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**The Binding of Oligopeptides to Cyclodextrins:
The Role of the Tyrosine Group**

by

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**The Binding of Oligopeptides to Cyclodextrins: The Role of the
Tyrosine Group**

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ABSTRACT

The formation of α -cyclodextrin (α -CD) and β -cyclodextrin (β -CD) inclusion complexes with free tyrosine and the tyrosine residues in two oligopeptides were investigated using steady-state fluorescence spectroscopy. The oligopeptides consist of five amino acids (pentapeptide) and the tyrosine residues are at the n-terminus of both peptides. The two peptides used in this study have specific biological applications and are known to bind selectively to specific receptors.

Cyclodextrins were used to model this receptor-peptide (protein-ligand) interaction. Equilibrium binding constants and the enthalpy and entropy of binding were recovered. Molecular size of the tyrosine-containing species and pH (7.0 vs 10.0) were found to have little effect on α -CD binding. However, tyrosine binding to β -CD was dependent on the size (free tyrosine vs peptide), structure, and pentapeptide conformation.

Introduction

Previous work from our laboratories has focused on producing specific cellular responses from minimal peptide sequences (MPS) covalently bound to fluoropolymer surfaces [1-3]. Minimal peptide sequences are the smallest sequential set of amino acids from a larger protein necessary to elicit a specific response. These MPS are known to promote cellular responses through the binding with cell-surface receptors (integrins) [4]. This is a specific receptor-ligand response, where the ligand (MPS) is a short, sequential sequence of amino acids along the primary backbone chain of a larger protein [5]. Adhesive proteins (e.g., fibronectin, vitronectin, fibrinogen) and extracellular matrices (e.g., laminin, collagen, von Willebrand factor) have several binding sites for integrin cell surface receptors [6]. These integrin receptors are proteins that connect the interior of the cell to its exterior environment and allow the cell to "see" and "feel" its environment and respond accordingly. Integrin receptors can recognize cell binding domains within large proteins as small as three to five sequential amino acids.

A MPS of particular interest to us is a peptide fragment of laminin, tyr-ile-gly-ser-arg (YIGSR). YIGSR has been shown to interact specifically with the integrin receptors on certain neuronal cells [1,3,7]. The MPS YGGFL (tyr-gly-gly-phe-leu) is an enkephalin that serves as the bodies naturally-formed opiate. Enkephalins bind to receptors on specific cells within the brain that also bind morphine and heroin [8]. Their function is to induce analgesia, deadening pain sensations [8].

The objective of this paper is to develop an understanding of how these MPS bind and the role of the tyrosine residue in this binding process. Toward this end, we have studied the binding of L-tyrosine, YIGSR and YGGFL to a simple surrogate of a protein, namely cyclodextrin [9-12].

The binding of β -cyclodextrin (β -CD) to aromatic amino acid residues has been shown to decrease the *in vitro* neurotoxicity of the β -amyloid peptide [13], which is the major component of senile plaque found in the brain of Alzheimer patients [14,15]. The β -amyloid peptide has been shown to have *in vitro* neurotoxic and neurotrophic properties, and possibly function as an adhesion molecule [16]. Camilleri, *et al.* [13] attributed the decrease in *in vitro* neurotoxicity to the binding of the β -CD to specific amino acid residues of the β -amyloid peptide. β -amyloid contains one tyrosine and three phenylalanine residues. Electrospray ionization mass spectroscopy (ESI-MS) was used to demonstrate multiple β -CD binding to the β -amyloid peptide. Additional work by Camilleri, *et al.* [17] reported on the formation of β -CD complexes with phenylalanine and tryptophan residues. However, their ESI-MS results did not suggest the formation of tyrosine/ β -CD complexes. This particular result may, of course, arise from the ESI-MS ionization process as other researchers have demonstrated the formation of tyrosine/ β -CD complexes and recovered binding and thermodynamic parameters [18-21].

The B-chain of insulin is well-known to form dimers and oligomers [22]. Interestingly, the B-chain contains three phenylalanine and two tyrosine residues, and addition of β -CD inhibits insulin aggregation in solution [23]. Several additional reports have appeared that document the effects of cyclodextrins in biological systems [24-31], however, there are few published reports discussing the binding of tyrosine and the like to model hosts like cyclodextrins [17-21]. This dearth of information is especially troubling considering that several previous publications [13,17,23] suggest an important role for tyrosine and other aromatic amino acid residues in several key binding processes.

Lewis and Hansen [18] were the first to report thermodynamic binding values for tyrosine

with α -cyclodextrin (α -CD). They used calorimetry to determine the binding of twenty molecules to α - and β -CD in water. Matsuyama, *et al.*, [19] improved on the initial results and used microcalorimetry to determine the binding of tyrosine, phenylalanine, and tryptophan to α - and β -CD. Inoue and coworkers [21] studied the binding of phenylalanine and tyrosine to α - and β -CD using ^1H and ^{13}C -NMR. More recently, ESI-MS [17] and competitive spectrophotometry [20] were used to follow the binding of the aromatic amino acids with cyclodextrins. In addition, a reverse phase-high pressure liquid chromatography method used α - and β -CD as mobile phase modifiers to improve the separation of aromatic amino acids, including tyrosine [32]. The addition of cyclodextrins to the mobile phase reduced the amino acid capacity factors and improved the separation factors by increasing the number of theoretical plates.

In the current work we report on the binding of free tyrosine and the tyrosine residues in YIGSR and YGGFL to α - and β -CD. Changes in the steady-state fluorescence spectra of tyrosine were used to determine the equilibrium binding constant (K) and thermodynamic parameters (enthalpy and entropy). YIGSR contains one tyrosine residue at the n-terminus. The pentapeptide YGGFL is slightly more complicated because it contains tyrosine *and* phenylalanine; individually both are known to bind with cyclodextrins [18-21]. Previous investigations of tyrosine-cyclodextrin binding were accomplished using techniques that required one to use moderately high-levels of tyrosine. Further, most of the published data were obtained using different techniques or conditions. As a result, there is significant deviation between individual sets of reported thermodynamic binding parameters. In this work we have studied tyrosine (5×10^{-6} M) binding at near physiological pH (7.0) and under more basic conditions (pH 10.0) and present the first thermodynamic binding parameters for cyclodextrin complexes with the tyrosine

residue in biologically important peptides (YIGSR and YGGFL).

