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Production and Properties of the α Core Derived from the Cyclic Adenosine Monophosphate Receptor Protein of *Escherichia coli*†

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ABSTRACT: Proteolytic digestion of the cyclic adenosine monophosphate (cAMP) receptor protein (CRP) in the presence of cAMP results in the formation of a resistant core molecule. The CRP core retains the dimeric structure and cAMP-binding properties of the native CRP, while cAMP-dependent DNA binding is lost. CRP consists of two identical polypeptides of molecular weight 22 500. The CRP cores formed after proteolytic digestion in the presence of 0.1 mM cAMP differ in molecular weight: subtilisin and trypsin CRP cores, ~12 500; chymotrypsin CRP core, ~13 000; and *Straphylococcus* V8 protease CRP core, ~18 000. In the absence of cAMP, CRP is relatively resistant to these enzymes. The αCRP core produced by subtilisin digestion has been characterized as follows: The sedimentation coefficient of the αCRP core is 2.7 S, consonant with a molecular weight of 25 000, which indicates that it consists of two polypeptides of molecular weight ~12 500. The hydrodynamic properties of CRP and αCRP core appear similar in gel-filtration studies with frictional ratios of 1.17 and 1.23, respectively. Chromatography on the cation-exchange resin, Bio-Rex 70, shows that the αCRP core eluted at a lower KCl concentration than CRP, suggesting that the αCRP core has a lower net positive charge than CRP. Titration of sulfhydryl groups with dithionitrobenzoic acid shows the presence of two accessible and two buried cysteinyl residues in the native CRP protomer. The αCRP core retains only the two buried cysteines which are titrated in the presence of sodium dodecyl sulfate.

Transcription of catabolite-repressible operons involves the cAMP-dependent binding of CRP[1] to a site within the promoter of these operons (Pastan and Adhya, 1976). Proteolysis of the cyclic AMP receptor protein (CRP) in the presence of cAMP produces the α core fragment (αCRP) whose subunit of ~12 500 molecular weight is derived from the native CRP subunit of 22 500 molecular weight (Krakow and Pastan, 1973). While the CRP shows cAMP binding and cAMP-dependent DNA binding at pH 8, the α core does not show this activity at pH 8, although its cAMP binding activity is retained. We have previously shown that cAMP produces a conformational change in the α core, resulting in an increased resistance to proteolysis and denaturation (Eilen and Krakow, 1977a). This event, in the native CRP, has been postulated to function as a conformational signal in effecting the formation of the DNA-binding site in the β region of the protein (Eilen and Krakow, 1977b). We present here a method for the purification of CRP and studies of the proteolytic production and properties of the αCRP core.

Materials and Methods

Materials. All biochemicals were of reagent grade. Subtilisin BPN′, trypsin, chymotrypsin, ovalbumin, and cAMP were products of Sigma Chemical Co. *Staphylococcus aureus* V8 protease was obtained from Miles Laboratories, and calf thymus DNA was from Worthington. Horse heart cytochrome *c* and sperm whale myoglobin were purchased from Schwarz/Mann. Bio Rex 70, Cellex N-1, and $NaDodSO_4$ were obtained from Bio-Rad Laboratories, and Sephacryl S-200 and Blue dextran 2000 were from Pharmacia. Phenylmethanesulfonyl fluoride, 5,5′-dithiobis(2-nitrobenzoic acid), and Brij 58 were products of the Pierce Chemical Co., and acrylamide was from Eastman Chemicals. Bisacrylamide and polyethylenimine (50%) were obtained from the Gallard-Schlessinger Chemical Co. Nitrocellulose filters (0.45-μm pore size) were obtained from Matheson Higgens and GF/C glass-fiber filters from Whatman.

E. coli KLF 41/JC 1553 diploid in the CRP structural gene was generously provided by Dr. Ira Pastan.

α Core Fragment of CRP. The α core protein used in these studies was prepared by subtilisin digestion of CRP in the presence of cAMP followed by chromatography on DNA-cellulose and Bio-Rex 70 (Eilen and Krakow, 1977a).

