was synthesized by the methods described in literatures.[9,10] The sodium salt of porphyrin ($Na_4H_2TPPS_4$) was purified by successive reprecipitation from an aqueous acetone solution (70% v/v) and by a column chromatography. The purity of $Na_4H_2TPPS_4$ was checked by a thin-layer chromatography and NMR spectroscopy.[11] Anionic exchange resin, Muromac 1-X-2 (100—200 mesh) of nitrate form, was used for preparation of porphyrin-loaded resin. Buffer solutions were prepared by mixing sodium citrate with hydrogen chloride or sodium hydroxide for pH=3.41, 4.32, 5.00, and 6.68, and by mixing sodium hydrogencarbonate with sodium carbonate or sodium hydroxide for pH=8.52, 9.21, and 11.21.[12] All other reagents were analytical reagent grade.

Preparation of Anion-Exchange Resin Modified with H_2TPPS_4. Anion-exchange resin (4.0 g) was added to a 1:1 acetone–water solution (200 cm^3) containing H_2TPPS_4 (0.1 mmol).[13] This mixture was shaken until the solution became colorless. The resin was filtered and dried in room. H_2TPPS_4 is strongly bound to the anion-exchange resin by ion-pair formation and physical adsorption. The porphyrin resin was stable for a few months, and H_2TPPS_4 was not eluated under the present experimental conditions at pH 3—11.

Determination of Formation Constants of Molecular Complexes of H_2TPPS_4 with Amino Acids. The formation constant was determined spectrophotometrically at pH 7, 25 °C, and an ionic strength of 0.1 mol dm^{-3} ($NaNO_3$) in the presence of various concentrations of amino acids. The absorption spectra were recorded by a Hitachi 323 UV-VIS spectrophotometer in a 1-cm cell with a thermostated water jacket.

Distribution Coefficients (K_d) of Amino Acids to the Anion-Exchange Resin Modified with H_2TPPS_4. The porphyrin-loaded resin (100 mg) was equilibrated with a 10-cm^3 solution involving 10^{-4} mol dm^{-3} amino acid at 25 °C, and for 30 min under a mild shaking. The concentration of amino acid in the aqueous solution was determined by the ninhidrin reaction.[14)]

Separation of Amino Acids Using the Porphyrin-Resin Column. A glass column (length 15 cm, i.d. 0.8 cm) was filled with a citrate buffer (pH 5.0), and packed with the porphyrin-modified resin (8 g). A 1-cm^3 sample solution containing amino acid was added to the top of the column, and eluted by citrate buffer (pH 5.0) at a flow rate of 1 cm^3 per min. The eluate was fractionated by 1-cm^3 portions with a Gilson FC 203 fraction collector. The fractionated solution was analyzed for the determination of amino acid by the ninhidrin method at pH 5.0.

^{1}H NMR Spectroscopy. The ^{1}H NMR spectra were recorded on a JEOL GX-270 spectrophotometer (270 MHz) at 25 °C in D_2O solvent, using the center peak of the tetramethylammonium ion as internal reference.[15)] All chemical shifts were converted to a sodium 3-(trimethylsilyl)-1-propanesulfonate (DSS) reference by adding 3.188 ppm. The use of DSS reference is unreliable, since ^{1}H NMR signals of trimethylsilyl group shift considerably by hydrophobic interaction between DSS group and aromatic moiety of ligands. A routine Fourier-transfer program was used to analyze the ^{1}H NMR spectra, where data were accumulated over 100 times.

Results and Discussion

Formation of Molecular Complexes of H_2TPPS_4 with Amino Acids. Absorption spectrum of H_2TPPS_4 changed considerably in the presence of amino acid as observed for the reaction of H_2TPPS_4 with metal–18-crown-6 complexes or 1,10-phenanthroline.[2,5)] The absorption maximun at 413 nm (Soret band) decreased with increasing of amino acid. The isosbestic points are 388 and 420 nm. Under the present experimental conditions (pH 7.0) porphyrin is free base form, because the protonation constants of H_2TPPS_4 to form H_3TPPS_4 and H_4TPPS_4 are $10^{4.99}$ and $10^{4.76}$, respectively, and amino acid is neutral form (HA).[16)] Thus the reaction of H_2TPPS_4 with amino acid may be given by

$$n\mathrm{HA} + \mathrm{H_2TPPS_4} \overset{K}{\rightleftharpoons} (\mathrm{HA}_n)\cdot\mathrm{H_2TPPS_4}, \tag{1}$$

and the equilibrium constant is defined by

$$K = [(\mathrm{HA}_n)\cdot\mathrm{H_2TPPS_4}][\mathrm{HA}]^{-n}[\mathrm{H_2TPPS_4}]^{-1}. \tag{2}$$

