# LIGAND-INDUCED CHANGE IN THE RADIUS OF GYRATION OF cAMP RECEPTOR PROTEIN FROM *ESCHERICHIA COLI*

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## 1. Introduction

In *Escherichia coli* positive control of the expression of catabolite-sensitive operons is mediated by the cAMP-dependent binding of the cyclic AMP receptor protein (CRP) to a promoter-associated site [1,2]. The CRP protomer consists of 2 apparently identical subunits of mol. wt 22 500 [3] and contains 2 domains which are functionally distinct. The amino-proximal domain is involved in cAMP binding and subunit–subunit interaction [4], and the carboxyl-proximal domain binds to DNA [5].

The stimulation of DNA binding elicited by cAMP is presumably mediated by conformational transitions in CRP. The relative position of the two available sulfhydryl groups in the DNA-binding domain is altered in the presence of cAMP. cAMP induces a dithionitrobenzoic acid-mediated, disulfide-bond crosslinking of the subunits within the CRP protomer [6]. Modification of CRP in the presence and absence of cAMP by the bifunctional reagent *o*-phenylenedimaleimide results in crosslinked forms indicative of trapped conformational states [7]. CRP covalently labeled with an environmentally sensitive fluorescent probe shows cAMP-induced alterations in fluorescence indicative of perturbations of the microenvironment of the probe [8,9]. CRP in the absence of cAMP is notably resistant to digestion by a variety of proteolytic enzymes. In the presence of cAMP, limited digestion occurs, resulting in a resistant core [4,10,11] and suggesting a ligand-induced conformational change in CRP.

Small-angle X-ray scattering of macromolecules allows the determination of gross morphologic parameters, such as the radius of gyration, axial ratio, and shape [12], which are sensitive to changes in tertiary structure [13]. We have used small-angle X-ray scattering to investigate the structural properties of CRP in the presence and absence of cAMP.

## 2. Materials and methods

CRP was purified by the procedure in [4] from *E. coli* KLF 41/JC 1553. The homogeneity of the preparation was verified by sodium dodecyl sulfate–polyacrylamide gel electrophoresis. cAMP was purchased from Sigma Chemical Co.

The small-angle X-ray scattering measurements were done with CuK$\alpha$ radiation in a small-angle goniometer with Kratky collimation. A solution flow cell with 4 $\mu$m mica windows and a 1 mm aluminum spacer were used. The entrance and receiving slits were 0.101 and 0.250 mm wide, respectively. The scattered intensities were measured over 1.0–9.0 Kratky $m$ values, in steps of 2. $m$ values correspond to the scattering angles, $2\theta$, by the relation tan $2\theta \doteq m/216$. The value 216 is the effective distance in mm of the detector arm. The intensities were recorded with a proportional counter. A programmed step-scanning device was used for automatic operation. All measurements were made at 20°C.

Scattering data from solutions of various protein concentrations in the presence and absence of cAMP (0.1 mM) and from corresponding buffer solutions were recorded. The difference curve (solvent scatter-

ing minus sample scattering) was smoothed and then desmeared by the procedure in [14].

The radius of gyration, $R$, was calculated from the slope of a plot of $\ln(I)$ versus $m^2$ by the Guinier equation:

$$I(h) = I(0)\frac{e^{-h^2R^2}}{3}$$

where $I(h)$ is the intensity at $h$, which is the scattering vector $4\pi\sin\theta/\lambda$. At low angles $h \simeq 2\pi/\lambda(m/216)$. Shape and axial ratio were deduced from matching a plot of $\log I$ versus $\log hR$ with theoretical curves [15].

## 3. Results and discussion

Guinier plots for three concentrations of CRP in the presence and absence of cAMP are shown in fig.1. The radius of gyration of the protein is 2.9 nm without the ligand and 2.5 nm with the ligand, showing that a ligand-induced conformational change decreases the radius of gyration. Eilen and Krakow [16] have shown that ligand binding increases the resistance of the $\alpha$-core region of CRP to proteolysis by subtilisin and lowers the titratability of the –SH groups by Ellman's reagent. They have interpreted these observations as indicative of a tightening of the molecule, rendering the sites for proteolysis and the –SH groups unavailable for attack by their respective reagents. Wu et al. [9] have reported in their nanosecond depolarization studies that the single rotational correlation time decreases from 55–52 ns in the presence of cAMP. The ligand-induced decrease in the radius of gyration reported here provides a direct physical evidence supporting the suggestion that cAMP binding results in an overall contraction of the CRP molecule.

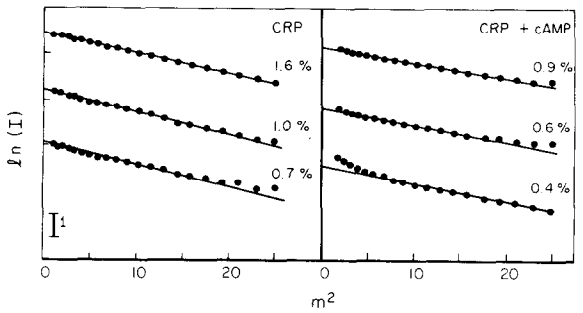


Fig.1. Guinier plots for the cAMP receptor protein in the absence and presence of 0.1 mM cAMP. Protein concentrations (%) in 0.05 M Hepes buffer (pH 8) were as indicated. The plots have been offset vertically for clarity. The radii of gyration given in the text are average values.

