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Application of fluorescence energy transfer and polarization to monitor *Escherichia coli* cAMP receptor protein and *lac* promoter interaction

(DNA–protein interaction/thermodynamic linkage)

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ABSTRACT **A fluorescence method was developed to study DNA–protein interactions in solution. A 32-base-pair (bp) DNA fragment of the *lac* promoter containing the primary binding site for *Escherichia coli* cAMP receptor protein (CRP) was chemically synthesized and labeled specifically at the 5′ end with fluorescent probe. Binding of cAMP receptor protein to this fragment can be conveniently followed by measuring changes in polarization of fluorescence of the labeled DNA or by measuring fluorescence energy transfer from protein tryptophan residues to the DNA label. Formation of protein–DNA complex was monitored as a function of cAMP concentration. Various equilibrium constants can be resolved to characterize the binding of cAMP to CRP and the subsequent binding of CRP–cAMP and CRP–$(cAMP)_2$ to DNA. These binding studies showed that the two ligated forms of CRP have significantly different affinities for specific-site DNA. These results show that, in principle, the fluorescence technique can yield thermodynamically valid equilibrium constants under essentially any solution conditions. This technique also has the potential of providing information regarding the structure of protein–DNA complexes.**

Quantitative structural studies on cAMP receptor protein (CRP) in conjunction with ligand-binding studies have shown clearly that CRP from *Escherichia coli* exhibits three conformational states, free CRP and two cAMP-dependent states, which correspond to the CRP–cAMP and CRP–$(cAMP)_2$ complexes (1). The binding properties of these two complexes to the *lac* promoter were investigated by gel-retardation technique, and the results showed that the formation of protein–DNA complex is a complicated function of cAMP concentration. At cAMP concentrations that favor the formation of CRP–cAMP, binding of the protein to DNA is favored. At high concentration of cAMP, which favors the formation of CRP–$(cAMP)_2$, a decrease in protein–DNA complex was seen. These results strongly suggest that the CRP–cAMP and CRP–$(cAMP)_2$ complexes have different affinities for the *lac* promoter (1). These conclusions are not consistent with the report of Takahashi *et al.* (2). These authors concluded that the CRP–cAMP complex exhibits essentially the same affinity for the *lac* promoter as that of the CRP–$(cAMP)_2$ complex.

The differences between the results of these two studies may be attributed to the differences in experimental conditions, as necessitated by the techniques chosen to monitor protein–DNA interaction. The gel-retardation technique (3, 4) dictates that the experiments be conducted at low-salt concentration, and because protein–DNA interactions are highly salt dependent (5), possibly the results of DNA-binding study are not applicable to that of the structure and ligand-binding studies, which are conducted at higher salt concentration (1). To acquire detailed valid thermodynamic data to define the linkages among the interactions of cAMP, CRP, and DNA, we looked for a simple and reliable approach that allows collection of a large amount of accurate data under well controlled solution conditions. To this end, a method using fluorescence energy transfer and polarization has been developed and applied to the *E. coli* CRP system. These results clearly show that the association constant of CRP–cAMP for *lac* promoter is significantly higher than that of CRP–$(cAMP)_2$. They also show that the approach developed is potentially a valuable and versatile tool applicable to any protein–DNA system.

METHODS

Materials. T4 polynucleotide kinase was purchased from Boehringer Mannheim, cystamine and 1-methylimidazol were purchased from Sigma, 7-diethylamino-3-(4′-maleimidylphenyl)-4-methylcoumarin (CPM) was from Molecular Probes; DEAE-Sephacryl (TSK-Gel Toyopearl DEAE-650 M) was from Supelco, and Sep-Pak C_{18} cartridges were purchased from Waters. CRP was purified as described (1). The following absorption coefficients were used: 30,000 $M^{-1}\cdot cm^{-1}$ at 387 nm for CPM (6), 14,650 $M^{-1}\cdot cm^{-1}$ at 259 nm for cAMP (7), and 20,400 $M^{-1}\cdot cm^{-1}$ at 278 nm for CRP monomer (8).