Experimental

Distilled deionized water was used to prepare all solutions and all materials were used as received. Cyclodextrins were from Sigma. Stock solutions (1.0×10^{-3} M) of the individual pentapeptides (YIGSR and YGGFL) and L-tyrosine (Sigma) were prepared in 0.01 M phosphate buffer, pH 7.0 or 0.01 M carbonate buffer, pH 10.0. All cyclodextrin stock solutions (α -CD, 0.100 M; β -CD, 0.014 M) were prepared in either 0.01 M phosphate buffer, pH 7.0 or 0.01 M carbonate buffer, pH 10.0 to maintain ionic strength. In the titration experiments, the cyclodextrin concentrations were systematically varied (0 - 0.100 M, α -CD; 0 - 0.014 M, β -CD) and the tyrosine, YIGSR, or YGGFL concentration was maintained at 5×10^{-6} M. All stock solutions were stored at 4 °C and used within 2 weeks of their initial preparation.

Steady-state fluorescence measurements were carried out using a SLM-Aminco model 48000 MHF spectrofluorometer [33,34]. The sample temperature was maintained at the desired value (± 0.1 °C). All samples were equilibrated at each temperature for at least 30 min prior to making measurements. An excitation wavelength of 275 nm was used and the emission was recorded from 290 to 400 nm. Excitation at 275 nm excites selectively tyrosine residues, with no interference from phenylalanine or any other amino acid residue present in these particular oligopeptides. All spectra were blank corrected and the integrated area under the individual emission spectra (290-350 nm) as a function of added cyclodextrin was used as the measure of the fluorescence intensity.

Results

The tyrosine emission is a strong function of the physicochemical properties of its local

environment. As a result, one can conveniently use changes in tyrosine fluorescence on titration with α - and β -CD as a means to determine the equilibrium binding constants (K) [35,36]. In this work, the Benesi-Hildebrand approach [36] was used to recover K. The enthalpy (ΔH) and entropy (ΔS) of binding were calculated from the corresponding van't Hoff plot. We assumed that the aromatic ring of tyrosine enters the cyclodextrin through the wider, secondary hydroxyl side of the cavity.

Figure 1 presents a typical series of emission spectra for 5×10^{-6} M tyrosine as a function of added α -CD. An enhancement in the tyrosine fluorescence on adding α -CD is clearly evident. This trend is also observed when titrating YIGSR and YGGFL with α - and β -CD (results not shown). Inclusion of tyrosine into the cyclodextrin cavity is thought to increase the fluorescence intensity by minimizing interactions that cause and/or promote non-radiative paths for excited-state relaxation (quenching and solvent relaxation) [37]. In addition, the fluorescence quantum yield and excited-state lifetimes of phenol and several phenol derivatives are known to increase on binding to cyclodextrins [37].

A series of temperature-dependent Benesi-Hildebrand plots for the tyrosine residue in YIGSR complexed to α -CD are shown in Figure 2. In general, Benesi-Hildebrand plots for tyrosine, YIGSR, and YGGFL titrated with either α - or β -CD exhibited good linearity ($r^2 \geq 0.97$). This result suggests a simple 1:1 binding stoichiometry for the tyrosine residue to cyclodextrin. Table I presents the recovered equilibrium constants for the binding of tyrosine, YGGFL, and YIGSR with α - and β -CD at pH 7.0 and 10.0 at 20 °C. There are several important aspects of these data that merit special mention. First, free tyrosine and the tyrosine residues in YGGFL and YIGSR have larger binding constants to β -CD relative to α -CD (a two-fold increase for free

tyrosine, ten-fold for YIGSR, and six-fold for YGGFL). Second, changes in pH (from 7.0 to 10.0) have little if any effect on the tyrosine binding to α -CD. Third, there is little difference in α -CD binding between free tyrosine and tyrosine at the n-terminus of either pentapeptide. Fourth, tyrosine residue/ β -CD binding is much stronger in the pentapeptides compared to free tyrosine. This may be a function of deeper and tighter penetration of the tyrosyl group into the β -CD cavity [21], with additional stabilization from hydrogen bonding between the oligopeptide backbone and secondary hydroxyl groups on the outer rim of the β -CD cavity. Finally, the equilibrium binding constant with β -CD is significantly higher for YIGSR than YGGFL.

The fact that tyrosine/ α -CD binding is unaffected by pH (7.0 and 10.0) is not surprising. Both pH values are significantly below the pK_a of the secondary hydroxyls on the outer rim of the cyclodextrin, which is 12 [9,38]. Although the pK_a of tyrosines phenolic hydroxyl function is 10.1, tyrosinate fluorescence, which is well-resolved from tyrosine fluorescence, was not observed in this work. It can thus be assumed that at pH 7.0 and 10.0, that tyrosine's phenolic group and the hydroxyls on the cyclodextrin remain fully protonated. It is thus not surprising that pH (7.0 vs 10.0) does not affect the binding constant significantly.

Interestingly, the binding constant for tyrosine with α -CD was, for the most part, unaffected by the size of the tyrosine-containing molecule. This result suggests that inclusion of tyrosine's aromatic ring structure into the α -CD cavity is geometrically limited. Accessibility of the aromatic ring and secondary stabilizing interaction (e.g., hydrogen bonding) issues that might be associated with the pentapeptides are apparently overridden and do not affect tyrosine residue binding to α -CD.

Results from temperature-dependent experiments (Tables II and III) were used to

calculate the enthalpy (ΔH) and entropy (ΔS) of binding for tyrosine, YGGFL, and YIGSR to α - and β -CD (Table IV). The magnitude of the α -CD binding constants were only a function of the temperature; pH had little effect. In the presence of β -CD, the recovered binding constants were found to be a function of guest molecule size and temperature (Table III). The binding of free tyrosine to β -CD is about 2-fold stronger than with α -CD. However, the tyrosine/ β -CD binding was essentially temperature invariant. YIGSR/ and YGGFL/ β -CD complexes were considerably stronger than the corresponding α -CD complexes and the tyrosine/ β -CD complex. In addition, the binding constants were statistically different for the two peptides, unlike with α -CD. However, in both oligopeptides, tyrosine is the n-terminal amino acid; therefore, any differences in binding must arise from differences in tyrosine ring accessibility and/or the amino acid sequence of the two peptides. The secondary and tertiary structures of the peptides could also effect additional stability to the inclusion complex, for example, through hydrogen bonding (*vide infra*).