Protein Determination. Protein was determined by the

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[1] Abbreviations used are: CRP, cyclic AMP receptor protein; αCRP, resistant core formed by subtilisin digestion in the presence of cyclic AMP; cAMP, 3′,5′-cyclic AMP; DTNB, 5,5′-dithiobis(2-nitrobenzoic acid); TNB, thionitrobenzoate; $PhCH_2SO_2F$, phenylmethanesulfonyl fluoride; $NaDodSO_4$, sodium dodecyl sulfate; DTT, dithiothreitol; EDTA, (ethylenedinitrilo)tetraacetic acid; Bistris, 2-[bis(2-hydroxyethyl)amino]-2-(hydroxymethyl)-1,3-propanediol.

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method of Schaffner and Weissmann (1973), except that the amido black stained protein was eluted from nitrocellulose filter disks with 0.6 mL of eluent solution (25 mM NaOH, 0.5 mM EDTA in 50% EtOH), and the absorbance at 630 nm was measured in a 0.5-mL quartz cuvette.

Sulfhydryl Titration with DTNB. Determinations of sulfhydryl groups were carried out by the method of Ellman (1959) using a Beckman Acta III recording spectrophotometer.

Polyacrylamide Gel Electrophoresis. Electrophoresis on 12.5% $NaDodSO_4$–polyacrylamide slab gels was performed by the method of Laemmli (1970) on an apparatus (Studier, 1973) manufactured by the Aquabogue Machine Shop, Aquabogue, N.Y. The gels were stained for 40 min at 60 °C with 0.1% Coomassie blue made up in the destaining solution of 25% isopropyl alcohol, 10% acetic acid.

Sedimentation Velocity Centrifugation. Sucrose density gradient sedimentation velocity studies (Martin and Ames, 1961) were carried out at 4 °C on a Beckman L3-50 ultracentrifuge using an SW-50 rotor. Fractions were collected on an ISCO gradient fractionator.

Stokes Radius Determination. The Stokes radius of αCRP was determined by gel filtration on Sephadex G-75 using the method of Siegel and Monty (1966). The Stokes radius of standard proteins was calculated using the classical equation:

$$f/f_0 = a\Big/\left(\frac{3\bar{v}M}{4\pi N}\right)^{1/3}$$

where M is the molecular weight, a is the Stokes radius, $\bar{v}$ is the partial specific volume, f/f_0 is the frictional ratio, and N is Avogadro's number. The elution data are presented in terms of the parameter K_{av}, which is determined as follows:

$$K_{av} = \frac{V_e - V_0}{V_t - V_0}$$

where V_0 is the void volume or elution volume of Blue dextran (mL), V_t is the gel bed volume (mL), and V_e is the elution volume (mL).

Binding Assays. Assays for the binding of [^{3}H]cAMP and [^{3}H]d(A-T)$_n$ to CRP were carried out as previously described (Krakow and Pastan, 1973).

Lysozyme Assays. Lysozyme was assayed by measuring the rate of decrease in turbidity at 550 nm of a 0.3 mg/mL suspension of *Micrococcus lysodeikticus* cell walls in 10 mM potassium phosphate buffer, pH 7.

Preparation of Chromatographic Materials. Bio-Rex 70 was suspended overnight in an equal volume of 1 M K_2HPO_4 followed by extensive washing with deionized water. The Bio-Rex 70 suspension (50%, v/v) was adjusted to pH 7 with HCl. After 15 min, the supernatant was decanted and the resin resuspended in an equal volume of buffer A.

Denatured DNA–cellulose was prepared by the method of Alberts and Herrick (1971). The preparations contained about 0.5 mg of DNA per mL of packed volume.

Solutions. All buffers were prepared with deionized water and stored at 4 °C. Stock solutions of 1 M potassium phosphate, pH 7, and 0.5 M EDTA, pH 7.6, were used to prepare the following solutions: buffer A, 50 mM potassium phosphate (pH 6.8) and 0.1 mM EDTA; buffer B, 10 mM potassium phosphate (pH 7), 0.1 mM EDTA, and 0.1 M KCl; buffer C, 20 mM potassium phosphate (pH 6.5 or pH 8), 1 mM EDTA, and 0.1 M NaCl. A stock solution of 0.2 M Bistris propane, pH 8, was used to prepare various reaction mixtures.