Gel-Shift Assay. The assays were done as described (1) using the 32-base-pair (bp) DNA fragment labeled with ^{32}P by the T4 polynucleotide kinase forward reaction (9).

Labeling of DNA with Fluorescent Probe. DNA used in this work is a 32-bp fragment of the *lac* promoter with the sequence 5′-CGCAATTAATGTGAGTTAGCTCACTCATTAGG-3′. This fragment contains the primary binding site for CRP (10). The underlined sequence highlights the conserved TGTGA motif and its inverted repeat common to all CRP binding sites (11).

Complementary strands of this fragment were synthesized on an Applied Biosystems 380A DNA synthesizer and were purified by denaturing PAGE. The strategy to label the 32-bp fragment of *lac* promoter DNA with CPM at the 5′ end is outlined in Fig. 1. The single-strand polynucleotide was phosphorylated with T4 polynucleotide kinase, as described by Maniatis *et al.* (9). Cystamine was added to the 5′ end of the single-stranded (ss) polynucleotide as described by Chu and Orgel (12), and the product was purified as described by Teare and Wollenzien (13). Typically ≈20 μg of phosphorylated ss DNA in 20 μl of water was mixed with 40 μl of 1 M

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Abbreviations: ss, single-stranded; ds, double-stranded; CRP, cAMP receptor protein; CPM, 7-diethylamino-3-(4′-maleimidylphenyl)-4-methylcoumarin; 32-bp-CPM, 32-bp fragment of *lac* promoter DNA labeled with CPM at the 5′ end.

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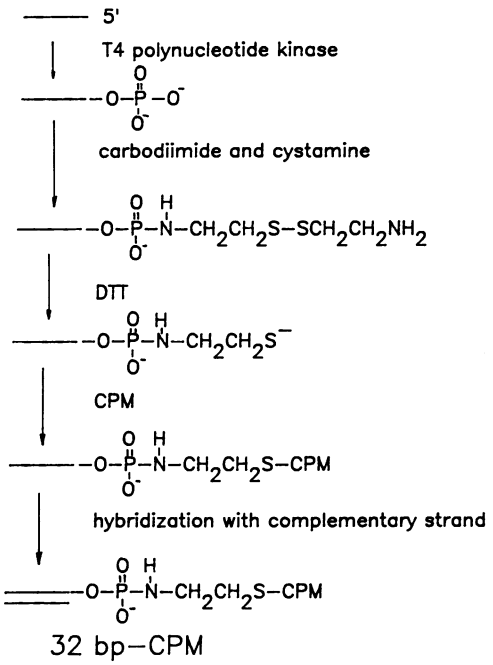

FIG. 1. The general strategy used to attach fluorescence probe to the 5′ end of ds DNA. DTT, dithiothreitol.