The enthalpy and entropy of binding are collected in Table IV. For α -CD binding, ΔH and ΔS values are all comparable for tyrosine, YGGFL, and YIGSR as a function of pH. This is not the case for β -CD binding, as tyrosine, YGGFL, and YIGSR exhibit significantly different ΔH and ΔS values. The equilibrium binding constant for tyrosine/ β -CD is essentially temperature independent; therefore, ΔS is close to zero. If the assumption that ΔS equals zero is made, ΔH is equal to -9 kJ/mol. This value is much lower than ΔH for tyrosine binding to α -CD and suggests that the near zero change in order in the tyrosine/ β -CD system compensates for the decrease in exothermicity.

Inspection of the results for the two pentapeptides binding with β -CD are especially interesting and require additional discussion. First, the enthalpy of binding becomes *more*

favorable as we progress from tyrosine (-9 kJ/mol), to YIGSR (-15 kJ/mole) to YGGFL (-36 kJ/mol). Second, the entropy of bind becomes *less* favorable as we progress from tyrosine (ca. 0 J/mol K), to YIGSR (-7 J/mol K) to YGGFL (-87 J/mol K). Third, the enthalpy of binding for YGGFL with β -CD is 2-fold more favorable compared to YIGSR and 4-fold greater than tyrosine alone. Together these results demonstrate their is a strong interplay between entropy and enthalpy that dictates the binding of these species to β -CD (*vide infra*).

When comparing results between α - and β -CD we note there is an order-of-magnitude change between ΔS for YIGSR binding to α - and β -CD and the entropy is significant less favorable with α -CD. Similarly, the entropy of binding is about 50% less favorable for YGGFL binding with α -CD compared to β -CD. We note also that the enthalpy for YIGSR binding to β -CD is actually less favorable compared to binding with α -CD (-15 vs -33 kJ/mol); however, the entropy is able to compensate strongly for this lack of favorable enthalpy.

A plot of ΔH vs ΔS provides a means to assess the level of ΔH - ΔS compensation and yields the equilibrium temperature. Figure 3 illustrates such a plot for all systems investigated showing the affects of pH, molecular size, and cyclodextrin. The linear relationship is given by $\Delta H = -(10.5 \pm 2.5) \text{ kJ/mol} + (264 \pm 36) \text{ K} (\Delta S)$. Others have reported similar ΔH - ΔS compensation with comparable slopes (equilibrium temperatures). For example, Lewis and Hansen [18] and Rekharsky, *et al.*, [39] report equilibrium temperatures of 265 K and 274 ± 60 K, respectively. Because our experiments were all conducted above 264 ± 36 K, changes in ΔH and ΔS are expected to compensate for one another.

Discussion

This study is designed to determine thermodynamic binding parameters for free tyrosine

and tyrosine residues in two pentapeptides with α - and β -CD and provide a comparison with published work (Table V). The binding of cyclodextrins with phenolic compounds involves non-specific van der Waals and hydrophobic interactions [37]. Intermolecular hydrogen bonding may also occur involving the phenolic hydroxyl function and/or other portions of the oligopeptides with the secondary and perhaps, primary hydroxyls on the outer rim of the cyclodextrin. This may help to further stabilize complex formation. There are two possible scenarios for cyclodextrin complex formation: (1) inclusion of the guest molecule inside the cavity, and (2) association of the guest with the perimeter of the cyclodextrin ring structure. In the case of the tyrosine residue, NMR studies have demonstrated inclusion of the phenolic side group *within* the cavity [21,40].

Inoue and coworkers [21,40] studied the binding of tyrosine and phenylalanine using ^1H - and ^{13}C -NMR using chemical shift differences between the complexed and uncomplexed states. The largest chemical shift differences were observed with β -CD and they suggest that the aromatic ring is deeply and tightly contained within the β -CD cavity. In the case of α -CD, where smaller chemical shift changes were observed, weaker binding compared to β -CD is suggested. This result is consistent with the geometric dimensions of α -CD [9-12], as deep and tight penetration of the aromatic ring is not possible. In addition, functional groups on the amino acid or oligopeptide, surrounding the aromatic ring, can interact (e.g., hydrogen bond) with hydroxyl groups on the cyclodextrin. This can occur more easily with β -CD when the aromatic ring is more deeply inserted [21], putting the peptide backbone in closer proximity to the cyclodextrin. With YIGSR and YGGFL, the tyrosine residues are at the n-terminus, allowing for the possibility that the n-terminal amine can hydrogen bond with the cyclodextrin hydroxyls.

The molecular dimensions of the cyclodextrins are well known [9-12] and the

conformation of the native peptides can be estimated using semiempirical molecular simulations [41,42]. Accessibility of the tyrosyl residues in the two peptides was assessed by simulating the conformation of YIGSR and YGGFL using the computer program SPARTAN[®] [41]. Final geometries for the model peptide structures were obtained by energy minimization using Dewar's Austin Model 1 (AM1) semiempirical Hamiltonian [42]. The energy minimized conformations are shown in Figure 4. In all cases, the aromatic tyrosine ring is accessible for cyclodextrin binding as there are no obvious steric interferences. In addition, the primary amine of free tyrosine and the n-terminus of the oligopeptides may be close enough to hydrogen bond with the secondary hydroxyls on β -CD.

Inclusion of the phenolic ring of tyrosine into the α -CD cavity is geometrically limited. The width of the phenyl ring (distance between the δ hydrogen atoms surrounding the hydroxyl function) is 4.3 Å. This dimension would allow the phenyl ring to fit into the wider opening of the α -CD, but not penetrate fully inside as the cavity narrows. However, the β -CD cavity is much wider [9-12] and would allow the entire aromatic ring to fit easily inside. In addition, the entire length of the tyrosine side chain (6.1 Å, distance between the phenolic proton and methylene carbon) can be included within a β -CD cavity. In this situation, additional stabilization for the complex through hydrogen bonding with the other portions of the oligopeptide are clearly possible.