Purification Procedure for CRP

Lysis and Cell Disruption. Frozen *E. coli* cells (500 g) (KLF 41/JC 1553) were suspended in 1.5 L of 75 mM Tris-OH and warmed to 20 °C with stirring. The suspension was rapidly brought to pH 8 with KOH followed by the addition of 50 mL of 0.5 M EDTA, pH 7.6. Lysozyme was added (300 mg in 50 mL of 20 mM Tris-HCl, pH 8) and the mixture stirred for 20 min. After cooling the lysate to 12 °C, the following additions were made: 50 mL of 1 M Tris-HCl, pH 8, 90 mL of 0.5 M $MgSO_4$, and 50 mL of Brij 58. After stirring for 10 min, the viscous lysate was dispersed in 400-mL batches for 3 min using a Tekmar Super Dispax SD 45 at 7500 rpm. After centrifugation at 12 000 rpm for 20 min in the JA-14 rotor of the Beckman J 21 centrifuge, the supernatant was readjusted to pH 8. All remaining steps were carried out at 4 °C.

Polyethylenimine Titration. Samples from the supernatant (2 mL) were titrated with 5% polyethylenimine (w/v, pH 8) to determine the amount required for the precipitation of nucleic acids and associated proteins without the loss of CRP as assayed by cAMP binding activity. After centrifugation for 10 min at 10 000 rpm, 20-μL samples of the supernatant were assayed for [^{3}H]cAMP binding. In general, up to 0.1 mL of 5% polyethylenimine/mL of supernatant could be added without affecting the cAMP-binding activity remaining in solution. The determined volume of polyethylenimine was then added dropwise to the extract with manual stirring over a period of 20–30 min. After centrifugation at 12 000 rpm for 20 min, the supernatant was decanted and adjusted to pH 7 with 1 M acetic acid.

Bio-Rex 70 Chromatography. The polyethylenimine supernatant was batch loaded by stirring with 300 mL of Bio-Rex 70 suspension for 10 min. The beads were then allowed to settle (20 min) and the supernatant was assayed for cAMP binding. Generally, less than 15% of the activity remained in the supernatant, which was discarded. The Bio-Rex beads were then batch washed four times with 2-L volumes of buffer A or until the absorbance at 280 nm fell below 0.1. The resin was then poured as a slurry into a glass column to form a bed measuring approximately 3 × 25 cm. Bound protein was eluted with a linear salt gradient (1 L total volume) from 0 to 1 M KCl in buffer A. The cAMP-binding activity elutes between 0.25 and 0.32 M KCl. The peak fractions were pooled and concentrated by adding ammonium sulfate to 60% saturation at a pH of 6.8–7.0. After centrifugation at 12 000 rpm for 20 min, the precipitate was redissolved in 5–10 mL of buffer B.

Sephacryl S-200 Chromatography. The concentrated fraction from the Bio-Rex 70 step was loaded onto a 2.5 × 90 cm column of Sephacryl S-200 equilibrated with buffer B. The protein was eluted at 30 mL/h, and the fractions containing CRP were pooled and adjusted to pH 6.5.

DNA–Cellulose Chromatography. The Sephacryl pool was adjusted to a conductivity of 3 mmho and loaded onto a 1.8 × 10 cm column of denatured DNA cellulose previously equilibrated at pH 6.5 with buffer C. The column was washed with 50 mL of buffer C, pH 6.5, followed by the elution of CRP with buffer C at pH 8. The column was stripped with buffer C and 2 M NaCl to remove other proteins that bind DNA but lack cAMP-binding activity. The fractions containing the stripped protein were discarded.

Bio-Rex 70 Concentration. The pooled DNA–cellulose fractions were loaded onto a 0.7 × 14 cm Bio-Rex 70 column equilibrated with buffer A containing 0.1 M NaCl. The protein was eluted in a salt step with buffer A containing 1 M NaCl. The peak fractions were pooled and stored at −20 °C.

