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Binding of Amino Acids in Water to a Highly Electron-Rich Aromatic Cavity of Pyrogallol or Resorcinol Cyclic Tetramer as π-Base

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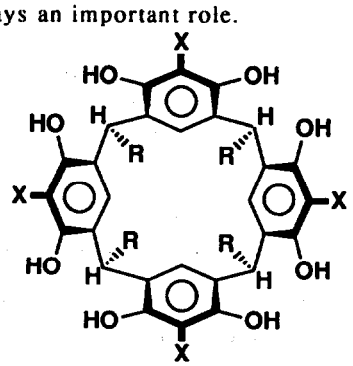
Key words: host-guest interaction; amino acid; hydrophobic effect; π-basicity; CH-π interaction

Abstract: *Tetrasulfonated derivatives of pyrogallol or resorcinol cyclic tetramer form a 1:1 complex in water with not only aromatic but also relatively hydrophobic aliphatic amino acids. The stability of the complex depends on both the hydrophobicity of the guest and the π-basicity of the host.*

Selective binding of biorelevant molecules such as amino acids through polar interaction in nonpolar organic media is a rapidly growing area of molecular recognition.[2] Such a polar interaction, however, is far less pronounced in water. In fact, amino acid binding in aqueous media has been limited, to the best of our knowledgement, to aromatic amino acids having good hydrophobicity as well as the capability of undergoing π-π stacking or charge-transfer interaction.[3] We have recently introduced a highly electron-rich aromatic cavity of water-soluble pyrogallol (**1a**) or resorcinol (**1b**) cyclic tetramer and opened a newer phase of aqueous host-guest association involving highly hydrophilic guests such as sugars.[4] We wish to report here that relatively hydrophobic aliphatic as well as aromatic amino acids can be bound to host **1**, where the *interaction* between the host as π-base and the guests as either σ- or π-acid plays an important role.



1a: R = $(CH_2)_2SO_3Na$; X = OH
1b: R = $(CH_2)_2SO_3Na$; X = H

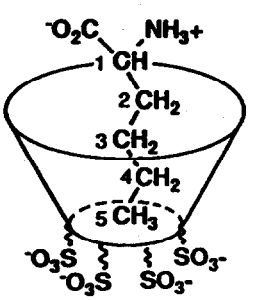


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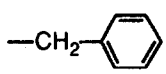
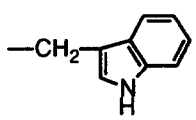
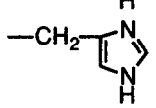
Table 1. Binding Constants (K) for the Complexation of Hosts **1a** and **1b** with Various Guests[a] and the Solubility of Guests[b]

guest	side chain of guest	host		solubility of guest (g/l)
		1a, K (M^{-1})	**1b**, K (M^{-1})	
Ser	$-CH_2OH$	~0	~0	422[c]
Thr	$-CH(CH_3)(OH)$	~0	~0	97[c]
Cys	$-CH_2SH$	~0	~0	*d*
Ala	$-CH_3$	~0	~0	167[e]
Val	$-CH(CH_3)_2$	4.6	~0	88.5[e]
norVal	$-CH_2CH_2CH_3$	6.0	<1	105[f,g]
Ile	$-CH(CH_2CH_3)(CH_3)$	7.1	<1	41.2[e]
Leu	$-CH_2CH(CH_3)_2$	12	2.0	24.3[e]
norLeu	$-CH_2CH_2CH_2CH_3$	17	2.5	11.5[f]
Met	$-CH_2CH_2SCH_3$	13	4.2	56[c]
Phe		64	10	29.6[e]
Trp		69	36	11.4[e]
His		50	–	41.9[e]

[a] [1] = 0.5~2 mM in D_2O at 25 °C. [b] In H_2O at 25 °C. [c] *CRC Handbook of Chemistry and Physics. 72nd. Ed.*; Lide, D. R. Ed.; CRC Press: U.S.A., 1992; section 7-1. [d] Freely soluble. [e] *The MERCK INDEX, 9th. Ed.*; Windholz, M. Ed.; MERCK & CO., Inc.: New Jersey, 1976. [f] *Jouyou Kagaku Binran*; Kishi, N.; Sano, R.; Suzuki, O.; Ito, Y. Eds.; Seibundou Shinkou, Inc.: Japan, 1960; pp. 326-327. [g] In H_2O at 15 °C.

Relatively hydrophobic amino acids were shown, on the same criteria as in the case of sugar complexation,[4] to form a 1:1 complex with host **1a** or **1b** in unbuffered D_2O at 25 °C. Evidence for this includes (1) characteristic guest-induced downfield shift (0.27~0.39 ppm at saturation binding in every case) of the aromatic 5-H NMR signal of the host, (2) characteristic host-induced upfield shifts of the ^1H NMR resonances for the alkyl side chain

of the guest, (3) saturation behaviors of these complexation-induced ^{1}H NMR shifts, and (4) continuous variation (Job) plot having a maximum at [host] = [guest]. The binding constants (K) evaluated by the Benesi-Hildebrand analysis of the titration data at constant [host] and varying [guest] are shown in Table 1,[5] together with the solubilities of amino acids in water as a rough measure of their hydrophobicities.