1-methylimidazol (pH 7.0), 200 μl of 1 M cystamine (pH 7.0), 160 μl of water, and 11.5 mg of 1-ethyl-3,3,dimethylaminopropylcarbodiimide. The use of carbodiimide from a freshly opened vial significantly improves the yield of cystamine derivative. After 2.5-hr incubation at 50°C, the reaction mixture was diluted to 10 ml with Tris/EDTA (TE) buffer (pH 7.5) and loaded on a DEAE-Sephacryl column (≈300 μl of the gel) equilibrated with TE buffer (pH 7.5). To separate derivatized DNA from reagents that have not reacted, the column was washed consecutively with 10 ml of TE buffer, 5 ml of 50 mM NaCl in TE buffer, and 3 ml of 200 mM NaCl in TE buffer. DNA was eluted with 3 ml of 1 M NaCl in TE buffer. To remove buffer and salt, the eluted DNA was applied directly to a Sep-Pak C_{18} column (Waters), which was then consecutively washed with 10 ml of water, 5 ml of 10% (vol/vol) methanol, and 3 ml of 15% methanol (vol/vol). DNA was eluted with 2 ml of 50% methanol. The cystamine derivative was dried in a Speed Vac vacuum and dissolved in 400 μl of TE buffer. To reduce the disulfide bond, 40 μl of 0.1 M dithiothreitol solution was added. After 1-hr incubation at room temperature, the mixture was applied to a Sep-Pak C_{18} column, which was then washed with 10 ml of water and 10 ml of 10% methanol. DNA was eluted with 2 ml of 50% methanol to a tube containing 40 μl of 1 mM dithiothreitol. To covalently attach CPM to the DNA derivative, 200 μl of 0.8 mg/ml solution of CPM in methanol was added immediately, and the mixture was incubated for 1 hr at room temperature in the dark. One hundred microliters of 0.1 M dithiothreitol were added to quench the reaction. Probe that did not react was removed from the mixture by diluting the sample to 15 ml with TE buffer and loading the solution onto a Sep-Pak C_{18} column. The column was washed with 10 ml of 10% methanol, and DNA was eluted with 2 ml of 50% methanol and subsequently dialyzed overnight against 50 mM Tris/1 mM EDTA, pH 7.8, in the dark. The final product (32-bp fragment of *lac* promoter DNA labeled with CPM at the 5′ end; 32-bp-CPM) contains 0.5–1.0 mol of probe per mol of DNA.

Hybridization of Complementary Strands. Concentration of modified DNA was measured by absorbance at 260 nm corrected for absorbance of the probe at this wavelength. Equimolar amount of phosphorylated complementary strand was added, and the mixture was heated for 2 min at 90°C before cooled down on ice. After 15-min incubation at 70°C, the water bath was turned off and allowed to cool down slowly to room temperature over a few hours. The double-stranded (ds) DNA was stored at −20°C in 50 mM Tris/1 mM EDTA, pH 7.8 buffer.

Fluorescence Measurements. All fluorescence was measured with a SLM 500C spectrofluorometer equipped with a polarization accessory in 50 mM Tris/1 mM EDTA, pH 7.8 buffer, containing appropriate KCl concentration. For experiments in which binding of CRP to DNA was monitored by fluorescence energy transfer, the excitation and emission wavelengths are 295 nm and 480 nm, respectively. When anisotropy measurements were involved, the excitation and emission wavelengths were 380 nm and 480 nm, respectively.

RESULTS

The absorption spectrum of 32-bp-CPM is characterized by peaks at 260 nm and 390 nm (Fig. 2*A*). From this spectrum, the stoichiometry of DNA labeling by CPM can be estimated (in this specific case, it is ≈0.6 mol of CPM per mol of DNA). The purity of this sample of 32-bp-CPM was analyzed by nondenaturing polyacrylamide gel. DNA was detected by silver staining. The result shows two bands corresponding to ds DNA in addition to a faint band of ss DNA contamination, which always amounts to <5% in the different preparations of 32-bp-CPM used. The two bands of ds DNA correspond to the unlabeled 32-bp DNA and the 32-bp-CPM DNA. The ratio of intensities of these bands provides another estimate of labeling efficiency. Fluorescence spectra (Fig. 2*B*) show excitation and emission maxima at 392 nm and 478 nm, respectively. These values are close to those reported for covalent thiol derivatives of CPM (14).

