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.

Acknowledgment. This work was supported by grants from National Science Foundation (Grant CHE 76-14283), the Hoffmann-La Roche Co., and the Merck Sharp & Dohme Co.

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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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Table I. Pentaammineruthenium Incorporation in RNase A and Effect on Enzymatic Activity

Reaction time, h	$[(NH_3)_5Ru\cdot His]$ $\times 10^4$ M, optical	$[Ru] \times 10^4$ M, AAS	$\frac{[(NH_3)_5Ru\cdot His]}{[RNase\ A]}$ optical	Relative enzymatic activity
0	0	0	0.0	100
6	2.7 ± 0.1	2.8 ± 0.1	0.9	65
12	4.4 ± 0.1	4.3 ± 0.1	1.5	38
18	5.6 ± 0.1	5.6 ± 0.1	2.1	22

amalgam which might lead to undesired side reactions. The reduced ruthenium complex readily diffuses through the tubing and reacts with the protein. The pentaammineruthenium(II) -histidine complexes thus formed are oxidized to the more stable trivalent state of ruthenium by placing the sample, still in the dialysis tubing, in 1 L of buffer and bubbling air through the solution external to the dialysis tubing for 12 h at 298 K. This latter procedure also removes unreacted ruthenium reagent which would interfer with subsequent analyses. Enzymatic activity was assayed by the method of Crook et al.[4]

The optical spectra of ruthenium labeled RNase A and the model compound pentaammineruthenium-histidine trichloride at pH 5.0 are shown in Figure 1A. In the labeled protein, a shoulder at ~300 nm and a broad, undefined absorption in the 400-500-nm region are observed that do not appear in the spectrum of the unmodified RNase A and match similar bands in the model compound (Figure 1A). The effect of changing the pH to 10.0 is shown in Figure 1B. At this pH, where the proton on the imidazole nitrogen has been titrated, an intense, sharp band at 370 nm and a broader, less intense band at ~600 nm are apparent in the labeled protein that again are not found in the unmodified protein. Bands of similar intensity and width are observed at 374 and 610 nm in the model compound (Figure 1B). Based on the similarities apparent in the optical spectra and the expected sensitivity of the spectra to pH, it is concluded that the pentaammineruthenium-histidine complex has been formed in RNase A in a low ionic strength, buffered aqueous solution at neutral pH and room temperature.

No change in the spectrum is apparent after extensive dialysis or storage at 277 K for 2 months at pH 7, indicating that the complex is very stable. This stability is consistent with the extremely slow ligand exchange rate that has been observed with other trivalent ruthenium complexes.[5]

To determine the specificity of this reaction for histidine residues, the level of incorporation was monitored both by optical spectroscopy and by atomic absorption spectroscopy of the ruthenium metal. Analysis by the optical method involves a simple calculation based on the Beer–Lambert law and uses the values of the absorbances at 278 and 303 nm and the values of the extinction coefficients of both the complex and the protein at these wavelengths.[6] The assumption is made that only the protein and any pentaammineruthenium-histidine complexes formed contribute to the optical spectrum. Reaction of the ruthenium reagent with other amino acids obviously will give rise to errors in the ruthenium content calculated by this method. Atomic absorption spectroscopy measures total ruthenium content of the modified protein, irrespective of the site of modification.

In Table I, it can be seen that the concentration of ruthenium in protein samples reacted for varying times is the same within experimental error when measured by these two independent methods. Therefore, for RNase A, it can be concluded that the ruthenium reagent is specific for the imidazole side chains of histidines under the conditions employed.[8,9] It is also evident that an increasing level of ruthenium incorporation results in a corresponding decrease in enzymatic activity. This effect was expected since it is known from previous studies[10] that the

