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Thermodynamics of Binding of Aromatic Amino Acids to α-, β- and γ-cyclodextrins

K. Matsuyama[1], S. El-Gizawy[2], J.H. Perrin[3]

1) Faculty of Pharmaceutical Sciences
Kyushu University
Fukuoka Japan

2) Faculty of Pharmacy
University of Assiut
Assiut Egypt

3) College of Pharmacy
University of Florida
Box J-494, J. Hillis Miller Health Center
Gainesville, Florida 32610

Abstract

The thermodynamics of the binding of aromatic amino acids, i.e., tryptophan, phenylalanine and tyrosine to α-, β- and γ-cyclodextrin were investigated by microcalorimetry. Heat was evolved following the interaction of tryptophan and tyrosine with α- and β-cyclodextrins but not with γ-cyclodextrin. Phenylalanine only appeared to react with γ-cyclodextrin. Human serum albumin contains these amino acids but the heat evolves following interaction with α and β-cyclodextrins was much lower than that evolved with the individual amino acids. No heat was evolved following the interaction of γ-cyclodextrin with the albumin.

Introduction

There have been many studies in recent years concerning the interaction of cyclodextrins (CyDs) with drugs in order to improve the stability or solubility characteristics of the drug (1-9). The bioavailability of flurbiprofen has been

increased following complexation (8), a prostaglandin complex, suitable for oral use has been marketed and the possiblity of CyDs as an aid to peritoneal dialysis have been investigated (10).

These studies make the investigation of the interaction of CyDs with peptides, particularly those containing aromatic amino acids such as phenylalanine (Phe), tryptophan (Trp) and tyrosine (Tyr) essential. CyDs may be important for stabilizing peptides or they may cause toxicological problems by unwanted reactions with endogenous proteins.

In the preliminary investigations, the interaction between α -, β- and γ-cyclodexin (CyD) and three aromatic amino acids as well as human serum albumin (HSA) are investigated by microcalorimetry.

Experimental

Materials - Human serum albumin (HSA), fraction V (lot no. 64F-9349) was obtained from Sigma Chemicals (St. Louis, MI). The α-, β- and γ -CyDs manufactured by Nihon Shokuhin Kako Co., Ltd., Tokyo, Japan were a gift of Professor K. Uekama (Kumamoto, Japan) and were used as supplied. Phenylalanine (Phe), Tryptophan (Trp) and Tyrosine (Tyr) were purchased from Fisher Scientific Company (FairLaw, N.J.). Dowex 50W-x8 cation exchange resin and 1 - x8 anion exchange resin were obtained from J.T. Baker Chemicals (Phillipsburgh, N.J.). All buffer materials were of reagent grade and deionised water was used throughout the study.

Methods - All measurements were made in the LKB Flow Microcalorimeter (Model 21707-121) (LKB Bromma, Sweden) housed in Tronac water bath (Tronac Inc., Orem, UT). This calorimeter is capable of giving a short term temperature stability of $\pm 0.0002^{o}C$ at $25^{o}C$. The two solutions (aromatic amino acid and CyD solution) were pumped to the mixing cell using two LKB 10200 peristaltic pumps, the flow rate was determined daily to within 0.01 ml h^{-1}. An accuracy known total flow rate of approximately 32 ml h^{-1} of the 0.1 M pH 7.4 phosphate buffer was used at $25^{o}C$.

The heat flux was obtained and interpreted in terms of 1:1 binding constant (K), the enthalpy change (ΔH) and the free energy change (ΔG) and entropy change (ΔS) as previously described (6).

