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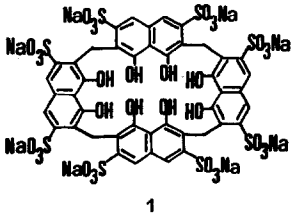
Complexation of Amino Acids by Cyclotetrachromotropylene in Aqueous Solution - Importance of CH-π and π-π Interactions

Bo-Long Poh* and **Chi Ming Tan**
School of Chemical Sciences, Universiti Sains Malaysia, 11800 Minden, Penang, Malaysia

Abstract: The stability constants K of the 1:1 host to guest complexes formed between the cyclic tetramer, cyclotetrachromotropylene, and amino acids in water at pD 7.0 and 25° C were determined by ^{1}H nmr spectroscopy. The results indicate that the interactions between the aromatic π-bonds of the host and the C-H bonds (for aliphatic amino acids) and aromatic π-bonds (for aromatic amino acids) of the guests are the major factors responsible for the complexation. There is a linear relationship between log K and the number of C-H bonds interacting with the hydrophobic host cavity.

X-ray crystal structures of proteins have revealed the importance of two hydrophobic interactions in stabilizing the structures of proteins and their complexes. One is the interaction between C-H and aromatic-π bonds, as found in several sugar-binding proteins.[1] For example, in a D-glucose-binding protein, D-glucopyranose is sandwiched between two aromatic amino acid residues (phenylalanine and tryptophan) and its C-H bonds interact with their aromatic-π bonds. The other is the interaction between aromatic-π bonds of the aromatic amino acid residues (such as phenylalanine, tyrosine and tryptophan) found im many proteins.[2] To gain an understanding of these two hydrophobic interactions, we studied the complexation of twelve amino acids with cyclotetrachromotropylene (1) in an aqueous solution at pD 7.0 using proton nmr spectroscopy. Ten aliphatic amino acids (2-11) provide the C-H bonds, and host 1 and the two aromatic amino acids (12-13) provide the aromatic-π bonds.



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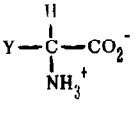
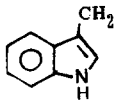


	Y		Y
2	H	8	CH_2CONH_2
3	CH_3	9	CH_2CO_2H
4	$CH(CH_3)_2$	10	$(CH_2)_2CO_2H$
5	$CH(CH_3)CH_2CH_3$	11	$(CH_2)_4NH_2$
6	$CH(OH)CH_3$	12	$CH_2C_6H_5$
7	$(CH_2)_2SCH_3$	13	

RESULTS AND DISCUSSION

The proton chemical shifts of the amino acids are shifted upfield in the presence of 1, indicating that the guest molecules are included in the host cavity. The induced shifts observed for the highest molar ratio of host to guest used are given in parentheses in the fifth column of Table 1 (difficulty in observing the guest proton signals limited the host to guest molar ratio used to ten). The change in the proton nmr spectrum of a guest in the presence of 1 is illustrated by isoleucine (5) in Figure 1.