Because CPM and tryptophan form a good donor–acceptor pair (14), an attempt was made to monitor DNA–protein interaction by measuring fluorescence energy transfer between the CPM residue at the 5′ end of 32-bp-CPM and

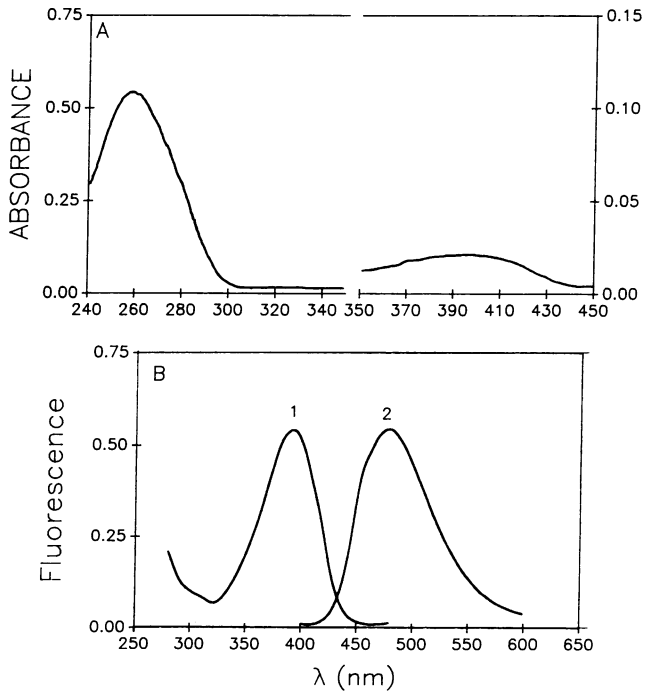

FIG. 2. (*A*) Absorption spectrum of 1.33 μM of 32-bp-CPM in 50 mM Tris/1 mM EDTA, pH 7.8. (*B*) Corrected fluorescence excitation (peak 1) and emission (peak 2) spectra of 46 nM of 32-bp-CPM in 50 mM Tris/25 mM KCl/1 mM EDTA, pH 7.8.

tryptophan residues of CRP. Fig. 3 shows the emission spectra of different 32-bp-CPM samples excited at 295 nm. The 32-bp-CPM, when excited at 295 nm shows some fluorescence intensity at 480 nm. Upon adding CRP alone, only a small increase in fluorescence intensity occurs—most likely due to some nonspecific binding under the experimental conditions. To induce formation of the specific DNA–CRP complex, 200 μM of cAMP was added to the cuvette. This addition resulted in ≈15% increase of fluorescence intensity at 480 nm, an increase that could be due to a change in the local environment of the probe or to an energy transfer from tryptophan to the probe. The former possibility was tested by monitoring probe emission (at 480 nm) with and without cAMP and/or CRP when the excitation wavelength was set for the probe only (at 380 nm). The results showed that under all experimental conditions tested, the presence of cAMP or CRP does not perturb fluorescence intensity of the probe. Hence, we concluded that the increase of fluorescence at 480 nm (with excitation at 295 nm) can be ascribed to fluorescence energy transfer between tryptophan residues of CRP and CPM of DNA. Fig. 3 also shows that the quantum yield of donors (tryptophan) increases upon CRP–DNA complex formation, an observation that has been reported (15). Besides monitoring DNA–protein interaction by energy transfer, preliminary measurements showed that the anisotropy of polarization of 32-bp-CPM in 25 mM KCl increases from 0.168 ± 0.0018 to 0.198 ± 0.0010 upon formation of the specific CRP–DNA complex. Anisotropy values for both free DNA and CRP–DNA complex depend slightly on salt concentration. From these results, we concluded that both fluorescence energy transfer and polarization can be used to monitor CRP binding to DNA.

An attempt was then made to measure binding under stoichiometric conditions by using both fluorescence energy transfer and anisotropy measurements. Fig. 4 shows that a linear increase in anisotropy or fluorescence intensity was seen with increased CRP/DNA ratio up till ≈1.0, after which both signals level off. Because both types of signals level off after a CRP/DNA ratio of 1.0, all of the CRP molecules are apparently active in specific binding to DNA. When stoichiometric binding to the 32-bp fragment is measured with gel-shift assay, the same result is obtained—i.e., CRP displays 100% activity. No binding was seen in the absence of cAMP under the conditions of this experiment. These results showed that the CRP samples used in this study are all thermodynamically active in binding to specific-site DNA with a stoichiometry of one CRP per DNA site. Another important conclusion is that the chemical modification of the

