Ultraviolet Resonance Raman Spectra of Cyclic AMP Receptor Protein: Structural Change Induced by Cyclic AMP Binding and the Conformation of Protein-Bound Cyclic AMP

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Cyclic AMP (cAMP) receptor protein (CRP), also known as catabolite gene activator protein, regulates the transcription of several catabolite-sensitive genes in *Escherichia coli*.[1] CRP itself interacts weakly and nonspecifically with DNA. However, its complex with cAMP recognizes a specific sequence of DNA and binds to the DNA strongly, suggesting a conformational change in CRP upon association with cAMP.[2,3] Recently, Heyduk and Lee have identified 1:1 and 1:2 complexes of CRP and cAMP (to be termed CRP–cAMP and CRP–cAMP$_2$, respectively), and shown that CRP–cAMP is active in the specific DNA binding.[4] More recently, DeGrazia et al. have studied the visible Raman spectra of aqueous CRP and solid CRP–cAMP$_2$ and analyzed the secondary structure of the protein.[5] Raman spectra of the complexes in aqueous solution, however, have not been reported yet because of their low solubility in water. In this study ultraviolet resonance Raman (UVRR) spectroscopy was employed in order to investigate CRP and CRP–cAMP in dilute solutions ($<10^{-4}$ M). It was possible to obtain some pieces of information on the environments and conformation of tryptophan side chains, structural change of the β-roll part of CRP induced by cAMP binding, and the conformation and the hydrogen-bonding (H-bonding) state of cAMP in CRP–cAMP.

Figure 1 shows the 240-nm resonance Raman spectra of dilute solutions of CRP (a) and mixtures of CRP and cAMP that contain the CRP–cAMP complex dominantly (b and c). CRP is a dimer of identical subunits, and each subunit consists of 209 amino acid residues including six tyrosines (Tyr), two tryptophans (Trp), and six prolines (Pro).[8] The spectrum of aqueous CRP is dominated by the resonance-enhanced bands of the main chain amide III mode, side chain modes of Tyr and Trp, and the imide II mode of X-Pro bonds (Figure 1a). For CRP–cAMP, three adenine ring modes are clearly observed in addition to the protein bands (Figure 1b, c).

The intensity ratio of Trp bands around 1360 and 1340 cm^{-1} is sensitive to the hydrophobicity of microenvironments of Trp side chains.[11] In figure 1a, the intensity ratio $I(1361)/I(1341)$ is practically the same as that of aqueous amino acid Trp, indicating that both Trp side chains are in hydrophilic environments, possibly at the surface of CRP as found in the crystal structure of CRP–cAMP$_2$.[12] The frequency of another Trp band around 1550 cm^{-1} (W3) is known to correlate with the absolute value of the torsion angle $\chi^{2,1}$ around the C_β–C_3 linkage.[13] The peak frequency 1553 cm^{-1} in the spectra of CRP and CRP–cAMP indicates a mean $|\chi^{2,1}|$ value of 100°. The W3 frequency differs from that (1547 cm^{-1}) in the visible Raman spectrum at a high concentration,[5] and the $|\chi^{2,1}|$ value is inconsistent with the crystal data of CRP–cAMP$_2$ (63° and 102° for Trp-13 and Trp-85, respectively, in subunit A, and 79° and 87° in subunit B).[12] The difference in $|\chi^{2,1}|$ may be ascribed to differences in intermolecular interactions. Since a calorimetric study has indicated oligomer formation from CRP dimers,[14] it is likely that, in solution at high concentrations or in crystals, intermolecular interactions between dimers alter the $\chi^{2,1}$ angles of Trp side chains on the protein surface.