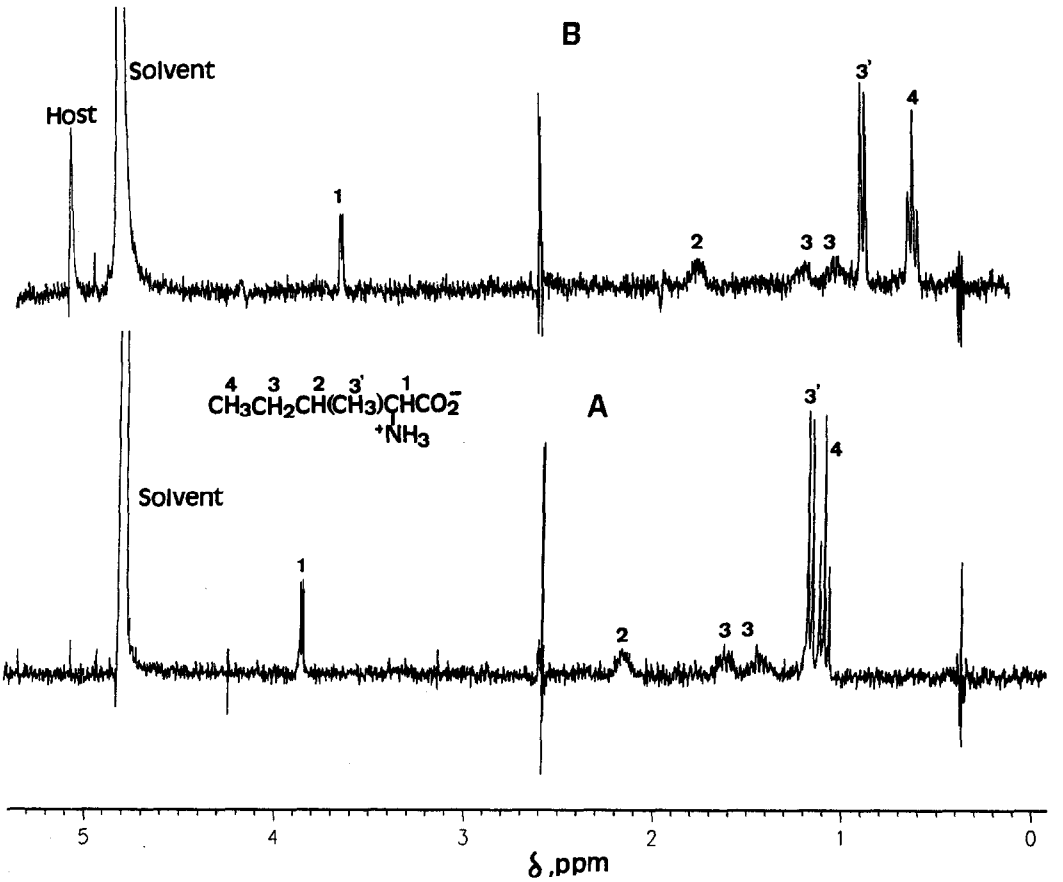


Figure 1. 300 MHz ^1H nmr spectra in D_2O at 25°C of 1.52×10^{-2} M of isoleucine (solvent peak at 4.80 ppm as internal reference); (A) no host, (B) in the presence of 1.54×10^{-2} M of 1. The aromatic proton peak of 1 not shown.

Table 1. Proton NMR Chemical Shifts of Amino Acids and Stability Constants K of their 1:1 Complexes with 1 in D_2O at 25°C, pD 7.0, I = 1.70 M.

No.	Amino Acid	Proton	δ_u[a], ppm	$\Delta\delta$[b], ppm	K[c], M^{-1}	sd[d], ppm
2	Glycine	H_1	3.67	1.67(0.07)[e]	0.4	0.002
3	L-Alanine	H_1	4.05	0.95(0.35)	2	0.02
		H_2	1.71	1.01(0.37)	2	0.02
4	L-Valine	H_1	3.80	1.60(0.54)	3	0.01
		H_2	2.44	1.94(0.65)	3	0.01
		H_3	1.20	2.10(0.70)	3	0.01
		H_3	1.15	2.15(0.64)	3	0.02
5	L-Isoleucine	H_1	3.86	1.06(0.81)	22	0.01
		H_2	2.17	1.97(1.53)	22	0.02
		$H_3(CH_3)$	1.17	1.42(1.10)	22	0.02
		H_3	1.63	2.08(1.65)	22	0.04
		H_3	1.45	2.05(1.58)	22	0.03
		H_4	1.10	2.20(1.68)	22	0.02
6	L-Threonine	H_1	3.79	0.89(0.24)	2	0.01
		H_2	4.43	0.83(0.24)	2	0.01
		H_3	1.49	1.09(0.30)	2	0.02
7	L-Methionine	H_1	3.87	1.02(0.85)	42	0.01
		H_2	2.18	1.48(1.23)	42	0.01
		H_3	2.65	1.75(1.45)	42	0.02
		H_4	2.15	2.00(1.65)	42	0.02
8	D,L-Asparagine	H_1	4.18	0.88(0.37)	4	0.02
		H_2	3.09	0.99(0.41)	4	0.02
9	D,L-Aspartic acid	H_1	4.19	1.49(0.56)	3	0.02
		H_2	3.11	1.41(0.47)	3	0.01
10	D,L-Glutamic acid	H_1	3.93	1.13(0.36)	3	0.02
		H_2	2.26	1.56(0.51)	3	0.03
		H_3	2.61	1.61(0.53)	3	0.04
11	L-Lysine	H_1	3.91	2.05(2.05)	120	0.15
		H_2	2.03	1.81(1.81)	120	0.13
		H_3	1.60	1.95(1.95)	120	0.15
		H_4	1.86	2.08(2.08)	120	0.15
		H_5	3.17	2.24(2.24)	120	0.16

Table 1 cont.