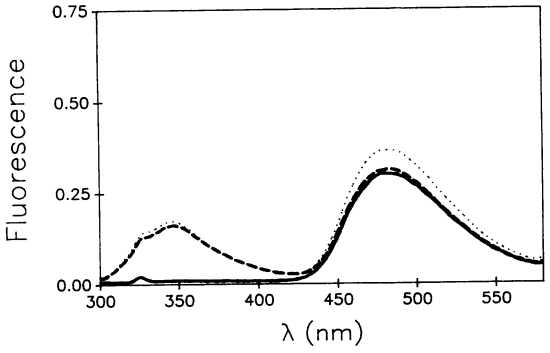

FIG. 3. Detection of fluorescence energy transfer between CRP and CPM of 32-bp-CPM upon formation of the specific protein–DNA complex. Excitation was at 295 nm. All spectra were recorded in 50 mM Tris/25 mM KCl/1 mM EDTA, pH 7.8, buffer. —, 0.66 μM 32-bp-CPM; – –, 0.66 μM 32-bp-CPM plus 0.90 μM CRP; ....., 0.66 μM 32-bp-CPM plus 0.90 μM CRP and 200 μM cAMP.

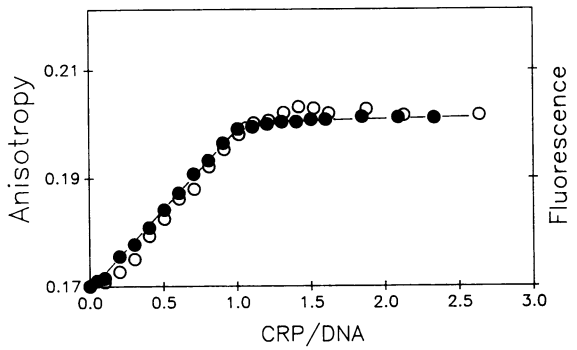

FIG. 4. Stoichiometric titration of 32-bp-CPM with CRP. Titration was performed in 50 mM Tris/25 mM KCl/1 mM EDTA/0.2 mM cAMP, pH 7.8. Concentration of 32-bp-CPM was 98.5 nM. Complex formation was monitored by energy transfer (○) and anisotropy (●). For presentation, fluorescence intensity data were normalized to the results of anisotropy measurements.

32-bp DNA by CPM does not alter the binding stoichiometry of CRP.

Having determined the stoichiometry, we used the same approaches to obtain equilibrium constants for protein–DNA interactions. All subsequent titrations were performed using anisotropy because at low DNA concentrations, anisotropy seems to yield more reproducible signals than fluorescence energy transfer.

Before conducting any extensive binding studies, it is necessary to establish the effect of modification of DNA by CPM on the binding constant of CRP to the DNA. In a control experiment unlabeled 32-bp DNA was used as a competitor in the titration of 32-bp-CPM with CRP. If 32-bp-CPM exhibits a significantly different affinity for CRP, then the apparent binding constants should depend on the presence or absence of 32-bp-DNA. No significant difference between the affinity of labeled and unlabeled DNA was detected; thus, covalently attached CPM alters neither the stoichiometry nor the binding constant of CRP to the 32-bp DNA. A detailed study on CRP–*lac* promoter interaction was therefore initiated.