The 1239-cm^{-1} band in Figure 1a, which disappears in D_2O solution, is assigned to the amide III vibration of the β-sheeted part of the protein. Although the visible Raman study on aqueous CRP has shown a conformational distribution of 44% α-helix, 28% β-sheet, 18% turn, and 10% undefined structure,[5] any bands assignable to the α-helical amide III are not identified in Figure 1a, presumably due to its much smaller Raman cross section than that of the β-sheet.[15] Upon binding of a cAMP molecule, the

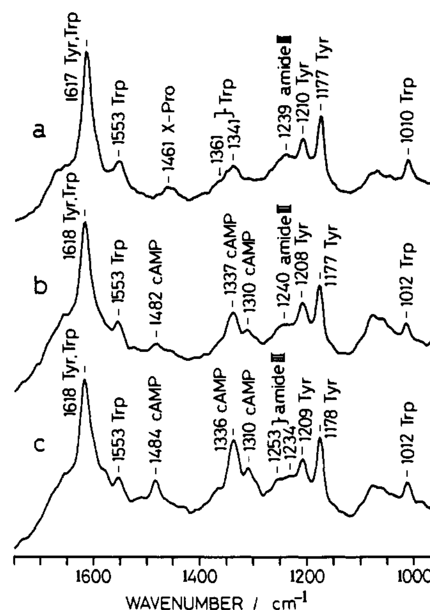

Figure 1. UVRR spectra of 45 μM CRP (a) and mixtures of 40 μM CRP with 52 μM cAMP (b) and 37 μM CRP with 110 μM cAMP (c) dissolved in 300 mM KCl + 50 mM potassium phosphate (pH 6.8). The sample solution in a cylindrical quartz cell was rotated at 3000 rpm; 240-nm excitation (1.0 mW) from an H_2-Raman-shifted pulsed (30 Hz) Nd:YAG laser was employed (the beam spot size at sample, ca. 0.1 × 0.2 mm). Under the same experimental conditions, Raman intensity saturation for aqueous amino acid tryptophan (1 mM) was less than 10%, being consistent with the data on cytochrome *c*.[6] The UVRR apparatus has been described elsewhere.[7] CRP was purified from *E. coli* strain pHA7/PP47[8] by the procedure of Eilen et al.[2] The concentration of CRP dimer was determined by using the extinction coefficient at 278 nm, $\epsilon_{278} = 4.1 \times 10^4$ M^{-1} cm^{-1}.[9] According to the binding constants,[10] $K_1 = 9.4 \times 10^4$ M^{-1} and $K_2 = 0.7 \times 10^4$ M^{-1}, 60% of CRP forms the CRP–cAMP complex, 9% forms CRP–cAMP$_2$, and 31% is free in spectrum b and CRP–cAMP:CRP–cAMP$_2$:CRP = 62:28:10 in spectrum c.

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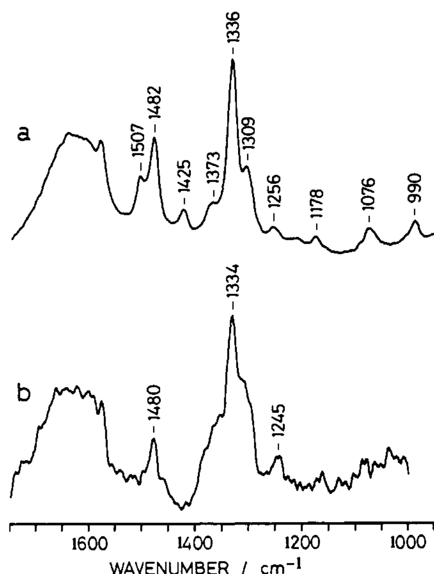

Figure 2. UVRR spectra of 55 μM cAMP (a) and a mixture of 40 μM cAMP and 65 μM CRP (b) with 266-nm excitation (1.5 mW). The binding constants[10] cited in the caption to Figure 1 predict that 70% of cAMP molecules are bound to CRP in the 1:1 complex form and 8% in CRP–cAMP$_2$, and 21% are free in spectrum b.

scattering in the amide III region clearly becomes weak and broad, indicating partial structural change in the β-sheet part of CRP–cAMP (a cAMP band around 1250 cm^{-1} is negligible in parts b and c of Figure 1). Change in the band shape of amide III on going from Figure 1b to Figure 1c is a result of increase and decrease in the content of CRP–cAMP$_2$ and free CRP, respectively, and suggests that the amide III frequency of CRP–cAMP$_2$ is also different from that of free CRP. According to the X-ray analysis of CRP–cAMP$_2$, each of the cAMP molecules is buried in the interior of the β-roll of each subunit.[12] Possibly, in solution also, the cAMP molecule is trapped in the β-roll part of CRP and distorts the β-roll structure of the subunit.

