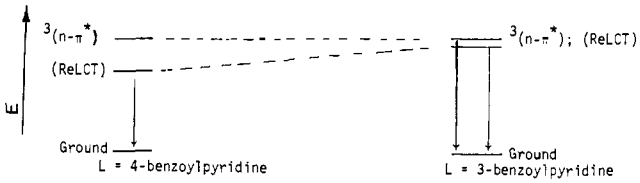
Scheme I. 77 K State Diagram



the 3-benzoylpyridine complexes at 77 K is longer lived than the 298 K emission, as usual, and we assign this component of the emission to the same CT transition as at 298 K. The long-lived, structured emission is attributable to a slightly perturbed n-π^* triplet state of the 3-benzoylpyridine. In every case the total emission quantum yield is high, but the relative amount of CT and n-π^* emission depends on X. For X = Cl and Br the relative yields are roughly equal, whereas for X = I the CT emission is much more prominent. It is noteworthy that the CT state is slightly lower for X = I than for X = Cl and Br from the emission data at 298 or 77 K for both 3- and 4-benzoylpyridine complexes (Table I).

The state orderings for the 3- and 4-benzoylpyridine complexes are shown in Scheme I. The facts that the CT state is at lower energy and that we observe no long-lived emission from the 4-benzoylpyridine under conditions used for the 3-benzoylpyridine complexes are consistent. For the 3-benzoylpyridine complexes it is apparent that the lowest Re L CT and n-π^* states are not thermally equilibrated at 77 K. Non-interconvertibility of the two emissive states is likely a consequence of significant differences in their geometry. Excitation spectra for the n-π^* and the Re L CT emission of the 3-benzoylpyridine complexes show that upper states relax to both of the emitting states; i.e., the relative efficiency of the n-π^* and the Re L CT emissions is independent of the excitation wavelength and the corrected excitation spectrum strongly parallels the absorption spectrum of the complex. The relatively weak n-π^* absorption associated with the diaryl ketone is just barely perceptible on the side of the Re $\rightarrow$ L CT absorption in the 3-benzoylpyridine complex, and likewise is not a well-defined feature in the excitation spectrum of the complex. The excitation spectrum of the free ligand is beautifully structured in the ~350-nm region, under the same measurement conditions.

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Importance of Apolar Binding in Complex Formation of Cyclodextrins with Adamantanecarboxylate

Sir:

We wish to report here the importance of apolar binding in complex formation of cyclodextrins (CDs). CDs have served as enzyme models for many years, mainly because they form complexes with substrates prior to reaction; this is consistent with enzymatic reactions.[1,2] Furthermore, complex formation of CDs with guest compounds such as drugs, insecticides, etc., causes new physicochemical features, leading to practical usages.[2] Thus, information on the binding forces of complex formation is obviously very important. The binding forces between CDs and guest compounds, however, are still the subject of controversy involving four proposals: (1) van der Waals interactions;[3,4] (2) hydrogen bonding;[5] (3) release of high energy water molecules from the cavity of CD;[3,6] and (4) release of strain energy in the macromolecular ring of CD.[7] Several studies were made to evaluate the contribution of the four kinds of binding forces.[4b,8] However, apolar binding, which is operative in the formation of enzyme-substrate complexes, was regarded as unimportant in the formation of CD-guest complexes, since complexation is usually associated with a favorable enthalpy change and an unfavorable (or slightly favorable) entropy change.[2,6] Usually apolar binding is characterized by a very favorable entropy change.[9] In this paper, we evaluate the role of apolar binding in complexation of CDs with guest compounds.

Complexation of 1-adamantanecarboxylate (**1**) with α-cyclodextrin (α-CD) is a good system to examine the contribution of apolar binding in complexation. A molecular model study in a previous paper[3] showed that **1** is too bulky to be included in the cavity of α-CD; rather **1** sits *on top* of the cavity. Thus, factors 1-4 can be minimized in complexation of **1** with α-CD. In the present paper, the enthalpy change (ΔH) and the entropy change (ΔS) for complexation of **1** with α-CD are determined. For comparison, the values of ΔH and ΔS for complexation of **1** with β-cyclodextrin (β-CD), which has a cavity large enough to almost completely accommodate **1** *inside*, are also measured.

The values of ΔH and ΔS were evaluated from the dissociation constants of the CD-**1** complexes (K_d) at different temperatures. K_d's were determined by competitive inhibition of the CD-accelerated cleavage of *m*-nitrophenyl acetate by **1**. As shown in a literature,[3] the Y intercept in the plot of [**1**] vs. $(k_2 - k_{obsd})/(k_{obsd} - k_{un})$, keeping [CD] constant, gave K_d.[10] Here, k_2 and k_{un} are the rate constants of the CD-*m*-nitrophenyl acetate complex and that of the ester in the absence of CD, both of which can be determined in the absence of **1**.

Table I shows K_d's of CD-**1** complexes at different temperatures, and the enthalpy and entropy changes for complexation of CDs with **1**. Experimental errors in K_d's (and in ΔH and ΔS) were estimated by consideration of the errors in k_2's as well as the deviations in the plots of [**1**] vs. $(k_2 - k_{obsd})/(k_{obsd} - k_{un})$ (see note 11). The errors in Table I are maximal errors.

Complexation of **1** with α-CD exhibited a quite favorable ΔS but only a small favorable ΔH; this is in contrast to a large favorable ΔH reported for many inclusion complexes of CDs.[2,6] The stabilization energy due to a favorable ΔS is >70% of the total stabilization energy, whereas a (favorable) ΔH showed <30% contribution. The large favorable ΔS can be attributed to a transfer of **1** from aqueous medium to more apolar medium such as the cavity of CD. This transfer requires breakdown of structural water molecules around **1**, resulting in a large favorable ΔS and a small unfavorable ΔH. This is obviously apolar binding.