Typical binding isotherms determined at different cAMP concentrations are shown in Fig. 5. The binding of CRP to the 32-bp-CPM fragment evidently depends on cAMP concentrations. At higher cAMP concentration, the binding isotherm is shifted to the left, implying a stronger interaction between CRP and DNA. Under experimental conditions, the concentration of complexes formed is significant as com-

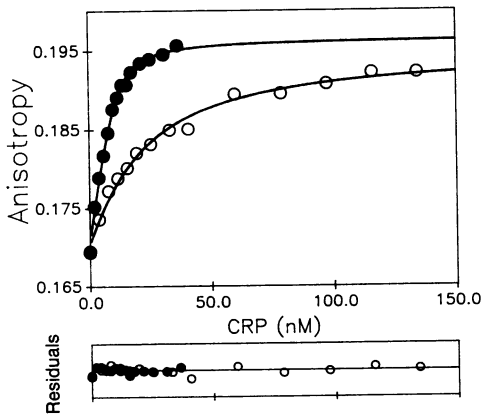

FIG. 5. Examples of isotherms of binding of CRP to 32-bp-CPM. Titration was done in 50 mM Tris/100 mM KCl/1 mM EDTA, pH 7.8. Concentration of 32-bp-CPM was 11.1 nM. ○, 0.5 μM cAMP; ●, 500 μM cAMP. Solid lines represent the best fit of the data to Eq. 3.

pared with total protein concentration—a fact that needs to be accounted for during data analysis. For a simple reaction,

$$P + DNA \overset{K}{\rightleftarrows} P\text{–}DNA, \quad [1]$$

the association constant K is expressed as follows:

$$K = [P\text{–}DNA]/([P]_T - [P\text{–}DNA])([DNA]_T - [P\text{–}DNA]), \quad [2]$$

where P and P–DNA are free protein and protein–DNA complex, respectively; $[P]_T$ and $[DNA]_T$ are the total concentrations of protein and DNA, respectively. Eq. 2 allows solution for [P–DNA]. Because each DNA species may exhibit a specific value of anisotropy,

$$A = A_{DNA_F} + (A_{P\text{–}DNA} - A_{DNA_F}) \left\{ \frac{K[DNA]_T + K[P]_T + 1 - \sqrt{(K[DNA]_T + K[P]_T + 1)^2 - 4K^2[DNA]_T[P]_T}}{2K[DNA]_T} \right\}, \quad [3]$$

where A is the measured value of anisotropy; A_{DNA_F} and $A_{P\text{–}DNA}$ are specific values of anisotropy associated with free DNA and P–DNA complex, respectively. Thus, the value of K can be obtained by fitting the experimental data to the equation by using nonlinear regression analysis.

For CRP, the system is more complicated than that expressed by Eq. 1. Nevertheless, the data can be analyzed in an analogous manner. Binding of CRP to DNA in the presence of cAMP can be described as follows.

$$\begin{array}{ccccc} P + cAMP & \overset{K_1}{\rightleftarrows} & P\text{–}cAMP + cAMP & \overset{K_2}{\rightleftarrows} & P\text{–}(cAMP)_2 \\ & & + & & + \\ & & DNA & & DNA \\ & & K_3 \uparrow\downarrow & & K_4 \uparrow\downarrow \\ cAMP + DNA\text{–}P\text{–}cAMP & & \overset{K_5}{\rightleftarrows} & & DNA\text{–}P\text{–}(cAMP)_2 \end{array}$$

Scheme I

K_1 and K_2 are equilibrium constants for CRP–cAMP and CRP–$(cAMP)_2$ formation, respectively. K_3 and K_4 are equilibrium constants for the binding of CRP–cAMP and CRP–$(cAMP)_2$ to DNA and $K_5 = K_2 K_4/K_3$. Within the concentration ranges of DNA and protein used, no nonspecific binding of CRP to DNA was assumed. This assumption is valid because without cAMP, no CRP binding to DNA was seen even at micromolar concentrations of CRP.