The purified CRP, which migrated as a single band on

TABLE I: Purification of CRP.

Stage of purification	Vol (mL)	Protein (mg)	Act. (units)	Sp act. (units/mg)	Yield (%)	A_{280}/A_{260}
Lysate supernatant	1800	41 400	270 000	6.5		0.50
Polyethylenimine supernatant	1300	22 100	163 000	7.4		0.72
Bio-Rex 70, I	200	260	352 000	1354	100	0.93
Sephacryl S-200	225	90	276 400	3070	79	1.12
DNA-cellulose	110	35.5	220 000	6300	63	1.70
Bio-Rex 70, II	6.5	32.5	210 000	6460	60	1.78

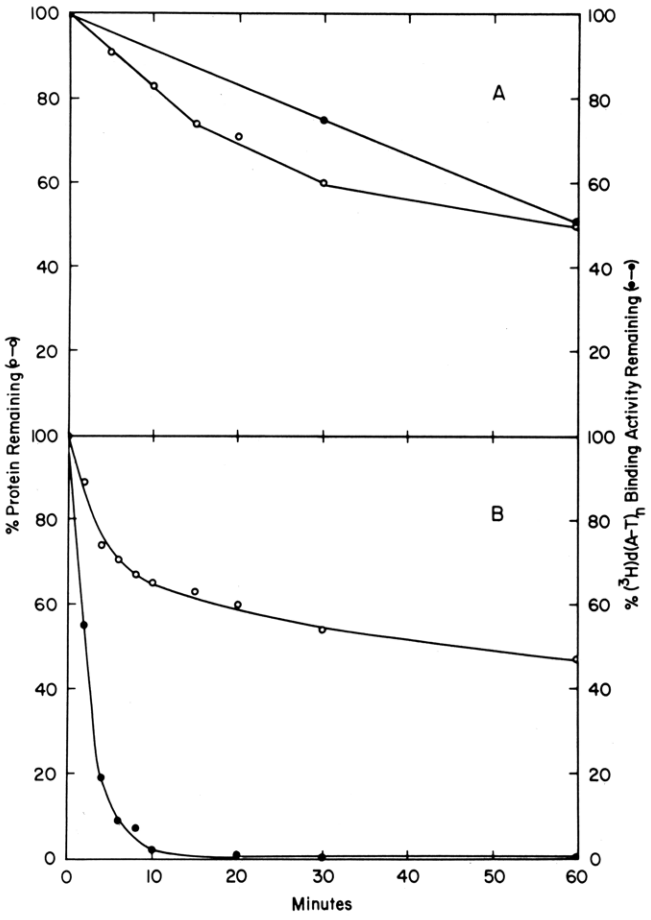

FIGURE 1: Effect of proteolysis on cAMP-dependent d(A-T)$_n$ binding by CRP. Samples containing (final volume 0.3 mL) 10 mM potassium phosphate buffer (pH 7), 1 mM EDTA, 0.1 M KCl, 290 μg of CRP, 10 μg of subtilisin BPN′ (A), and 10^{-4} M cAMP (B) were incubated at 37 °C. At the indicated times, aliquots of 25 μL were removed and incubated 5 min at 37 °C in a mixture containing (final volume 0.1 mL) 40 mM Bistris propane buffer, pH 8, and 0.25 mM $PhCH_2SO_2F$. Samples from the $PhCH_2SO_2F$-containing mixture of 50 μL for protein determination (○), 10 μL for d(A-T)$_n$-binding assays (●), and 5 μL for $NaDodSO_4$-gel electrophoresis were removed and treated as described under Materials and Methods.