Our fluorescence data indicates that the YIGSR/ β -CD complex is more stable than the YGGFL/ β -CD complex. The optimized conformations (Figure 4) suggest several possible explanations for the increased stability of the YIGSR/ β -CD complex. First, the n-terminal amine of YIGSR is more accessible for hydrogen bonding with the cyclodextrin. The carbonyl function

β to the n-terminal amine is on the opposite side of the peptide chain, much too far away for hydrogen bonding to occur. Therefore, the n-terminal amine is completely available to hydrogen bond with the cyclodextrin and is certainly close enough if the entire phenol group is included within the β -CD cavity. For YGGFL, the β -carbonyl is on the same side of the peptide backbone as the amine and is only 2.3 Å away from one of the amine protons. The proximity of the carbonyl oxygen to the amine protons would undoubtedly allow intramolecular hydrogen bonding. This already established hydrogen bond would compete with any intermolecular hydrogen bonds between the cyclodextrin and the YGGFL residues. Second, the tyrosyl group of YIGSR appears to be completely free of any steric hindrance. Whereas, YGGFL has a more folded conformation and the aromatic ring of the phenylalanine residue is only 8 - 10 Å away from the tyrosine aromatic ring. Third, the additional aromatic ring in YGGFL may also form an inclusion complex with α - and β -CD [18-21,39,43-45]. Matsuyama, *et al.*, [19] reported that the phenylalanine/cyclodextrin binding constant is similar to that of tyrosine/cyclodextrin. Lewis and Hansen [18] and Inoue and coworkers [21] reported the phenylalanine/ β -CD complex is more stable and favored over the tyrosine/ β -CD complex. However, if multiple cyclodextrin binding were to occur to YGGFL, it would be difficult for one YGGFL molecule to bind *two* β -CD molecules simply because the outer diameter of β -CD is 15.3 Å and the two aromatic groups are only 8 - 10 Å apart. For two β -CD molecules to complex simultaneously with a single YGGFL oligopeptide, a great deal of additional system order would be required as the results indicate in Table IV (compare entropy values of -7 J/mol K for YIGSR binding to β -CD with -83 J/mol K for YGGFL binding to β -CD). Fourth, in YIGSR, the basic guanidino group of arginine (amino acid at the c-terminus of YIGSR) is 10 - 13 Å away from the tyrosyl group. The guanidino group

contains one secondary amine and two primary amines (in resonance with each other) that are capable of forming hydrogen bonds with the cyclodextrin thus adding additional stability to the complex. The basic character of the guanidino group may also lead to an ionic stabilization. Finally, the amino acid side chains on YGGFL are all hydrophobic and would not provide additional hydrogen bond stabilization of the tyrosyl/ β -CD complex. The conformations suggested in Figure 4 are used only to estimate the conformation of the two peptides and show the accessibility of tyrosine's aromatic ring.

Our recovered thermodynamic constants (Tables I-IV) are similar to several previously published values [19-21] (Table V). For the tyrosine/ α -CD complex, our binding constant value is in agreement with Matsuyama, *et al.* [19] who used microcalorimetry and similar experimental conditions (0.1 M phosphate, pH 7.4, 298 K). Matsuyama, *et al.*, [19] also reported a higher binding constant for the tyrosine/ β -CD complex than the tyrosine/ α -CD complex. Their reported tyrosine/ β -CD value is, however, about 35% lower than our recovered value, but, the trend is consistent.

Horsky and Pitha [20] reported a higher binding constant for the glycine-tyrosine/ β -CD complex than the glycine-tyrosine/ α -CD complex. This agrees well with our results of significantly higher binding constants for oligopeptide/ β -CD complexes than oligopeptide/ α -CD complexes. The binding constants recovered for glycine-tyrosine/cyclodextrin systems cannot, unfortunately, be compared to our oligopeptide/cyclodextrin systems because of the differences in size, conformation, and functional groups. However, the trend is, again, consistent.

The large difference in the tyrosine/ α -CD binding constant obtained by Lewis and Hansen [18] may be explained as follows. First, neither ionic strength nor pH were apparently controlled

in these experiments. Second, due to the nature of the measurement scheme, these authors used a relatively high tyrosine (0.04 M) and *lower* concentrations of α -CD (0.009 M) and β -CD (0.003 M). Thus, they were unable to approach anything close to saturation.

Our enthalpy and entropy of binding values are much different than those previously reported (compare Table IV and Table V). However, our values are similar to those reported for the binding of phenylalanine with cyclodextrin [39,43-45]. Our results indicate that the inclusion of tyrosine in the cyclodextrin cavity is an exothermic reaction that is not favored entropically. Figure 3 shows that the unfavorable entropy is, however, compensated by a more favorable enthalpy term. The closeness of ΔH and ΔS for α -CD binding suggests that similar forces are responsible for the complexation. This is in agreement with partial inclusion of the tyrosine ring into the α -CD cavity in all three tyrosine systems.

Very different results are obtained for β -CD. The ΔS value for tyrosine/ β -CD is not as unfavorable as it is in the other systems. Comparing the two oligopeptide systems, the additional binding site on YGGFL (i.e., phenyl residue of phenylalanine) would require more order in the system, hence a more negative ΔS value than in YIGSR. In addition, the ΔH compensation for the unfavorable ΔS of binding values is greatest for YIGSR/ β -CD. This is demonstrated by $\Delta H/\Delta S$ for YIGSR/ β -CD equal to 2143, compared to 430 ± 32 for all α -CD inclusion systems studied here and YGGFL/ β -CD.

Conclusions

We have reported on the thermodynamic binding parameters of free tyrosine and tyrosine residues in two oligopeptides with α - and β -CD. The binding of tyrosine (free or residues) to α -CD is independent of the size, amino acid sequence, and pH (7.0 vs 10.0). This is attributed to

geometric limitations of α -CD cavity. In contrast, size and amino acid sequence/structure have significant effects on the tyrosine binding to β -CD. This was demonstrated clearly when comparing YIGSR and YGGFL. The pentapeptide, YGGFL contains a phenylalanine residue which may compete for β -CD binding, affecting tyrosine residue/ β -CD binding. In addition, differences in the ability of the individual oligopeptides to form intermolecular hydrogen bonds with the cyclodextrin are also partially responsible for the increased stability of the YIGSR/ β -CD complex. Thermodynamically, the favorable ΔH of binding offsets the unfavorable ΔS value.

We have demonstrated that a non-specific receptor, β -CD, has very different binding affinities for the tyrosine residue in oligopeptides and that this is a function of the oligopeptides 1°, 2°, and 3° structures. In addition, β -CD has an increased affinity for the tyrosine residue in the two pentapeptides studied here when compared to free tyrosine. This result arises because the non-included residues within the individual pentapeptides are able to interact favorably with the exterior of the cyclodextrin cavity and provide secondary stabilization effects. This general trend might increase with the number of amino acid residues until one reaches the point where the accessibility of tyrosine aromatic ring itself becomes occluded by the other amino acid residues.

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Table I. Equilibrium binding constants for tyrosine, YIGSR, and YGGFL to α - and β -CD at 20°C.