UVRR spectra of aqueous cAMP and CRP-bound cAMP excited at 266 nm are shown in parts a and b of Figure 2, respectively. Since the excitation wavelength is close to an absorption of cAMP at 260 nm, Figure 2b is dominated by the bands of the adenine ring of trapped cAMP and none of the protein bands are clearly observed. The S/N ratio of the spectrum is rather poor due to overlapping fluorescence from CRP. Certainly, however, the 1256-cm^{-1} band of free cAMP shifts to 1245 cm^{-1} upon complexation with CRP and the same shift is observed in a 253-nm-excited spectrum (not shown). This large downshift is not explained by H-bonding interaction between cAMP and CRP because the frequency is not very sensitive to the H-bonding state of the adenine ring.[16] On the other hand, UVRR bands of adenosine in the 1250–1150-cm^{-1} region show large shifts on ribosyl C(1′)–D substitution (e.g., the 1253-cm^{-1} band shifts to 1248 cm^{-1}), which suggests that adenine vibration modes in this frequency region are coupled with the ribose ring modes and their frequencies are sensitive to the adenine–ribose glycosidic-bond conformation.[17] Indeed, a downshift (−5 cm^{-1}) of the adenine Raman band at 1254 cm^{-1} has been observed[18] in conjunction with the B → Z transition of poly(dA-dT) where the glycosidic-bond conformation of adenosine changes from anti to syn.[19] Hence, the downshifted frequency in Figure 2b suggests a conformational change from anti of free cAMP to syn of CRP-bound cAMP. This result is consistent with those of ^{1}H NMR studies[20,21] in solution but inconsistent with the structure of cAMP in crystalline CRP–cAMP$_2$.[12]

Other differences between parts a and b of Figure 2 are the disappearance of the 1425-cm^{-1} band and the increase of the intensity ratio $I(1480)/I(1507)$ in part b. Thomas and Peticolas have reported that the adenyl 1424-cm^{-1} band is absent in a triple helix poly(dT)·poly(dA)·poly(dT) with Hoogsteen type base pairing, in which the adenine N(7) and C(6)–NH$_2$ are involved in H-bonding.[22] Since this band is not affected by H-bonding at NH$_2$,[16] the disappearance of the 1425-cm^{-1} band must be due to formation of an H-bond at N(7) that is stronger than that with water. On the other hand, the increase of $I(1480)/I(1507)$ indicates strong H-bonding at C(6)–NH$_2$, because the intensity ratio increases with increasing H-bonding strength at C(6)–NH$_2$ when excited at 266 nm.[16] It is concluded that the cAMP molecule in an aqueous CRP–cAMP complex is stabilized, at least partially, by H-bonds at N(7) and C(6)–NH$_2$, as is found in crystalline CRP–cAMP$_2$ by X-ray crystallography.[12]

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Registry No. cAMP, 60-92-4; Trp, 73-22-3.

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Selective Enrichment of the Metal Carbonyl Anion in Reactions between Metal Carbonyl Cations and Anions

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Reactions of cations and anions continue to occupy the attention of organic and organometallic chemists.[1-3] Electron transfer to metal carbonyl cations from anions has been shown to occur through two mechanisms: (1) a single electron transfer that leads to odd-electron complexes and ultimately to bimetallic complexes and (2) a CO^{2+} transfer that generates a new cation and a new anion.[2,3] We have previously suggested that these reactions proceed by nucleophilic attack of the anion on the carbon of a carbonyl of the cation.[3c] We now report new evidence consistent with this scheme.

The reaction of $Re(CO)_6^+$ with $Mn(CO)_5^-$ occurs by a single electron transfer and does not exhibit a CO^{2+} transfer.[4] However,

$$Re(CO)_6^+ + Mn(CO)_5^+ \rightarrow MnRe(CO)_{10} + Mn_2(CO)_{10} + Re_2(CO)_{10} + CO$$

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