No.	Amino Acid	Proton	δ_u[a], ppm	$\Delta\delta$[b], ppm	K[c], M^{-1}	sd[d], ppm
12	D,L-Phenylalanine	H_1	4.09	0.44(0.38)	44	0.02
		H_2	3.37	0.62(0.57)	44	0.03
		H_2	3.21	0.71(0.68)	44	0.04
		H_o	7.41	1.00(0.88)	44	0.02
		H_m	7.48	1.28(1.07)	44	0.01
		H_p	7.48	1.43(1.19)	44	0.03
13	L-Tryptophan[f]	H_1	4.18	0.46(0.46)	140	0.03
		H_2	3.59	0.79(0.79)	140	0.05
		H_2	3.43	0.63(0.63)	140	0.05
		H_4	7.46	0.91(0.91)	140	0.05
		H_6	7.68	1.28(1.28)	140	0.05
		H_7	7.40	1.29(1.29)	140	0.06
		H_8	7.33	1.22(1.22)	140	0.06
		H_9	7.85	1.09(1.09)	140	0.05

[a]Chemical shift of free amino acid. [b]Difference between the chemical shifts of free and complexed amino acid (for amino acids which were not completely complexed even at the highest molar ratio of host to guest used, the chemical shifts of their complexed form were obtained from non-linear regression fitting of their proton nmr titration curves); positive value indicates upfield shift. [c]Calculated by non-linear regression fitting. [d]Standard deviation between experimental and calculated chemical shifts. [e]Values in parentheses are the chemical shift changes when the molar ratio of host to guest is ten. [f]Chemical shift assignments according to Baichi, A., Rizzo, V., Skrabal, P., and Luisi, P.L., *J. Am. Chem. Soc.*, 1979, *101*, 5170.

For a host to guest molar ratio of ten used, only two amino acids, lysine (11) and tryptophan (13), reached complete complexation, as indicated by their chemical shift titration curves. All the proton chemical shift titration curves of these two amino acids show the two tangents meeting at a point where the molar ratio of host to guest is unity, indicating the complexes are of 1:1 host to guest stoichiometry.[3,6] A typical example is shown in Figure 2 for the H_1 and H_3 chemical shift titration curves of tryptophan (the subscript in H indicates the carbon bonded to the proton, the carbon atom with the α-amino group is numbered 1). We shall assume that the

complexes of the other amino acids in this work are also of 1:1 host to guest stoichiometry. Our assumption is justified by the good agreement between the observed and calculated chemical shifts. The stoichiometry of weak complexes cannot be reliably deduced by the method of drawing tangents (we simulated several series of titration curves with different values of stability constant for 1:1 host to guest stoichiometry and found that the method of drawing tangents to determine stoichiometry is reliable only when the stability constant is greater than 100 M^{-1}).

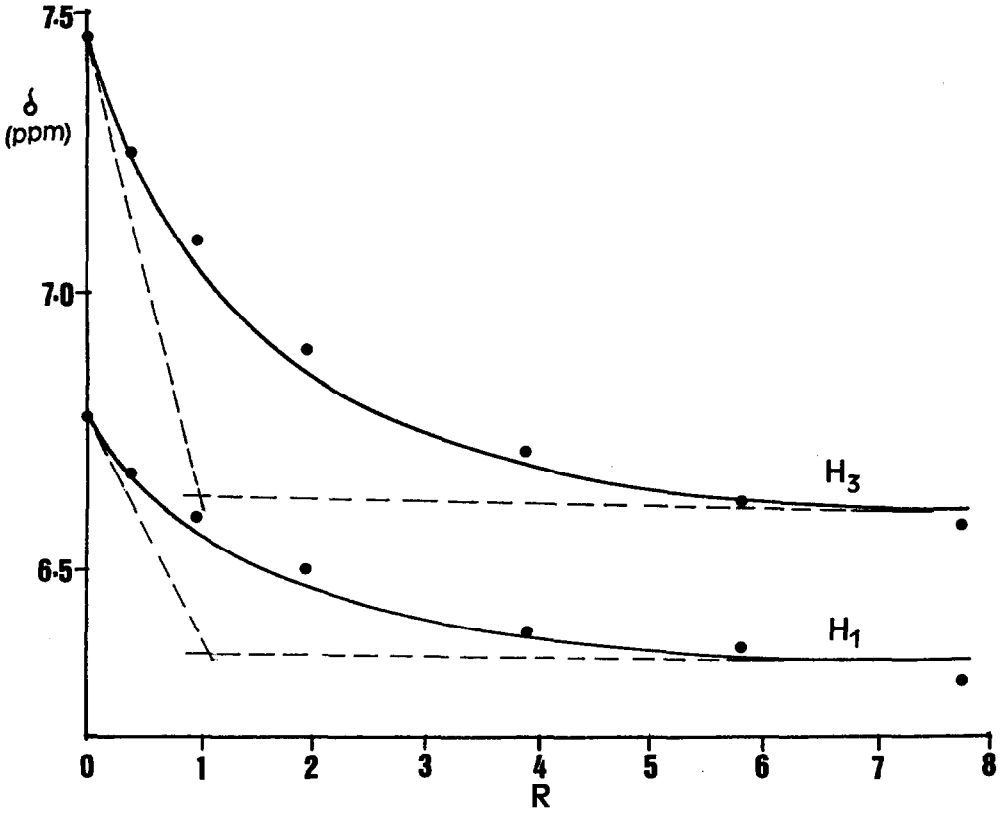
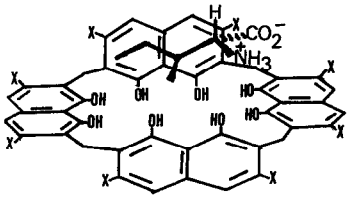