Data for CRP binding to DNA can be analyzed by Eq. 3 to yield apparent association constant (K_{app}) which, in accordance to Scheme I, can be expressed as follows:

$$K_{app} = \frac{(K_1K_3[cAMP] + K_1K_2K_4[cAMP]^2)}{(1 + K_1[cAMP] + K_1K_2[cAMP]^2)}. \quad [4]$$

The solid lines in Fig. 5 represent the best fit of the data to Eq. 3. A series of such titration at different cAMP concentrations provides information on the dependence of K_{app} on cAMP concentration, as shown in Fig. 6. At higher concentrations of cAMP, the value of K_{app} actually decreases. These results were fitted to Eq. 4. From this fit, values for the association constants for the binding of CRP–cAMP and CRP–$(cAMP)_2$ to DNA complexes were obtained. K_3 and K_4 assume values of $(8.4 \pm 1.2) \times 10^8\ M^{-1}$ and $< 10^7\ M^{-1}$, respectively. Only the upper limit of K_4 is reported because any value $<10^7\ M^{-1}$ does not lead to any changes in values for other Ks and summation of error. The upper limit of K_4 is possibly underestimated—e.g., at 200 μM cAMP, K_{app} decreases by 40% in 10 mM phosphate, implicating the presence of a nonspecific effect of high concentration of phosphate-containing components on CRP binding to DNA. Nevertheless, these values imply that in comparison with the singly ligated CRP, CRP–$(cAMP)_2$ essentially has much lower affinity for specific DNA. This result corresponds well with our previous report that CRP–cAMP and CRP–$(cAMP)_2$ differ significantly in their conformation and affinity to specific DNA (1).

The fitting also yielded values of $(2.8 \pm 1.8) \times 10^5\ M^{-1}$ and $(8.6 \pm 5) \times 10^2\ M^{-1}$ for K_1 and K_2, respectively. These results compare favorably with values of $0.92 \times 10^5\ M^{-1}$ and $8.5 \times 10^2\ M^{-1}$ determined by direct measurements of cAMP binding (1). However, the significant amount of error in estimating K_2 in this study renders the comparison between values of K_2 rather questionable. Nevertheless, at least the same order of magnitude has been derived by two completely different experiments.

DISCUSSION

Both gel-shift and fluorescence experiments demonstrated clearly that the CRP samples used in this study are 100% active in binding to the 32-bp fragment. This observation is interesting because a number of laboratories have reported that CRP exhibits only fractional activity (ranging from 20 to 100%) in specific-site DNA binding (3, 4, 15–18). Fractional activity has also been observed in this laboratory (≈20%) when stoichiometric titrations were performed using the gel-shift assay with a 203-bp fragment of *lac* promoter. This result raises an intriguing possibility that fractional activity of CRP (and possibly some other DNA-binding proteins) may not necessarily reflect an activity loss by the protein but may be an intrinsic property of the system or the methods used in the measurement.

This fluorescence approach provided information on the equilibrium constants of cAMP binding to CRP and the differential affinities of the CRP–cAMP and CRP–$(cAMP)_2$ species for specific-site DNA. The CRP–$(cAMP)_2$ form exhibits at least a 100-fold decrease in affinity. These observations completely agree with the gel-shift experimental results (1); however, they contrast to the results of Takahashi *et al.*

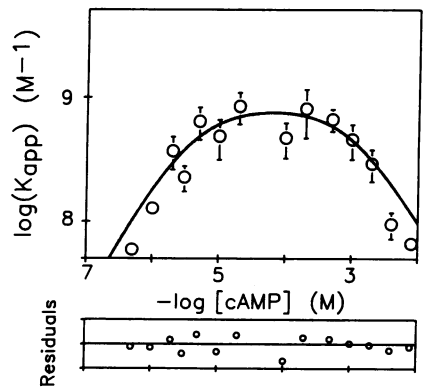

FIG. 6. Dependence of the apparent association constant (K_{app}) for the binding of CRP to 32-bp-CPM on cAMP concentration. Solid line represents the best fit of the data to Eq. 4.