Equation 2 can be rearranged in logarithmic form as follows:

$$\log K = \log([(\mathrm{HA}_n)\cdot\mathrm{H_2TPPS_4}]/[\mathrm{H_2TPPS_4}]) - n\log[\mathrm{HA}]. \tag{3}$$

The first term in right-hand side of Eq. 3 was determined from the absorbances in the various concentrations of amino acid, and is plotted against logarithmic concentration of amino acid in Fig. 1. The plot gives a straight line with a slope of unity (n=1), and shows that one molecule of HA reacts with an H_2TPPS_4. The formation constants of molecular complexes of H_2TPPS_4 with amino acids were determined from Eq. 3, and summarized in Table 1. The formation constant increases in the order Val< Leu<Phe<Tyr<Trp. The formation constants of H_2TPPS_4 with glycine, and L-alanine were not able to be determined, because the formation constants were very small. In Fig. 2 the formation constants are plotted against the hydrophobicity-scale[17)] of amino

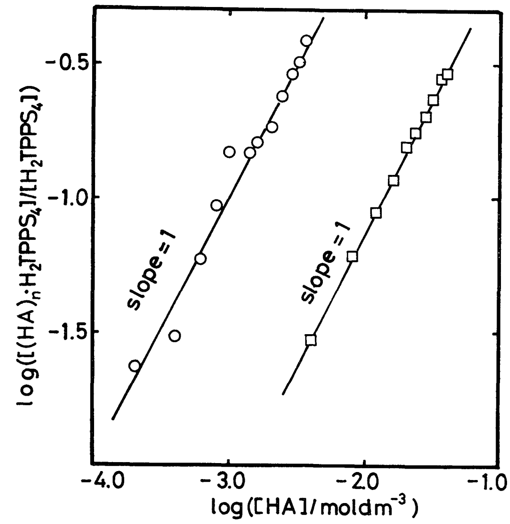

Fig. 1. Plots of log($[\mathrm{HA}]_n\cdot\mathrm{H_2TPPS_4}]/[\mathrm{H_2TPPS_4}]$) vs. log([HA]/mol dm^{-3}) for the reaction of H_2TPPS_4 with L-tryptophan (○) and L-phenylalanine (□) at pH 7.0, 25 °C, and I=0.1 mol dm^{-3} ($NaNO_3$). $C_{\mathrm{H_2TPPS_4}}$= 1.95×10^{-6} mol dm^{-3}.

Table 1. Formation Constants of Molecular Complexes of ($HA\cdot H_2TPPS_4$), Distribution Coefficients (K_d) of Amino Acids, and Their Retention Volumes ($V_{1/2}$) to a Column of Porphyrin-Loaded Resin

Amino acids	log $K^{a)}$	$K_d$$^{a)}$	$V_{1/2}$$^{a)}$	$\Delta G^{a,b)}$
Trp	2.00±0.05	18.5	48.4	14.2
Tyr	1.43±0.03	8.8	16.5	9.6
Phe	0.83±0.02	7.9	8.6	10.5
Leu	−0.41±0.07	3.8	5.0	7.5
Val	−0.96±0.09	0.0	4.5	6.3

a) Units of the constants are mol^{-1} dm^3, cm^3 g^{-1}, cm^3, and kJ mol^{-1} for K, K_d, $V_{1/2}$, and ΔG, respectively. b) Hydrophobicity scale was determined from the solubility of amino acids in various mixed aqueous solutions of ethanol or dioxane (Ref. 17).

acid side-chain. Figure 2 suggests that the stability of molecular complex increases with the hydrophobicity of amino acid side-chain. The result is in agreement with the observation in the enhanced stability for the ternary complexes with amino acids and in the kinetic effect of amino acid on metalloporphyrin formation.[7,8]

We notice the high stabilization for L-tyrosine compared with other amino acids. The extra stabilization for L-tyrosine was also observed in the distribution coefficient to the porphyrin resin described below, and in the catalytic effect of L-tyrosine on the metalloporphyrin formation. In these case, the extra stabilization may result from other factors such as a hydrogen bonding between hydroxyl group of L-tyrosine and porphyrin pyrrole nitrogen atom. Nevertheless, in this case too, the hydrophobicity of the side-chain has an influence on the stability increase.