The CRP protomer is composed of 2 identical subunits with a single binding site for cAMP [3]. It was of interest to determine the extent of interaction between subunits in the absence of the ligand. From a Soulé-Porod plot ($Im^4$ versus $m^4$) of protein X-ray scattering data it is possible to recognize the presence of intersubunit interference [17]. This suggests that one can discern the difference between molecules which have extensive intersubunit contacts and those in which the intersubunit contacts are minimal.

A clear minimum in the Soulé-Porod plot is predicted for a model of spheres in contact with each other and is experimentally observed with a multisubunit protein like L-asparaginase [17,18]. The $Im^4$ versus $m^4$ plot for CRP (fig.2) shows no such minimum. Therefore the electron density between the two component subunits of the CRP protomer is homogeneously distributed, indicating extensive contacts between them.

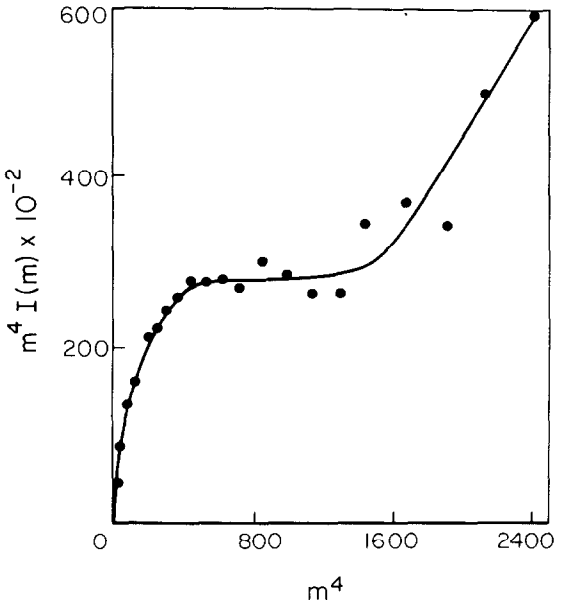


Fig.2. Soulé-Porod plot for CRP in the absence of cAMP. Data are taken from the 1.6% protein curve of fig.1.

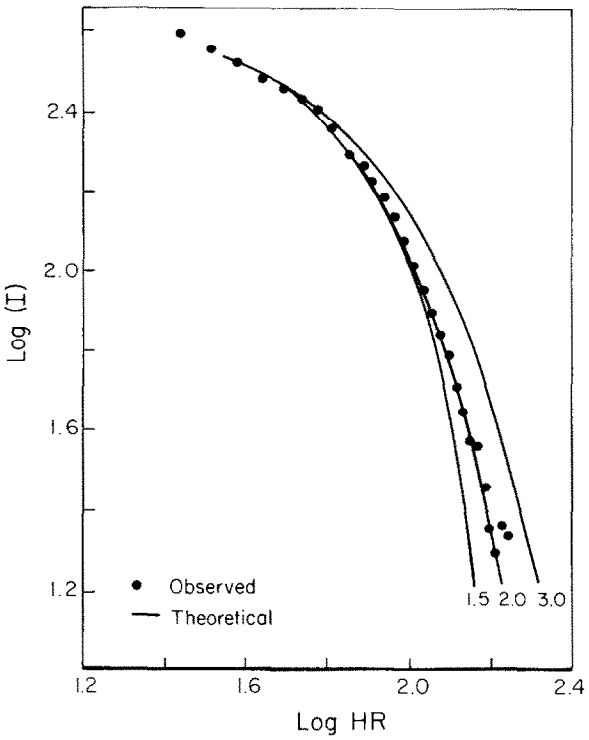


Fig.3. Comparison of the observed log $I$ versus log $hR$ for a 1.6% solution of CRP in the absence of cAMP, at pH 8, in 0.05 M Hepes buffer with theoretical curves for models with axial ratios of 1.5, 2.0 and 3.0.

The overall shape of the CRP molecule in the absence of the ligand was determined by comparing the experimental scattering curve with curves calculated for various models (fig.3). The theoretical model which best matched the experimental data was that of a prolate ellipsoid with an axial ratio of 1:2. We could not determine the shape of the ligand-bound CRP because solutions of $>0.9\%$ resulted in insoluble aggregates, from which reliable data at higher angles (which are required for shape analysis) could not be obtained. It is interesting that a related gene regulatory molecule, the *lac* repressor, a tetramer of mol. wt 150 000, is also a prolate ellipsoid with an elongated shape and a dumbbell-like projected profile [19].

The observed ligand-induced reduction in the radius of gyration may play an important role in organizing the DNA-binding domain of CRP for interaction with its cognitive site. β-Sheet structures of DNA-binding proteins have been implicated in binding to DNA. Several regions of the protein assume β-sheet structures when bound to helical DNA were reported [20]. The cohesive amino- and carboxyl-terminal polypeptides of each *lac* repressor subunit were proposed [21,22] to form antiparallel β-sheet regions which contribute to operator binding. X-ray crystallographic studies [23] showed that the DNA-binding site of human prealbumin is constructed almost entirely from a symmetry-related pair of β-sheets. The ligand-induced contraction of CRP reported here may facilitate the construction of the DNA-binding domain by bringing the carboxyl-proximal region of the two subunits into an antiparallel β-sheet orientation. The migration of readily titratable sulfhydryl groups, located in the carboxyl terminus of each subunit, to close proximity with each other [7,16] and the susceptibility of the DNA-binding region of the ligand-bound CRP to proteolytic digestion suggest that the antiparallel β-sheet structure may be formed as a result of cAMP binding.

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