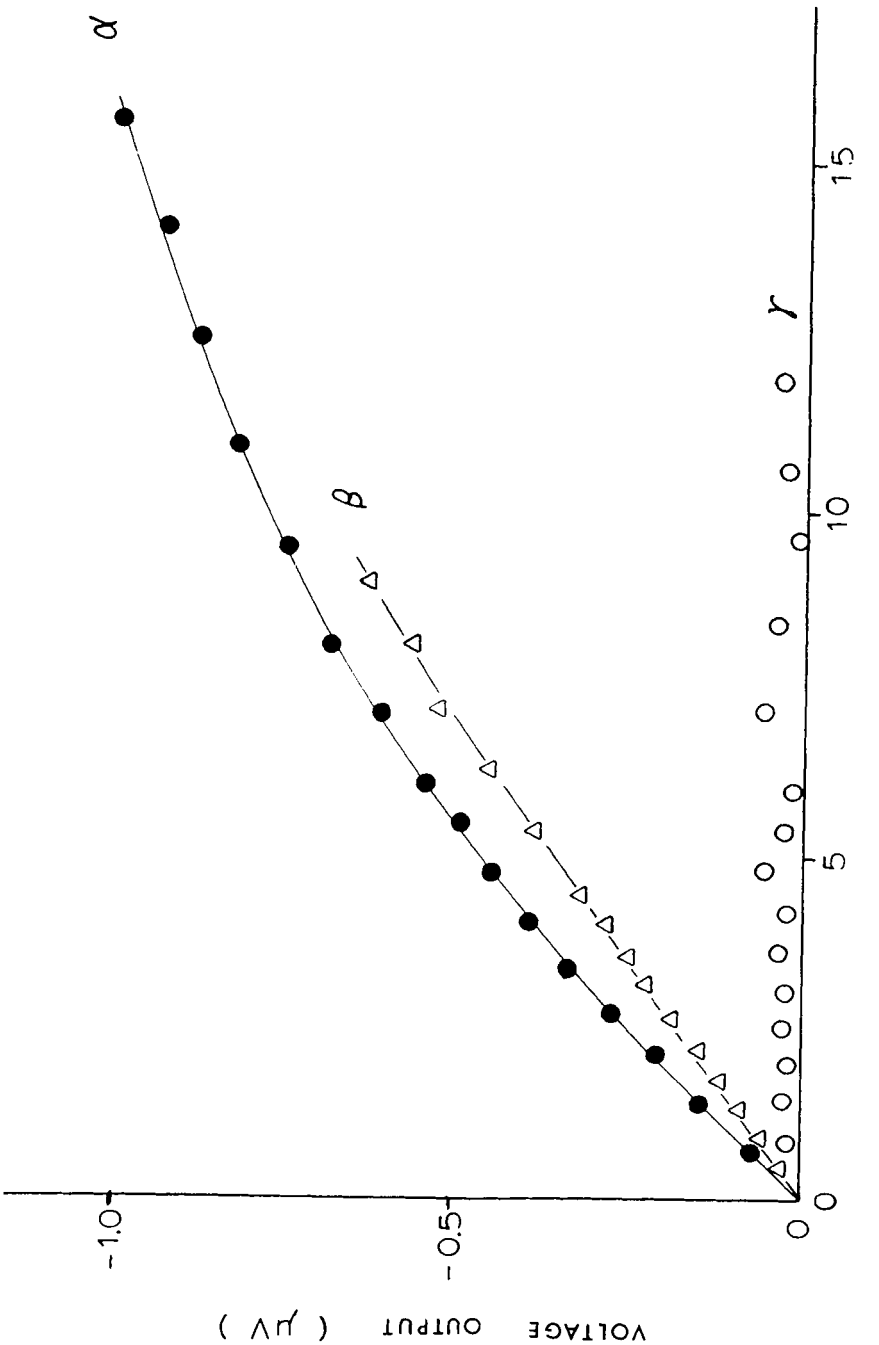

FIGURE 1

Table 1 - Equilibrium Constants and Derived Thermodynamic Parameters for Interaction between Aromatic Amino Acids and Cyclodextrins at 25°C.

K is in mol^{-1} liter, ΔG and ΔH are in Joules mol^{-1} and ΔS is in Joules $mole^{-1}$ K^{-1}

ΔG is obtained from K expressed on a unitless mole fraction scale

		-CyD	-CyD	-CyD
	K	21.2	213.7	-
	ΔH	-965	-763	-
L Trp	ΔG	-17524	-23253	-
	ΔS	55.5	75.4	-
	K	27.4	33.3	-
	ΔH	-2131	-3825	-
L Tyr	ΔG	-18160	-18644	-
	ΔS	53.8	49.6	-
	K	42.3	-	-
	ΔH	-1195	-	-
L Phe	ΔG	-19237	-	-
	ΔS	60.5	-	-

The fraction V HSA was deionised to ensure reproducible procedure (11). HSA was used in a concentration of 2.5×10^{-4} M, taking the molecular weight to be 66.500 G mol^{-1}.

Results and Discussion

β-CyD gave significant heats of reaction with Trp and Tyr but no measurable heat with Phe. γ-CyD gave no measurable heat with any of the aromatic amino acids investigated but α-CyD reacted with all the aromatic acids. The derived thermodynamic parameters shows low binding constants, small amounts of heat evolved on the interactions and significant gains in entropy following complexation, the reactions appear to be entropy driven. Although the interaction of α- and β-CyD with Tyr seem to be similar, β-CyD has almost ten-times the affinity for Trp as does α-CyD. Microcalorimetry shows no interaction between Phe and β-CyD, the smaller molecule possibly forming no stable complex with the β-CyD. In the case of α-CyD, the CyD with the smallest cavity of 6 A, the binding

constants were similar for the three amino acids. The larger cavity of the γ-CyD does not appear to be suitable for complexing those amino acids.

HSA was chosen as a protein model, because of its abundance in the body should the CyDs be introduced into the blood. HSA contain 31 Phe, 18 Tyr residues and a single Trp residue.

The figures shows the voltage output as HSA is titrated with α- and β-CyD, no heat of reaction was observed with γ-CyD. Many groups on the albumin molecules have potential to react with CyDs, and no separated heats of reaction or binding constants can be estimated, however the heat of reaction of the binding of the first CyD molecule to HSA can be estimated from the tangent at the origin of figure 1 and seems to be an order of magnitude lower than those obtained for the amino acid interactions. The binding constant can also be expected to be lower. Even if the binding constants are of the same order as those of the individual amino acids i.e., 10^2 M^{-1}, the competition with drug and endogenous materials is likely to be insignificant.

The data of the table does suggest that CyDs may well have application in the protection of peptides from enzymatic hydrolysis. This matter is being investigated further.

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