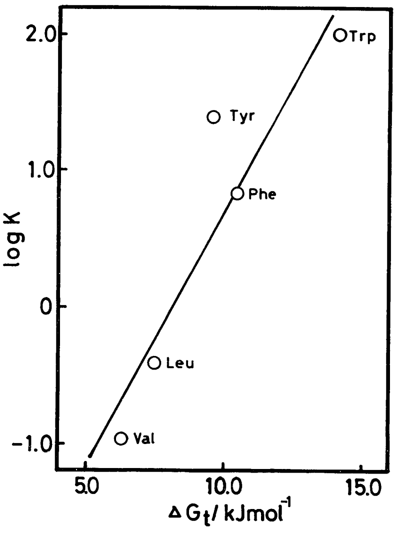

Fig. 2. Formation constants of molecular complexes of amino acids with H_2TPPS_4 are plotted against the hydrophobicity scale of amino acid residue.

Distribution Coefficient (K_d). Distribution coefficients of amino acids to the porphyrin-resin were calculated using the following equation:

$$K_d = \frac{(\text{Amount of amino acid in resin}) \times (\text{Volume of aqueous phase (cm}^3\text{))}}{(\text{Amount of amino acid in aqueous phase}) \times (\text{Weight of resin (g)})} \qquad (4)$$

Logarithmic value of distribution coefficient is plotted against pH in Fig. 3. The distribution coefficients are constant at pH 4—7, and increase with pH. Amino acids deprotonate to form the anionic amino acids at higher pH. Since the formation constant of molecular complex of amino acid with porphyrin is independent of pH at pH 6—9, the dependence of distribution coefficient on pH shows the higher stabilization of the molecular complex at higher pH by ion-pair formation between the anionic form of amino acid and the remaining cationic groups in the porphyrin-loaded resin, in addition to the hydrophobic interaction between the side-chain of amino acid and porphyrin plane.

The distribution coefficients determined at pH 5.0, where the following column-chromatographic separation was carried out, are given in the third column of Table 1. The distribution coefficients of amino acids increase in the order: Val<Leu<Phe<Tyr<Trp. It is expected that the distribution coefficient may depend on the formation constant of molecular complex. As shown in Table 1, the distribution coefficient increases with the formation constant of molecular complex. Amino acids with high hydrophobicity are adsorbed strongly to the porphyrin-loaded resin.

Retention Volume of Amino Acids to Porphyrin-Loaded Resin. Formation constant of the molecular complex and distribution coefficient of amino acid suggest the separation of amino acids on column of the porphyrin-adsorbed resin. A column of the resin modified with porphyrin was equilibrated with citrate buffer of pH 5.0. A 1 cm^3 of amino acid solution containing citrate buffer (pH 5.0) was added to the top of the column, and amino acid was eluated with the same buffer solution. The elution using citrate buffer of pH 5.0 enables us to determine amino acid without any pH adjustment before analysis of amino acid by the ninhidrin method. Void volume of the column was 5 cm^3, which was determined by loading a sodium chloride solution (234 ppm) and by a flame spectro-

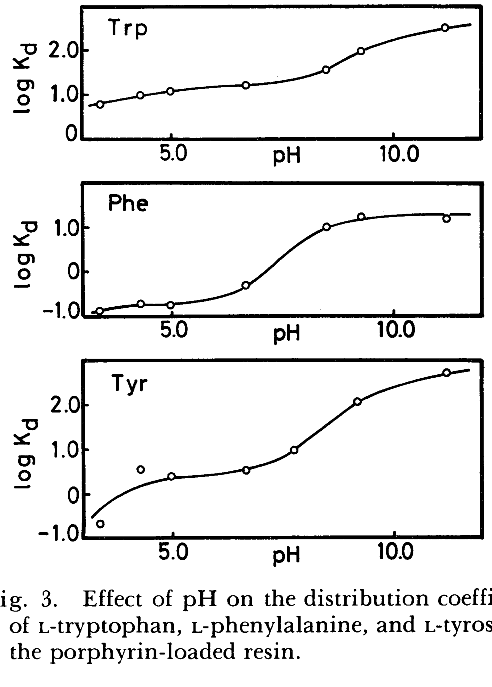

Fig. 3. Effect of pH on the distribution coefficients of L-tryptophan, L-phenylalanine, and L-tyrosine to the porphyrin-loaded resin.