Guest	α -CD binding, K (M^{-1})		β -CD binding, K (M^{-1})
	pH 7.0	pH 10.0	pH 7.0
L-tyrosine	27 ± 4	23 ± 5	48 ± 5
YIGSR	20 ± 4	19 ± 4	224 ± 8
YGGFL	20 ± 3	20 ± 2	123 ± 18

Table II. Temperature-dependent equilibrium binding constants for tyrosine, YIGSR, and YGGFL with α -CD.

pH	Guest	<u>Equilibrium Constants, M⁻¹</u>			
		283 K	293 K	303 K	313 K
7.0	tyrosine	36 ± 6	27 ± 4	16 ± 4	13 ± 3
	YIGSR	33 ± 5	20 ± 4	15 ± 4	8 ± 2
	YGGFL	32 ± 4	20 ± 3	16 ± 2	11 ± 3
10.0	tyrosine	33 ± 5	23 ± 5	19 ± 4	n.c.
	YIGSR	27 ± 6	19 ± 4	13 ± 4	n.c.
	YGGFL	25 ± 4	20 ± 2	14 ± 3	n.c.

n.c. Experiments not conducted

Table III. Temperature-dependent equilibrium binding constants for tyrosine, YIGSR, and YGGFL with β -CD.

Guest	<u>Equilibrium Constants, M⁻¹</u>			
	283 K	293 K	303 K	313 K
tyrosine	47 ± 8	48 ± 5	51 ± 9	33 ± 6
YIGSR	284 ± 15	224 ± 18	178 ± 17	151 ± 13
YGGFL	197 ± 21	123 ± 18	87 ± 11	43 ± 9

Table IV. Binding enthalpy and entropy for α - and β -CD binding with tyrosine, YIGSR, and YGGFL.

Guest	<u>α-CD, pH 7.0</u>		<u>α-CD, pH 10.0</u>		<u>β-CD, pH 7.0</u>	
	ΔH (kJ/mol)	ΔS (J/mol K)	ΔH (kJ/mol)	ΔS (J/mol K)	ΔH (kJ/mol)	ΔS (J/mol K)
L-tyrosine	-26 ± 3	-63 ± 10	-20 ± 3	-41 ± 11	--	--
YIGSR	-33 ± 4	-81 ± 15	-26 ± 2	-65 ± 14	-15 ± 2	-7 ± 3
YGGFL	-25 ± 2	-61 ± 8	-21 ± 3	-46 ± 11	-36 ± 4	-83 ± 23

-- The slope is ill-defined and we estimate ΔH and ΔS to be -9 kJ/mol and 0 J/mol K, respectively.

Table V. Published thermodynamic values for the binding of tyrosine to α - and β -CD.

	K, M ⁻¹	ΔH (kJ/mol)	ΔS (J/mol K)
tyrosine / α -CD ^a	794	-4 \pm 4	42
tyrosine / α -CD ^b	27.4	-2.1	53.8
tyrosine / β -CD ^b	33	-4	50
glycine-tyrosine / α -CD ^c	13 \pm 0.2	--	--
glycine-tyrosine / β -CD ^c	101 \pm 2	--	--
tyrosine / β -CD ^d	108	--	--

- a. From reference 18; calorimetry, 298 K.
- b. From reference 19; microcalorimetry, 298 K, 0.1 M phosphate, pH 7.4.
- c. From reference 20; competitive spectrophotometry, 296 K, phosphate-buffured isotonic saline, pH 7.4.
- d. From reference 21; ¹H and ¹³C-NMR, 304 K, pH 11.

Figure Captions

Figure 1. Steady-state fluorescence spectra of tyrosine (5×10^{-6} M) as a function of added α -CD.

Figure 2. Temperature-dependent Benesi-Hildebrand plots of YIGSR (5×10^{-6} M) titrated with α -CD at pH 7.0.

Figure 3. Enthalpy vs entropy plot for YIGSR, YGGFL, and tyrosine binding to α - and β -CD.

Figure 4. Simulated structures of L-tyrosine, YGGFL, and YIGSR obtained using the computer program SPARTAN[®] [50]. Structures were determined by energy minimization using Dewar's Austin Model 1 (AM1) semiempirical Hamiltonian [51].