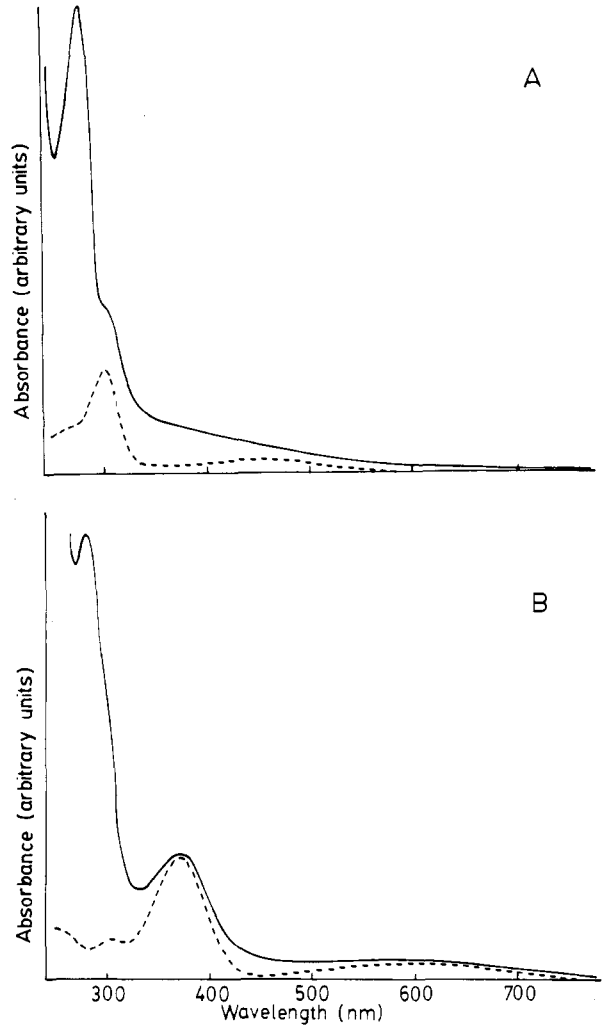

Figure 1. (A) The optical spectrum of pentaammineruthenium-RNase A (—) and pentaammineruthenium-histidine trichloride (- - -) at pH 5.0, 298 K. (B) The optical spectrum of pentaammineruthenium-RNase A (—) and pentaammineruthenium-histidine trichloride (- - -) at pH 10.0, 298 K. The reaction of the chloropentaammineruthenium dichloride with RNase A was run for 18 h as described in the text and resulted in the formation of 2.0 pentaammineruthenium-histidine complexes per protein molecule.

histidine residues at positions 12 and 119 are involved in the catalysis.

Our interest in synthesizing the pentaammineruthenium complex in proteins is based upon the observation that the charge-transfer optical absorption bands of similar aromatic heterocycles are sensitive to the polarity of the environment.[11] Changes in the absorption maxima of these bands would provide a method of following gross structural changes in the vicinity of the complex. We are currently studying the utility of this property of the probe in solving the mechanism(s) involved in reversible protein folding.[12]

There are several other interesting aspects of the modification reaction. Under the conditions used in these experiments, the ruthenium reagent reacts only with the imidazole moiety of histidine residues in RNase. Although other nucleophilic groups in RNase A, such as lysine (amino), aspartic or glutamic acid (carboxyl), and methionine (thioether) must be considered as potential candidates for complex formation, under the conditions used here, only the imidazole moiety of the histidines reacted. Studies on model compounds by Kuehn and Taube[13] indicate that sulfhydryl groups, not present in RNase A, can also form complexes. Although similar specificity for histidines may not be found in other protein systems, the fact that reaction with other ligands would result in

charge-transfer bands at different wavelengths[13,14] means that the type of ligand can be deduced from the optical spectrum. Another aspect of the reagent that should increase its value is its ability to form stable complexes with purine and pyrimidine bases in DNA.[15] The chloropentaammineruthenium reagent may therefore prove to be of general utility in studies of the structural and dynamic properties of both amino and nucleic acid polymers.

Acknowledgments. The authors thank Dr. Henry Taube for helpful discussions, Dr. Michael Clarke for discussions and permission to read his manuscript prior to publication, and the Research Corporation, the National Institutes of Health through Grant GM-23303, and The Pennsylvania State University for financial support.

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Selective Photoaggregation of Metal Atoms to Small Bimetallic Clusters of Known Size. The Chromium-Molybdenum System Cr_nMo_m

Sir:

Cryophotoclustering of metal atoms,[1] a new technique whereby matrix entrapped transition metal atomic species are photomobilized and photoaggregated at low temperatures, shows great promise for achieving cluster size distributions not hitherto accessible by conventional metal deposition-bulk thermal annealing matrix procedures.[2] By selectively exciting metal atomic species isolated in low temperature weakly interacting solids, at energies corresponding to their atomic resonance transitions, one can observe (for example, by optical spectroscopy) a highly controlled photoclustering phenomenon, whereby metal atom absorptions gradually decay and metal cluster absorptions concomitantly grow in.[1]