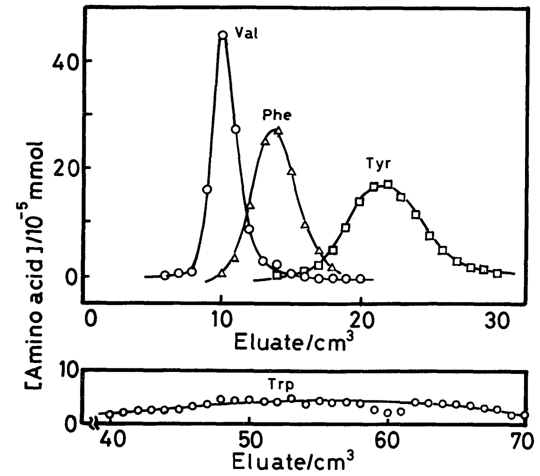

Fig. 4. Separation of L-valine, L-phenylalanine, L-tyrosine, and L-tryptophan from a column of the porphyrin-loaded resin by elution of citrate buffer (pH 5.0).

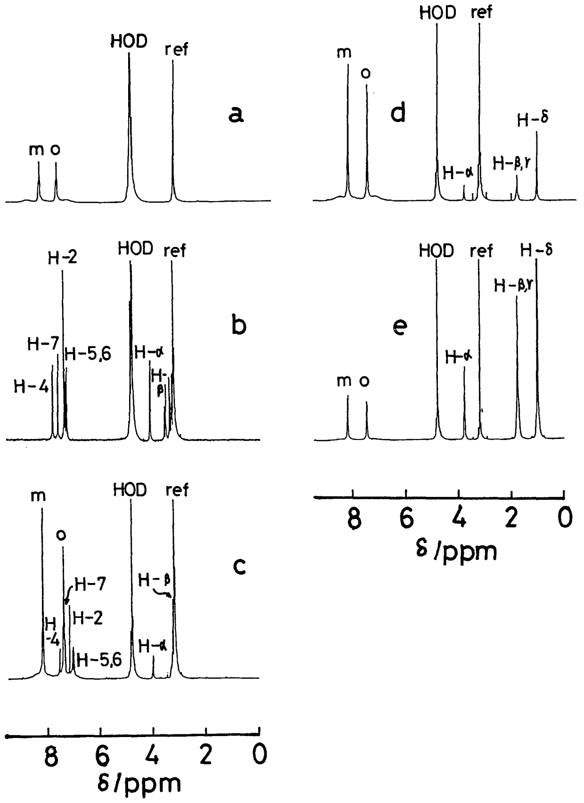

Fig. 5. ¹H NMR spectra of H_2TPPS_4 (a), L-tryptophan (b), 1:1 H_2TPPS_4/L-tryptophan (c), 1:0.5 H_2TPPS_4/L-leucine (d), and 1:10 H_2TPPS_4/L-leucine (e) at pD 6.70 (a), 6.40 (b), 6.42 (c), 6.43 (d) and 6.38 (e), and at 25 °C and I=0.1 ($NaNO_3$). Concentration of H_2TPPS_4 is 10^{-2} mol dm^{-3}. Chemical shifts are given as a standard of 3-(trimethysilyl)-1-propanesulfonate (DSS).

photometry of sodium in fractions. The elution graphs of amino acids on a column of the porphyrin-loaded resin are shown in Fig. 4. The retention volumes ($V_{1/2}$) are given in Table 1. Amino acids were eluted in the order of the distribution coefficients and of the formation constants of the molecular complexes. Broad elution of tryptophan is owing to strong retention of tryptophan to the porphyrin-adsorbed resin.

¹H NMR. Figure 5 illustrates ¹H NMR spectra of H_2TPPS_4, L-tryptophan, and mixtures of H_2TPPS_4 and L-tryptophan or H_2TPPS_4 and L-leucine in D_2O. The ¹H NMR spectrum of a 10^{-2} mol dm^{-3} H_2TPPS_4 shows two doublets at δ 7.577 and 8.232 due to the ortho and meta protons at pD 6.70. Two broad peaks are attributed to the pyrrole protons and are largely due to a slow rate of tautomerism of the porphyrin imine protons.[18] The addition of 10^{-2} mol dm^{-3} L-tryptophan to a solution of H_2TPPS_4 results in upfield shifts to δ 7.386 and 8.180 for ortho and meta phenyl protons. Similarly, the resonances of ortho and meta phenyl protons of H_2TPPS_4 are shifted to δ 7.482 and 8.203 by addition of 10^{-1} mol dm^{-3} L-leucine.