On the other hand, complexation of **1** with β-CD, in which **1** can be almost perfectly included in the larger cavity of β-CD,

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Table I. Dissociation Constants (K_d) and Enthalpy and Entropy Changes (ΔH and ΔS) of the Complexation of α-CD and β-CD with **1**[a]

CD	Temp, °C	K_d (10^{-4} M)	ΔH (kcal/mol)	ΔS (eu)
α-CD	15	7.2 ± 0.7		
	25	8.2 ± 0.7		
	32	8.0 ± 0.9	−1.2 ± 0.4	+10 ± 2
	40	10.0 ± 1.0		
	47	9.8 ± 1.1		
	55	11.0 ± 1.2		
β-CD	15	3.4 ± 0.5		
	25	5.2 ± 0.3		
	32	6.0 ± 0.5	−4.7 ± 0.8	−1 ± 3
	40	6.1 ± 0.7		
	47	7.2 ± 0.6		
	55	8.4 ± 0.9		

[a] pH 9.0 Borax buffer, I = 0.2 M (KCl); acetonitrile 1.5%.

showed a quite favorable ΔH and a small unfavorable ΔS.[12]

A comparison of the formation of the β-CD-**1** complex (large favorable ΔH and a small unfavorable ΔS) with the formation of the α-CD-**1** complex (small favorable ΔH and a large favorable ΔS) indicates that the inclusion reaction (change of the guest from sitting on top of the cavity to accommodation within the cavity) is accompanied by a large favorable ΔH and a large unfavorable ΔS. This is consistent with an increase of factors 1-4 on the "deeper" inclusion of the guest, accompanied by a loss of rotational freedom.

The above argument is supported by favorable ΔH (−9.6 and −7.6 kcal/mol) and unfavorable ΔS (−18 and −16 eu) on complexation of benzoic acid both with α-CD and with β-CD.[13] Benzoic acid can be included both in the cavities of α-CD and β-CD.

The stabilization energy due to apolar binding amounts to ~⅔ the total stabilization energy of the β-CD-**1** complex, when the amount of apolar binding in the β-CD-**1** complex is taken as equal to that in the α-CD-**1** complex. However, this value should be larger, since the larger apolar surface of **1** is transferred from the aqueous medium to the apolar cavity of CD on complexation with β-CD than with α-CD. Thus, the stabilization energy by apolar binding of the β-CD-**1** complex can be close to the total stabilization energy of the complex.

The importance of apolar binding in the complexation of CDs is consistent with the stronger binding of "capped" CD than native CD with guests.[14] Capping the primary hydroxyl group side of the cavity of CD with apolar groups increases the apolar nature of the cavity, which enhances apolar binding with guests.

In conclusion, a favorable entropy change due to apolar binding is largely responsible for the stabilization of complexes of CD with apolar guest compounds. However, inclusion complexes are accompanied by several other factors showing favorable enthalpy changes and unfavorable entropy changes. Thus, favorable enthalpy changes are usually observed on complex formation, though apolar binding has a predominant role. The present result reinforces the view that CDs can be good models of enzymatic binding as well as enzymatic reactions.

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(11) The plots of [**1**] vs. $(k_2 - k_{obsd})/(k_{obsd} - k_{un})$ do not deviate much from linearity. Therefore, experimental errors in K_d's derived from the deviations of the plots are less than ±3%. The k_2's used in these plots have experimental errors (around ±3%), since they are determined from the plots of $(k_{obsd} - k_{un})$ vs. $(k_{obsd} - k_{un})/[CD]$ in the absence of **1**.
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Synthesis of Pentaammineruthenium–Histidine Complexes in Ribonuclease A

Sir:

We have been interested in obtaining a protein modification reagent that can be detected by optical spectroscopy and which is sensitive to structural and dynamic properties of proteins. Recent investigations have centered on the synthesis of a stable paramagnetic transition-metal complex between chloropentaammineruthenium(III) dichloride and the imidazole side chains of histidine-containing proteins. Reactions involving the ruthenium reagent and the model compounds imidazole[1] and histidine[2] show that octahedral pentaammine-imidazole and pentaammine-histidine complexes readily form in an aqueous solvent at acidic pH, are extremely stable at neutral to acidic pH values, and contain charge-transfer absorption bands in the near-UV to visible region of the optical spectrum. In this communication, we report the synthesis of pentaammineruthenium(III)-histidine complexes in a protein, bovine pancreatic ribonuclease A (RNase A, EC 2.7.7.16), at neutral pH and room temperature.

Chloropentaammineruthenium dichloride was synthesized from hexaammineruthenium trichloride (Matthey Bishop, Inc.) by a published procedure.[3] Pentaammineruthenium-histidine trichloride was synthesized by the method described by Sundberg and Gupta.[2] The reaction of the ruthenium reagent with RNase A was performed in a manner similar to that for histidine with the following modifications. The protein was made 2-4 × 10^{-4} M in a small volume of solution buffered at pH 7 with 0.1 M tris-Cl and then placed in dialysis tubing. This sample was placed in 10 vol of the buffer containing fresh Zn amalgam and made up to 10^{-2} M in chloropentaammineruthenium dichloride. The latter compound only dissolves upon reduction by the amalgam. Sequestering the protein in this manner prevents it from coming into contact with the Zn

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