Figure 2. Variation of H_1 and H_3 chemical shifts of tryptophan (1.27×10^{-2} M) with the molar ratio (R) of the host (1) to guest used in D_2O at 25°C. The chemical shifts of H_1 have been moved up by 2.60 ppm. The titration curves were calculated using the values of K and δ of the free and complexed guest given in Table 1.

Host 1, in the chair conformation[3,7] (see 14), has only one vertical naphthalene wall to shield the guest molecule. The other naphthalene wall is antiparallel to it and situated about half the height of a naphthalene unit below it. The remaining two naphthalene units are in a horizontal position. All the amino acids, based on their pK_a values,[8] exist in the dipolar ionic form at pD 7.0.

The almost equal changes in the chemical shifts (Table 1) of the different kinds of protons in valine (4) and isoleucine (5) indicate that the aliphatic amino acids with an alkyl chain lie horizontally in the host cavity. For example, in the case of isoleucine, the observed induced chemical shift changes for a host to guest molar ratio of ten are (in ppm) H_2 1.53, two methylene H_3 1.65, 1.58, and H_4 1.68. However, the H_1 proton is significantly less shielded (0.81 ppm), indicating the hydrophilic dipolar ionic end is away from the hydrophobic naphthalene wall. The H_3 protons of the methyl unit are also significantly less shielded (1.10 ppm) than the two methylene H_3 protons, suggesting the longer C_2-C_4 unit (instead of the shorter C_2-CH_3 unit) faces the naphthalene wall for greater hydrophobic attraction. The inclusion of isoleucine in the cavity of the chair conformation of 1, depicted in 14, is analogous to that of 2-butanol in the same host.[3] The almost equal induced proton chemical shift changes in glutamic acid (10) and lysine (11) also indicate horizontal inclusion for these two guest molecules. However, the trend in the induced proton chemical shift changes in methionine (7), $H_4 > H_3 > H_2 > H_1$, is more consistent with a predominant vertical inclusion of the guest molecule in the cavity, the methyl end facing downward and the dipolar ionic end upward. We do not have an explanation for the different mode of inclusion for methionine.



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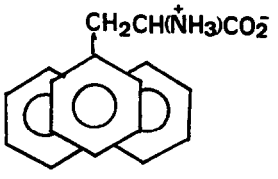

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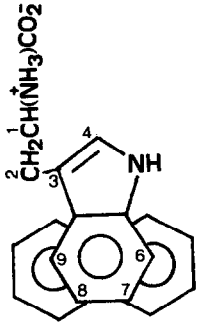

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In the case of phenylalanine (12), the larger shielding effects on the aromatic protons compared with those on the H_1 and H_2 protons and the trend in the shielding effects on the aromatic protons of $H_p > H_m > H_o$ (1.43, 1.28 and 1.00 ppm respectively) suggest that phenylalanine is included vertically in the host cavity, the hydrophobic phenyl ring downward and the hydrophilic dipolar ionic end upward (depicted in 15, only the vertical naphthalene wall of the host 1 shown). A similar mode of inclusion was observed for phenols as guest.[9] For tryptophan (13), the four aromatic protons, H_6 to H_9, are almost equally shielded and they are also more shielded than H_1 and H_2. An axial inclusion of the indole ring (see 16) is consistent with the nmr results.