The upfield shifts were also observed for ¹H NMR spectra of L-tryptophan in the presence of H_2TPPS_4. The ¹H NMR chemical shifts (δ/ppm) for free L-tryptophan are 7.323 (H-2), 7.743 (H-4), 7.235 (H-5,6), 7.546 (H-7), 4.067 (H-α), 3.472 (H-β) at pD 6.40. The ¹H NMR chemical shifts (ppm) for L-tryptophan move to 7.163 (H-2), 7.545 (H-4), 7.022 (H-5,6), 7.316 (H-7), 3.990 (H-α), and 3.242 (H-β) in a mixture of 1:1 H_2TPPS_4 and L-tryptophan at pD 6.42. The signals for β-protons overlap on the shoulder of signal for tetramethyl ammonium (internal standard) in the presence of H_2TPPS_4.

Since the formation constant of molecular complex between H_2TPPS_4 and L-leucine is low ($\log K = -0.41 \pm 0.09$), ¹H NMR spectra of L-leucine gives a slight shift in a mixture of H_2TPPS_4 and L-leucine as shown in Fig. 5. Chemical shifts (ppm) for L-leucine in the presence of H_2TPPS_4 are 3.786 (H-α), 1.764 (H-β,γ), and 0.998 (H-δ) for a 1:0.5 mixture of H_2TPPS_4 and L-leucine, and 3.771 (H-α), 1.751 (H-β,γ), and 0.989 (H-δ) for a 1:10 mixture of H_2TPPS_4 and L-leucine at pD 6.38. The upfield shifts for L-leucine by addition of H_2TPPS_4 is small, but the signals for porphyrin protons (especially ortho phenyl proton) clearly shift to upfield in the presence of L-leucine.

The ¹H NMR experiments on the H_2TPPS_4/Trp and H_2TPPS_4/Leu systems indicate directly the interaction between the side-chains of amino acids and the porphyrin plane. Large change in chemical shifts for indole protons shows the strong interaction for indole moiety of L-tryptophan with the porphyrin plane. In contrast, alkyl-side chain of L-leucine interacts very weakly with the porphyrin. Furthermore, change in chemical shifts of α- and β-protons of L-tryptophan by addition of H_2TPPS_4 are much smaller than that of indole protons.

The above upfield shifts support the hydrophobic interaction between side-chain of amino acid and the porphyrin plane. A possible structure for the molecular complex of H_2TPPS_4 with L-tryptophan or L-leucine is that the side-chain of amino acid sits above the porphyrin plane, and that the amine and carboxyl groups out of the porphyrin plane.

One of the most interesting features of present study is the interaction between the aliphatic side-chain of amino acid (e.g. L-leucine) and the porphyrin plane. The extent of the interaction is small but not negligible. We have previously proposed the importance of the aromatic–aliphatic interaction in the enhanced stability of ternary copper(II) complexes.[8] The aromatic-aliphatic interaction was also proposed in the ternary complexes of (His)Cu(Val), (phen)Cu(Leu) and (phen)Zn(Leu) from potentiometric studies, where His denotes L-histidine.[19,20] The interaction was directly confirmed by the upfield shifts in ^{1}H NMR for (phen)Zn(Leu) and (GlyPhe)Pd(aliphatic amine), where GlyPhe refers glycylphenylalanine.[20,21]

Aromatic-ring stacking has so far received much attention in the ligand–ligand interaction. However, the aromatic–aliphatic interaction also stabilizes the formation of molecular complexes, the extent is small compared with the aromatic-ring stacking though. The ligand–ligand interaction is favored for aromatic or bulky aliphatic-side chains with high hydrophobicity, while it is not favored for small aliphatic-side chains with low hydrophobicity. Thus the formation constants of the molecular complexes are correlated to the hydrophobicity even for somewhat structurally different amino acids as shown in Fig. 2. This observation corresponds to the enhanced stability of binary- or ternary metal complexes by the hydrophobic interaction between the bound ligands, and to the catalyzed reaction by amino acids for the metalloporphyrin formation.[7,8]

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