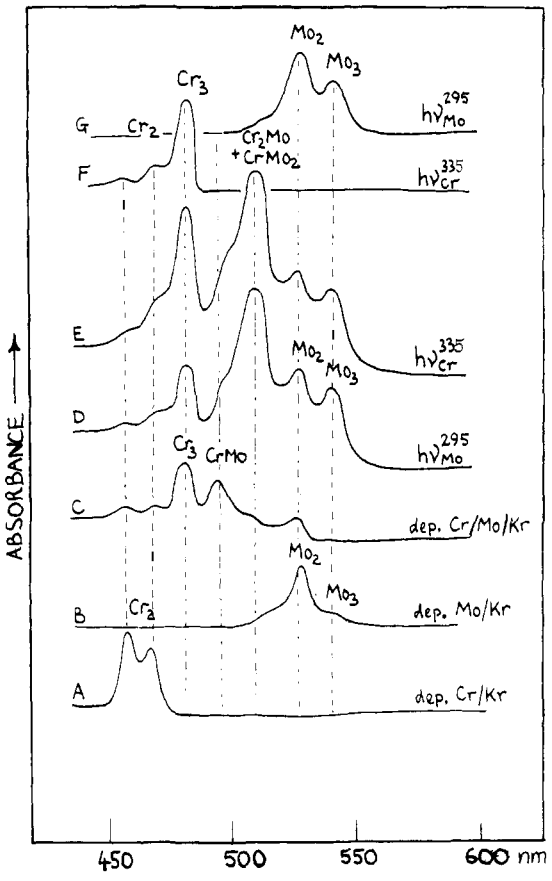

Figure 1. The low energy optical spectra of (A) Cr/Kr = $1/10^3$, (B) Mo/Kr = $1/10^4$, and (C) Cr/Mo/Kr = $1/1/10^3$ mixtures all deposited at 12K showing the characteristic absorptions of Cr_2, Cr_3, CrMo, Cr_2Mo, $CrMo_2$, Mo_2, and Mo_3; (D) the result of 10 min $h\nu_{Mo}^{295}$ photolysis of sample C; (E) the result of 5 min $h\nu_{Cr}^{335}$ photolysis of sample D; (F) the result of 3 min $h\nu_{Cr}^{335}$ photolysis of sample A; (G) the result of 30-min $h\nu_{Mo}^{295}$ photolysis of sample B. (dep. ≡ deposition at 12K.)

The process can be visualized as a reaction of a photomobilized metal atom with the substrate: $M^\dagger + M \rightarrow M_2$; $M^\dagger + M_2 \rightarrow M_3$; $M^\dagger + M_3 \rightarrow M_4$ etc. In all of the above, $M^\dagger$ represents the photomobilized metal atom which is assumed to diffuse in the system. Thus the photomobilization and the diffusion processes would control the rate of growth of metal clusters. In this communication we provide information on the question of selective photomobilization of metal atoms. The mixed metal cluster system Cr_nMo_m[1b,3] (where $m = 3$ to 0 and $n = 0$ to 3) was selected for study because details of the optical spectra of the various metal atom combinations in an argon matrix are known[1b,3] (see Figure 1). (Spectra in a krypton matrix are essentially the same except for a small frequency shift.)

In experiments described here a dilute mixed metal/krypton matrix of atom ratio Cr/Mo/Kr ≃ $1/1/10^4$ and a more concentrated atom matrix of composition Cr/Mo/Kr ≃ $1/1/10^3$ were irradiated with light of 335 nm. Such radiation should selectively photomobilize Cr atoms. Radiation at 295 nm used in a separate experiment should selectively photomobilize Mo atoms. In Kr the $Cr(3d^54s^1) \rightarrow Cr(3d^44s^14p^1)$ band maximum lies at roughly 341 nm while the $Mo(4d^55s^1) \rightarrow Mo(4d^45s^15p')$

Table I. Low Energy Optical Absorptions of Cr_nMo_m in Solid Argon and Krypton Matrices

	Cr_2	CrMo	Mo_2	Cr_3	Cr_2Mo	$CrMo_2$	Mo_3
Ar	460/469	486/489	418/524	477	494	498	534
Kr	457/468	496	510/526	479	496	496	538

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