The stability constant K of each 1:1 host to guest complex was obtained by a non-linear regression fitting procedure.[6] The K values obtained from different protons of the same amino aicd are in good agreement with one another (Table 1). Figure 2 shows two representative calculated titration curves together with the experimental chemical shifts for the case where complete complexation was observed, and Figure 3 for the case where complete complexation was not observed even at the highest host to guest molar ratio of ten used.

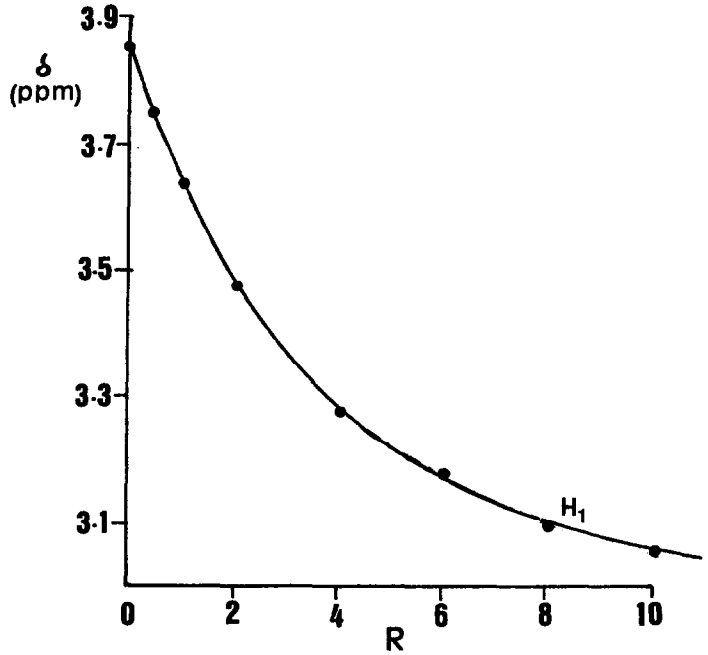

Figure 3. Calculated H_1 chemical shift titration curve of isoleucine in D_2O at 25°C. R is the molar ratio of the host to guest used and the points are experimental values. The K and δ values of the free and complexed guest used for calculating the titration curve are given in Table 1.

If the interaction between the C-H bonds of the aliphatic amino acids and the aromatic-π bonds of the naphthalene wall of 1 is the driving force for their complexation, a linear correlation between log K and the total number of C-H bonds (N) involved in the interaction is expected.[3] A plot of the log K and N values (for the branched amino acid 5, the longer four carbon unit chain having seven C-H bonds is used to calculate N instead of the shorter three carbon unit chain, consistent with 14 above) for the ten aliphatic amino acids given in Table 2 indeed shows a satisfactory linear correlation (Figure 4, correlation coefficient 0.921, slope 0.30 and intercept -0.78). The absence of any large deviation from the linear plot for amino acids 9, 10 and 11 which have a charged group in the side chain (9 and 10 have a CO_2^- group and 11 a NH_3^+ group) indicates that electrostatic interaction between the host and guest is small. Thus, the inclusion of the aliphatic amino acids inside the cavity of 1 is mainly due to the interaction between their C-H bonds and the aromatic-π bonds of the host.