Inspection of Table 1 reveals the following aspects. First, highly hydrophilic amino acids such as Ala, Ser, Cys, and Thr are scarcely bound. Second, more hydrophobic aliphatic homologs are bound, where the binding constants increase with increasing chain lengths (Ala << Val < norVal < Ile < Leu < norLeu). This order of amino acids is also the order of their decreasing solubilities in water. The selectivity among hydrophobic and hydrophilic amino acids indicates that the alkyl side chain in the former provides the primary site of interaction with the host; polar interaction, if any, involving the ammonio or carboxylate group of the guest is not important. The complexation-induced upfield shifts for the ^{1}H NMR resonances of the alkyl group of norLeu at saturation binding with **1a** as a host are 0.56 (1-H), 0.71 (2-H), 1.17 (3-H), 1.90 (5-H), and 1.92 ppm (4-H).[6] This is consistent with the binding mode of norLeu with its alkyl terminus deeply incorporated in the aromatic cavity of the host and its polar head groups exposed to bulk solvent, as schematically shown in structure **2**.

The third point of interest is the enhanced affinities of aromatic amino acids (Phe, Trp, and His).[7] However, there seems to be no *special* preference for aromatic guests over aliphatic ones, judging from their hydrophobicities. Fourth, and most importantly, every guest is bound more tightly to the pyrogallol host **1a** than to the resorcinol host **1b**. The former having an additional OH group on the benzene rings has a more electron-rich and less hydrophobic aromatic cavity, as compared with the latter. Thus, the π-basicity of the host is an important factor. This particular result indicates that the present amino acid binding in water, as in the case of mono[8] and polyol[4a] complexation, is not simply due to the so-call hydrophobic effect but there is substantial stabilization arising from the CH-π *interaction*[9] between the guests as σ-acid and the host as π-base. It is also interesting to note that the present aromatic guests having a phenyl, indolyl, or imidazolyl ring are sensitive to the basicity of the host in a similar manner as the aliphatic guests. They thus behave as either σ- or π-acid allowing either edge-to-face[10] or face-to-face[11] guest-host aromatic-aromatic interaction. Further work is now under way to make this point clearer.

REFERENCES AND NOTES

1. Present address: Section of Bioorganic Chemistry, Department of Bioengineering, Nagaoka University of Technology, Kamitomioka, Nagaoka, Niigata 940-21, Japan.
2. (a) Rebek, J., Jr.; Askew, B.; Nemeth, D.; Parris, K. *J. Am. Chem. Soc.* **1987**, *109*, 2432-2434. (b) Aoyama, Y.; Asakawa, M.; Yamagishi, A.; Toi, H.; Ogoshi, H. *J. Am. Chem. Soc.* **1990**, *112*, 3145-3151. (c) Galán, A.; Andreu, D.; Echavarren, A. M.; Prados, P.; de Mendoza, J. *J. Am. Chem. Soc.* **1992**, *114*, 1511-1512 and references cited therein.
3. (a) Tabushi, I.; Kuroda, Y.; Mizutani, T. *J. Am. Chem. Soc.* **1986**, *108*, 4514-4518. (b) Goodnow, T. T.; Reddington, M. V.; Stoddart, J. F.; Kaifer, A. E. *J. Am. Chem. Soc.* **1991**, *113*, 4335-4337. (c) Murakami, Y.; Ohno, T.; Hayashida, O.; Hisaeda, Y. *J. Chem. Soc., Chem. Commun.* **1991**, 950-952.
4. (a) Kobayashi, K.; Asakawa, Y.; Kato, Y.; Aoyama, Y. *J. Am. Chem. Soc.* **1992**, *114*, 10307-10313. (b) Kobayashi, K.; Asakawa, Y.; Aoyama, Y. *Supramolecular Chem.* in press.
5. The Benesi-Hildebrand analysis of the titration data at constant [norLeu] and varying [**1a**] gave the binding constant of 14 M^{-1}, which was in agreement with that of 17 M^{-1} (Table 1) obtained from the data at constant [**1a**] and varying [norLeu].
6. These values were evaluated by the Benesi-Hildebrand analysis.
7. For example, Trp and His are more strongly bound than norLeu and Met, respectively, although each pair has similar solubilities.
8. Kobayashi, K.; Asakawa, Y.; Kikuchi, Y.; Toi, H.; Aoyama, Y. *J. Am. Chem. Soc.* in press.
9. Nishio, M.; Hirota, M. *Tetrahedron* **1989**, *45*, 7201-7245.
10. Muehldorf, A. V.; Van Engen, D.; Warner, J. C.; Hamilton, A. D. *J. Am. Chem. Soc.* **1988**, *110*, 6561-6562
11. Ferguson, S. B.; Sanford, E. M.; Seward, E. M.; Diederich, F. *J. Am. Chem. Soc.* **1991**, *113*, 5410-5419.

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