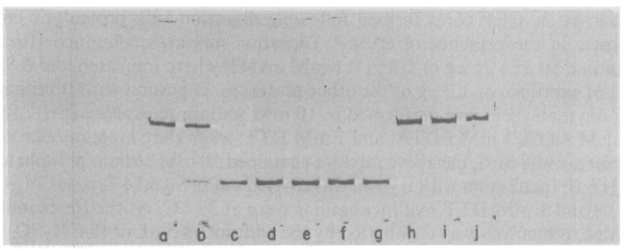

FIGURE 2: $NaDodSO_4$-gel electrophoresis of proteolytically digested CRP. Aliquots of 5 μL from the $PhCH_2SO_2F$-containing mixtures described in the legend to Figure 1 were adjusted to 0.1% $NaDodSO_4$, 10 mM DTT, and 10% sucrose (final volume 30 μL) and incubated for 10 min at 60 °C. The samples were layered on an $NaDodSO_4$-polyacrylamide slab gel followed by electrophoresis as described under Materials and Methods. The samples are as follows: Proteolysis in the presence of 10^{-4} M cAMP: (a) 0, (b) 2, (c) 5, (d) 10, (e) 15, (f) 20, and (g) 30 min. Proteolysis in the absence of cAMP: (h) 0, (i) 15, and (j) 30 min.

$NaDodSO_4$-polyacrylamide gels, had a specific activity of 6460 units/mg (Anderson et al., 1971) with a 60% recovery of cAMP-binding activity (Table I). The procedure can be completed in 5 days.

In preparing for polyethylenimine titrations, small samples (1 mL) should first be titrated with the polycation to ensure that cAMP-binding activity remains in the supernatant. Careful attention to pH adjustment of both the reagent and the lysate is required so that CRP remains dissociated from the DNA. It is not unusual for cAMP-binding activity to appear depressed during the initial stages of the purification. This may be due to residual polyethylenimine, an inhibitory factor or a cyclic nucleotide phosphodiesterase which may be removed by chromatography on Bio-Rex 70. For this reason, yields have been calculated starting with the first column step (Table I). Chromatography on Bio-Rex 70 is more convenient than phosphocellulose (Anderson et al., 1971) when dealing with a large volume, since the Bio-Rex 70 beads can be mixed with the extract and allowed to settle out. Chromatography on DNA-cellulose takes advantage of the cAMP-independent binding of CRP to DNA at pH 6.5 and its low affinity for DNA at pH 8 in the absence of cAMP (Krakow and Pastan, 1973). This effect, which may be due to the interaction of positively charged groups on CRP with the phosphodiester backbone of DNA, may be overcome by raising the pH in order to lower the positive charge on the protein. When required, the protein may be concentrated on a small Bio-Rex 70 column with minimal losses (Table I).

The yield of CRP represents 60% of the activity in the pooled peak of the Bio-Rex I chromatography step (Table I). This would indicate a nearly 1000-fold purification from the dispersed material or a 765-fold purification if the initial specific activity is calculated on the basis of 352 000 total units.

Results

Incubation of CRP with subtilisin in the absence of cAMP did not affect the specific activity of cAMP-dependent d(A-T)$_n$ binding (Figure 1A). Even after 40% of the total protein had been digested, only the native subunit of molecular weight 22 500 appeared on $NaDodSO_4$ gels (Figure 2). In the presence of 10^{-4} M cAMP, however, proteolysis resulted in a sharp drop in cAMP-dependent d(A-T)$_n$ binding with 50% of the activity lost after the digestion of only 13% of the protein (Figure 1B). By 30 min, the CRP subunit was replaced by the αCRP subunit of molecular weight ~12 500 (Figure 2).

Incubation of CRP plus cAMP with trypsin or chymotrypsin also resulted in loss of cAMP-dependent d(A-T)$_n$ binding (Krakow and Pastan, 1973). Shown in Figure 3 are the Na-