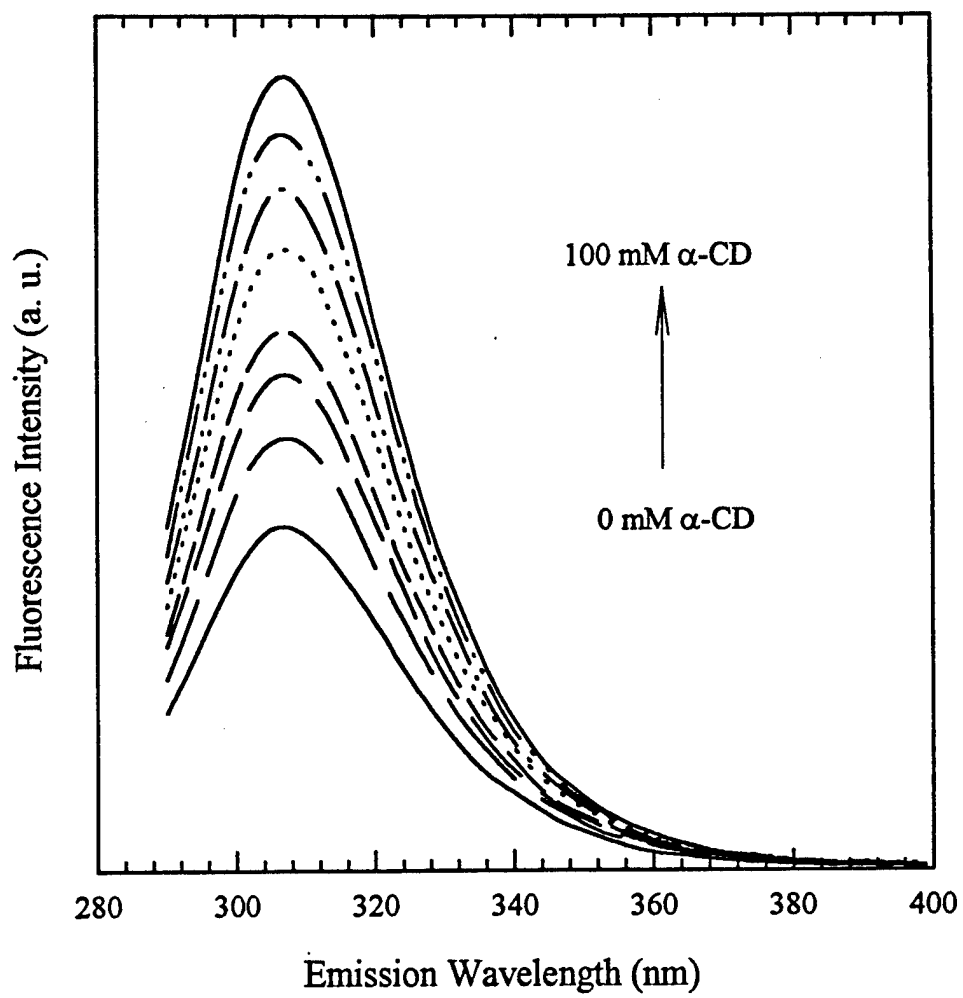


Figure 1

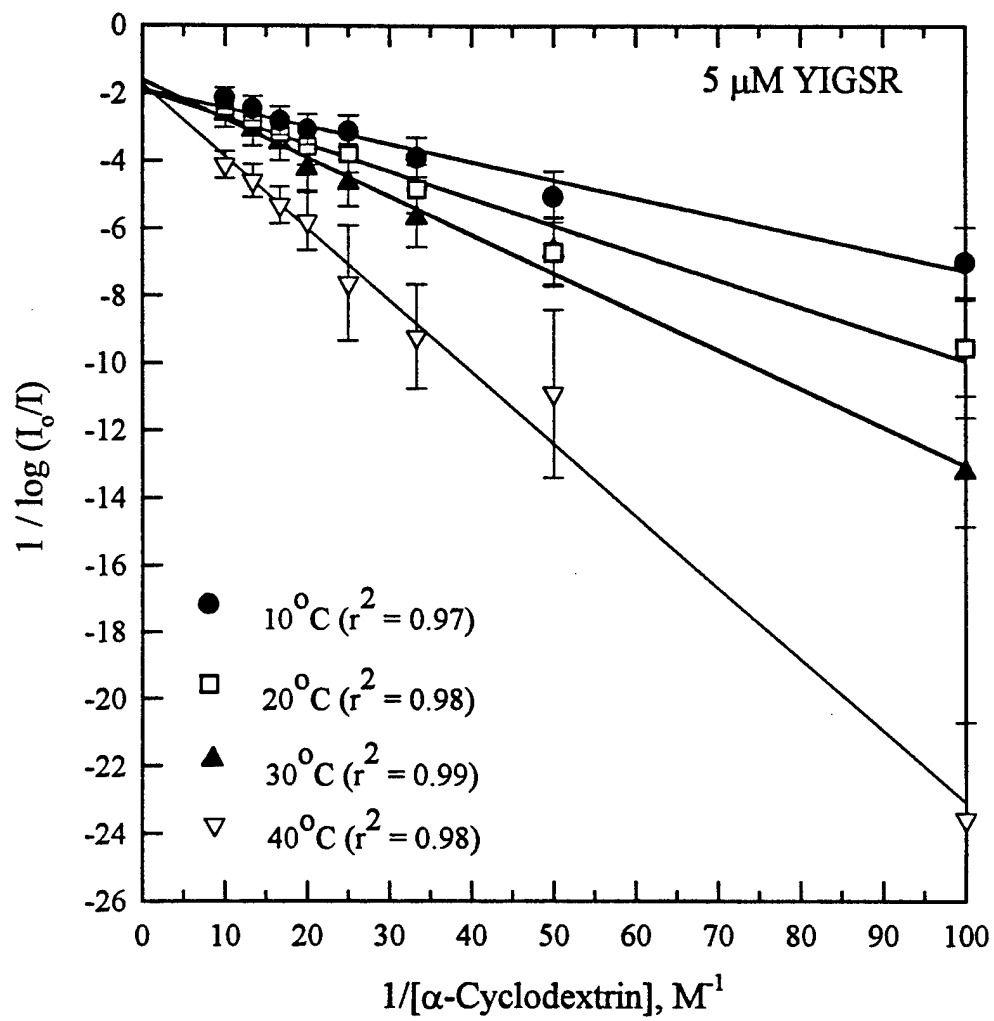


Figure 2