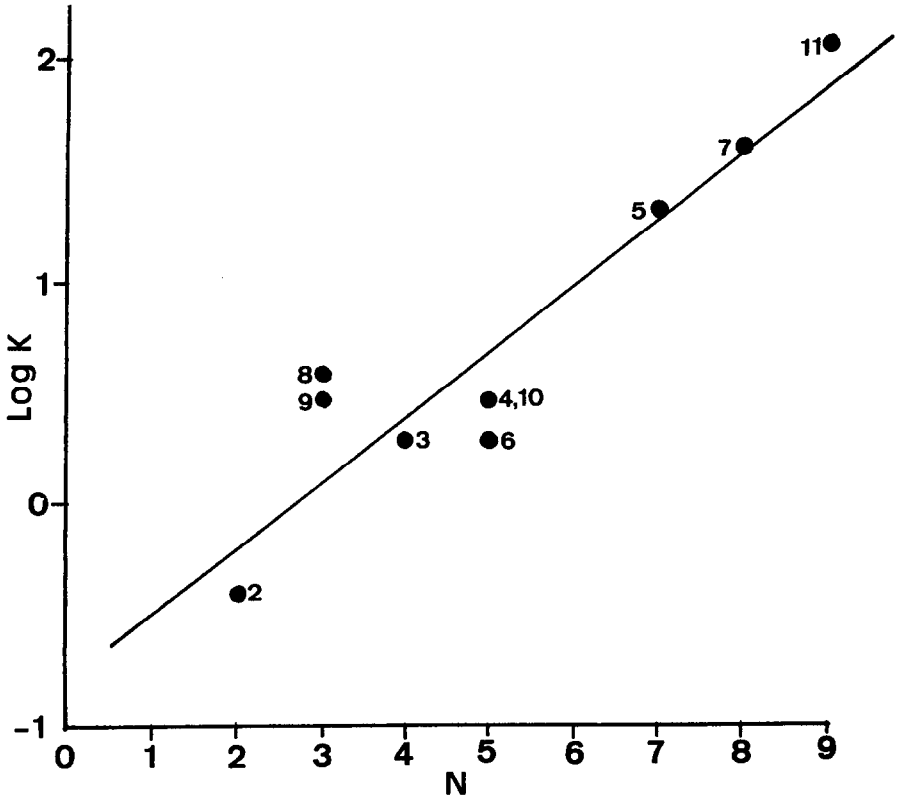

Figure 4. Relationship between log K and N (correlation coefficient 0.921, intercept -0.78 and slope 0.30). The numbers refer to the amino acids in Table 2.

Table 2. Log K and N of 1:1 Complexes of Aliphatic Amino Acids with 1

No.	Amino Acid	Log K	N
2	Glycine	-0.40	2
3	L-Alanine	0.30	4
4	L-Valine	0.48	5
5	L-Isoleucine	1.34	7[a]
6	L-Threonine	0.30	4
7	L-Methionine	1.62	8
8	D,L-Asparagine	0.60	3
9	D,L-Aspartic acid	0.48	3
10	D,L-Glutamic acid	0.48	5
11	L-Lysine	2.08	9

[a]The longer alkyl unit used (see text).

To estimate the interaction energy between a C-H bond and the π-bonds of a naphthalene ring, we compare the log K value of glycine (2) with the other three amino acids with an alkyl side chain (3 to 5). We obtain an average increase of 0.33 ± 0.02 log K unit per C-H bond, equivalent to a free energy of -0.5 Kcal per mole (a similar calculation of the log K data[3] of the complexes of 1 with the aliphatic alcohols gives a value of -0.8 Kcal per mole for the same kind of interaction).

The interaction between aromatic-π bonds is the main driving force in the complexation of aromatic guests.[4,5,9] To estimate the interaction energy between a phenyl ring and a naphthalene ring, we compare the log K values of phenylalanine (12) and alanine (3). The difference in their log K values (1.34) plus 0.33 (this value accounts for one less C-H bond in 12 ,compared with 3) gives a value of -2.3 Kcal per mole as the interaction energy between a phenyl ring and a naphthalene rings. Burley and Petsko[2] have calculated a value of -1 to -2 Kcal per mole for the interaction between two phenyl rings in several proteins. The interaction energy between two aromatic rings seems to be dependent on the size of the aromatic ring, since it is -3.0 Kcal per mole between a naphthalene ring and an indole ring (calculated by comparing the log K values of tryptophan and alanine).

EXPERIMENTAL

Materials. All the amino acids were commercial samples. The host 1 was prepared as described earlier[6].

^{1}H nmr spectra in D_2O at 25°C, pD 7.0 and ionic strength of 1.70 M were recorded with a 300 MHz Brucker AC300 Superconducting NMR spectrometer. The solvent peak (unaffected by the concentration variation of the host and guest compounds) at 4.80 ppm was used as the internal reference. In all the chemical shift titrations, the concentration of the amino acids was kept constant at about 2×10^{-2} M while the concentration of the host 1 varied.

Buffer solutions in D_2O of pD 7.0 and ionic strength of 1.70 M were prepared as described previously.[9]

Calculations of the stability constant K of the 1:1 host to guest complexes using the non-linear regression fitting of the proton chemical shift titration curves were carried out as reported earlier.[6] The K values obtained have an estimated error of 10%.

ACKNOWLEDGEMENT

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