(2); these authors could not detect a difference in the affinities of these two species for specific-site DNA. The apparent discrepancy most likely is the consequence of three simple factors. (*i*) Takahashi *et al.* monitored the cAMP-modulated CRP–DNA interactions by changes in fluorescence intensity of the tryptophan residues in CRP. The observed changes are composites of perturbations due to cAMP and DNA binding; thus, it is difficult to ascribe signal changes to a specific reaction being monitored. For example, the formation of CRP–cAMP does not generate any significant changes in the tryptophan emission signal, but CRP–$(cAMP)_2$ does (1). Thus, to ascribe exclusively the observed change in fluorescence intensity to DNA binding may be invalid. (*ii*) The concentration range of cAMP used by Takahashi *et al.* is limited to 1 mM, whereas in this study, it is extended to 10 mM (see Fig. 6) to ensure the presence of a significant amount of CRP–$(cAMP)_2$. Thus, these authors may have had difficulty detecting the signal reflecting on CRP–$(cAMP)_2$ binding to DNA. (*iii*) The concentration of DNA fragment we used is $\approx 10^{-8}$ M, whereas a 10-fold higher concentration was used by Takahashi *et al.* (2). Because the equilibrium constant of DNA binding is $\approx 10^{8}$ M^{-1}, under the conditions used by Takahashi *et al.*, the CRP–cAMP species would predominate, and the binding of that species to DNA would be the dominant reaction. Hence, we conclude that the apparent discrepancy between the two studies is only the consequence of technical complexity and distribution of the CRP–$(cAMP)_2$ species. The fact that both studies showed an equilibrium constant of $\approx 10^{8}$ M^{-1} for CRP–cAMP binding to DNA is a support of the general method.

Recently, Brown and Crothers (19) reported that CRP dissociates into monomers with a dissociation constant of 3.3×10^{-11} M. The kinetics of dissociation are cAMP and DNA dependent. The lowest concentration of CRP used in this study is ≈60-fold higher than the reported dissociation constant—i.e., CRP exists essentially only as dimers in the experimental conditions used. Consequently, the monomer–dimer equilibrium was not included in the data analysis in this study. The fact that the titration curves of DNA with CRP are apparently not sigmoidal seems to validate the appropriateness of this assumption. Nevertheless, the elegant work by Brown and Crothers (19) emphasizes the importance that in any consideration about the mechanism of CRP action, one must include the quantitative linkages among protein–ligand, protein–protein, and protein–DNA interactions.

The approach we described here seems a valuable addition to the existing methods of studying DNA–protein interactions—e.g., gel-shift assay (3, 4), filter binding (20), quantitative DNase footprinting (21), spectroscopic (e.g., refs. 2 and 22), and transport (e.g., refs. 23–25) techniques. The example of CRP shows that even for relatively complicated systems involving linked equilibria between ligand–protein binding and protein–DNA binding, quantitative information can be obtained by this fluorescence technique. The method is simple and can possibly be applied to any DNA–protein system, both specific and nonspecific. Necessary modification of DNA with a fluorescence probe can be performed in 1 day, and the properties of the probe can be tailored for a specific task. The method is compatible with any solution conditions (pH, temperature, ionic strength, etc.) provided that they do not interfere with fluorescence measurements. Large volumes of data can be conveniently acquired.

The most severe limitation of this approach is that it cannot be applied to systems where interactions are too tight. It can be estimated that association constants $>10^{10}$ M^{-1} may not be measurable by this approach. However, this limitation can be overcome by using higher salt concentrations to weaken the interactions under study. We note that the ability to alter salt concentration at will in this experimental design is definitely an advantage over filter binding or gel-shift assay, which cannot be performed in too high salt concentrations, where interactions are too weak for measurement by these techniques.

Our approach has several interesting prospective applications. It seems well suited for studying the kinetics of protein–DNA interaction because complex formation can be continuously monitored. Furthermore, some low-resolution structural data for protein–DNA complexes can be obtained—e.g., measuring distance by fluorescence energy transfer between the ends of DNA and different sites on the protein to determine distances between interesting sites of the complex.

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