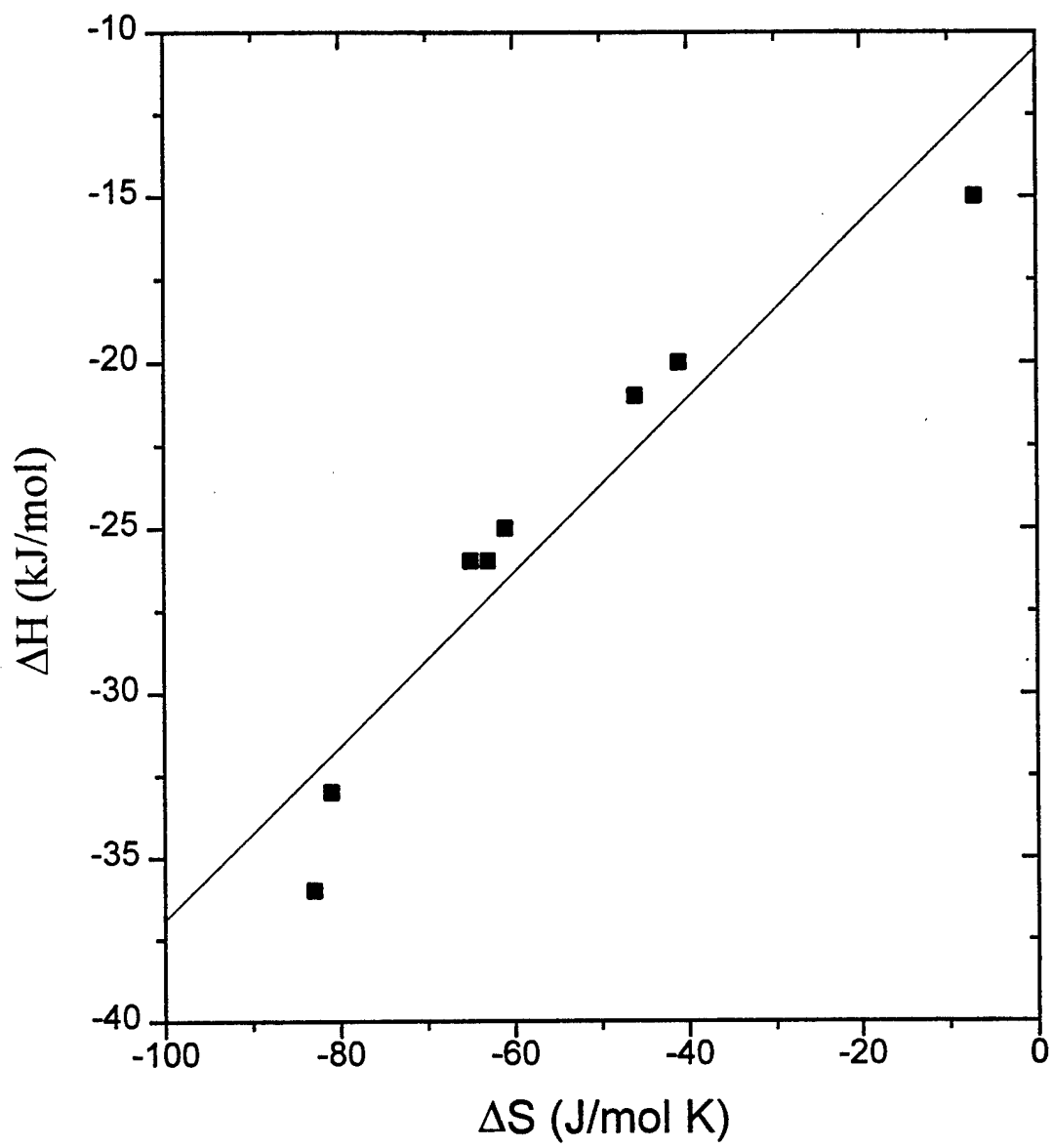
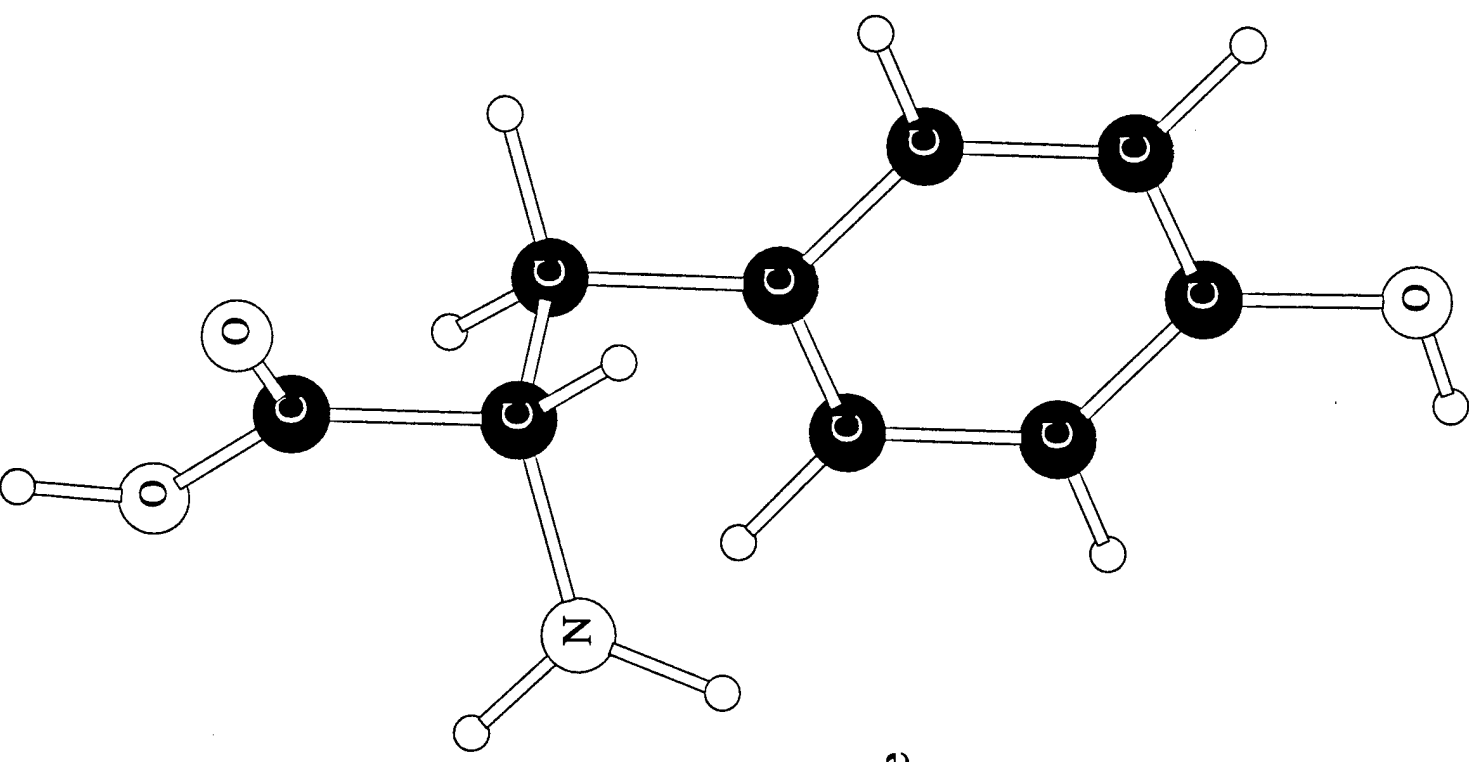
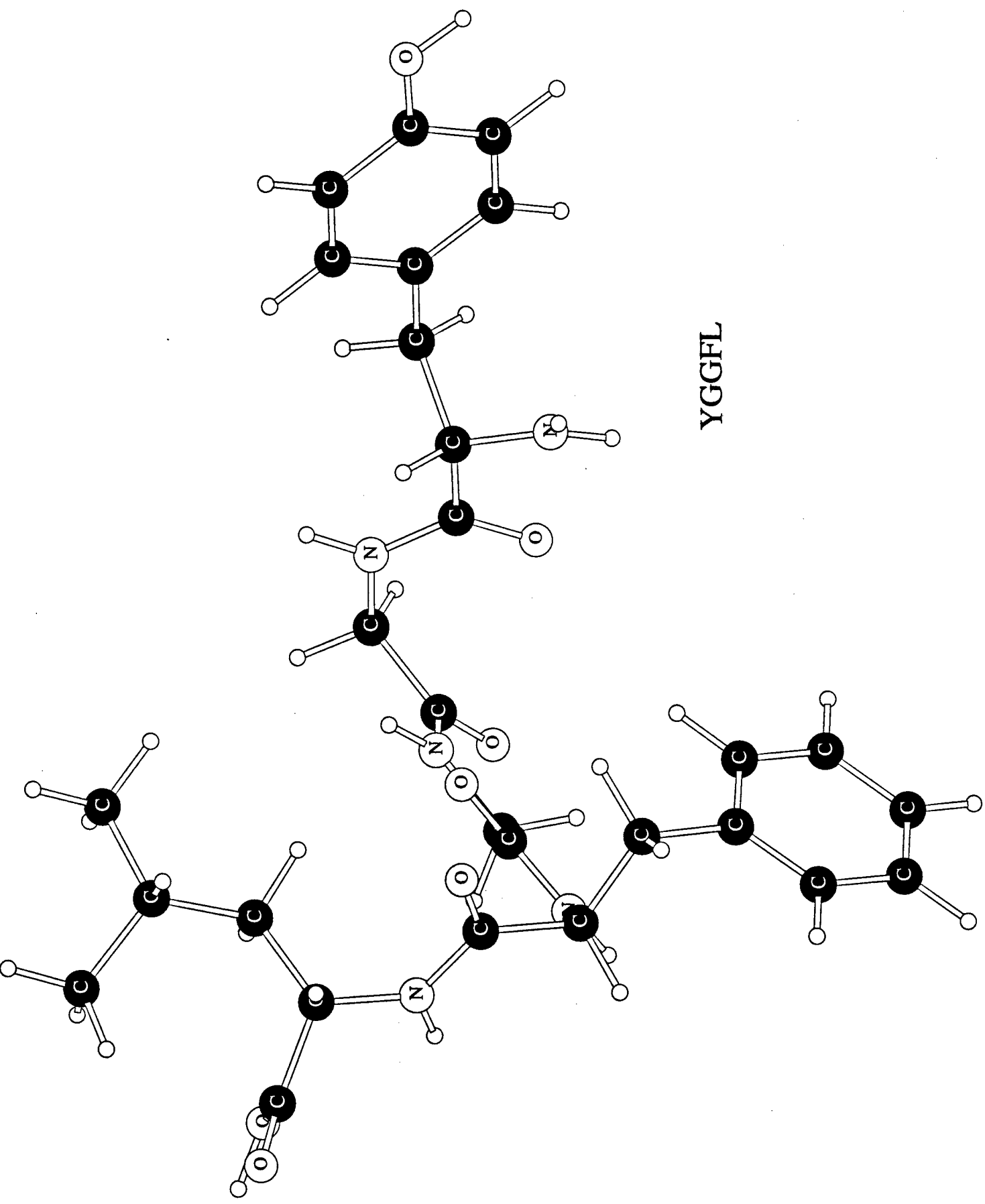


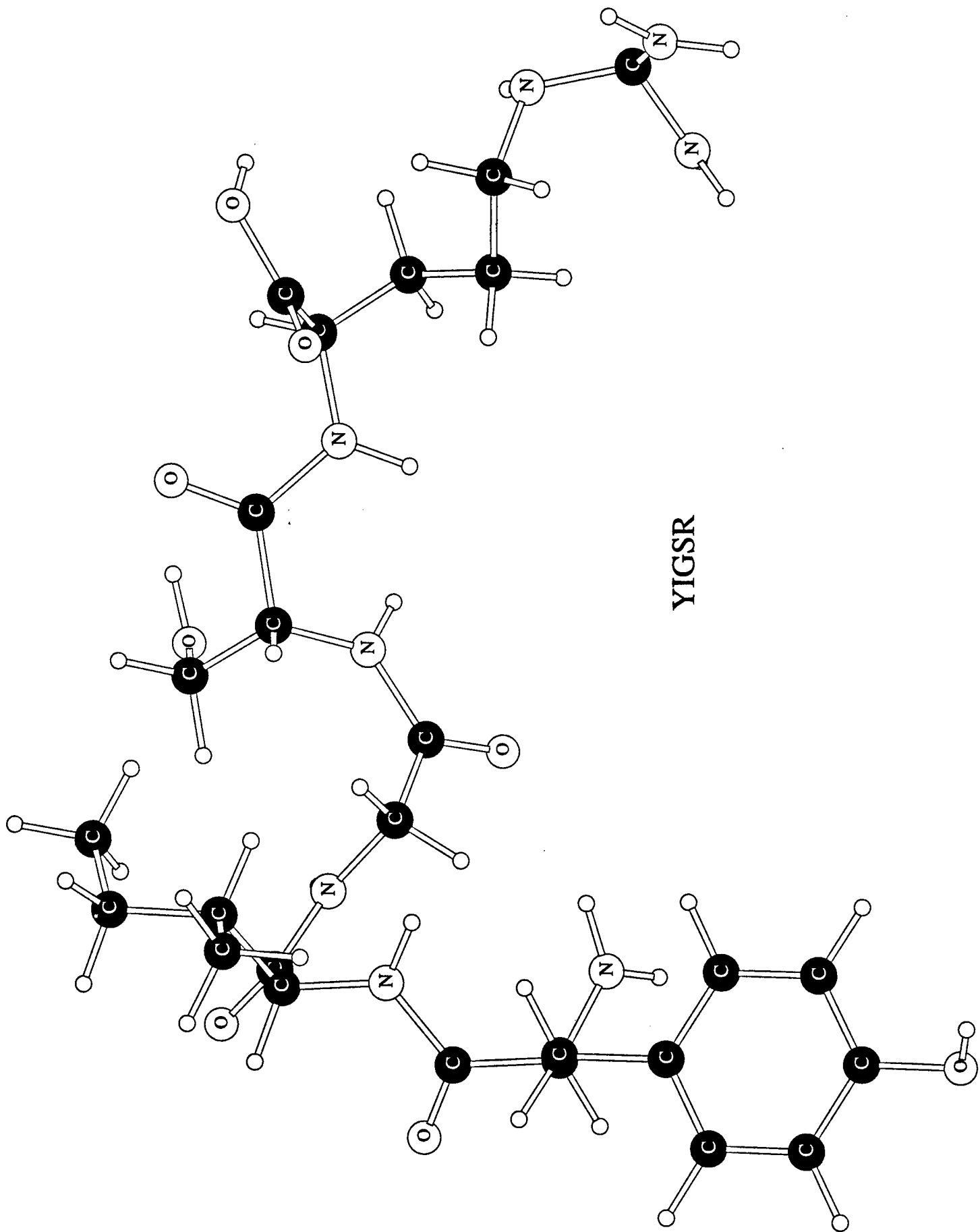
Figure 3



L-tyrosine



YGGFL



YIGSR

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