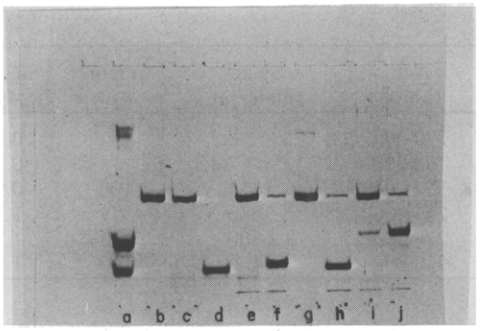

FIGURE 3: CRP cores formed following digestion with proteolytic enzymes in the presence of cAMP. Digestion mixtures contained (final volume 50 μL) 21 μg of CRP, 0.1 mM cAMP where indicated and 0.85 μg of subtilisin or 1.7 μg of the other proteases. Digestion with subtilisin or chymotrypsin was performed in 10 mM sodium phosphate (pH 7.0), 0.1 M NaCl, 1 mM EDTA, and 1 mM DTT; when the *Staph. aureus* V8 protease was used, the above mixture contained 10 mM sodium phosphate, pH 8.0. Incubation with trypsin was carried out in 50 mM Tris-HCl (pH 8.0) and 1 mM DTT. All incubations were at 37 °C. At the times indicated, proteolysis was terminated by the addition of 5 μL of $PhCH_2SO_2F$ to give a concentration of 0.1 mM. The samples were brought to 0.1% $NaDodSO_4$ and 20% sucrose (final volume 200 μL) and heated for 10 min at 60 °C. Aliquots (40 μL) were resolved by $NaDodSO_4$–polyacrylamide gel electrophoresis (12% slab gel): (a) ovalbumin, mol wt 46 400, myoglobin, mol wt 17 000, cytochrome *c*, mol wt 12 600; (b) untreated CRP; (c) subtilisin plus CRP (15 min); (d) subtilisin plus CRP in the presence of cAMP (15 min); (e) chymotrypsin plus CRP (30 min); (f) chymotrypsin plus CRP in the presence of cAMP (30 min); (g) trypsin plus CRP (30 min); (h) trypsin plus CRP in the presence of cAMP (30 min); (i) *Staph. aureus* protease plus CRP (120 min); (j) *Staph. aureus* protease plus CRP in the presence of cAMP (120 min).

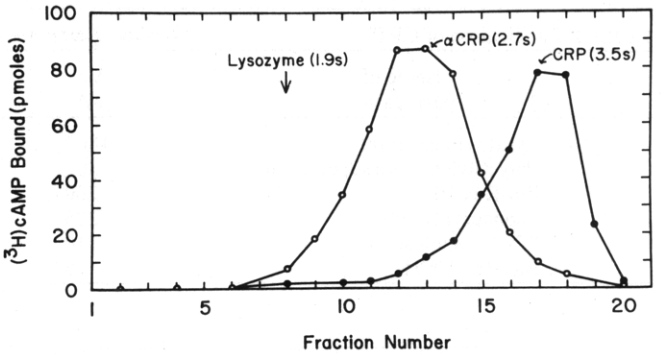

FIGURE 4: Sedimentation profiles of CRP and αCRP. Samples containing 60 μg of CRP (●), 60 μg of αCRP (○), or 100 μg of lysozyme were prepared in 10 mM potassium phosphate buffer, pH 7.5, containing 1 mM DTT and 2% sucrose in a final volume of 0.1 mL. The samples were layered on 5–20% sucrose gradients and centrifuged at 45 000 rpm for 40 h at 4 °C. Assays for cAMP binding and lysozyme were performed as described under Materials and Methods.

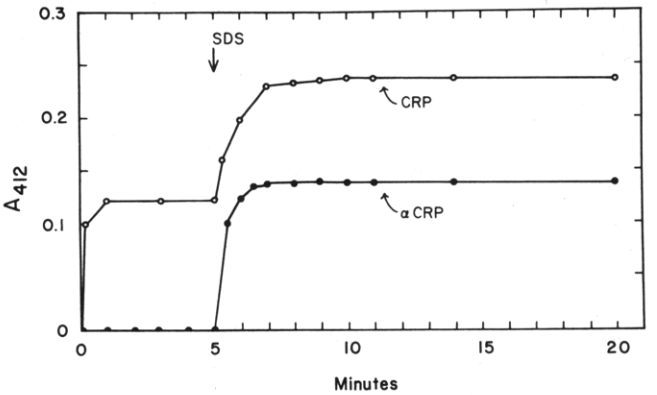

FIGURE 5: DTNB titration of CRP and αCRP. The reaction was carried out at 24 °C in a 0.5-mL quartz cuvette containing 0.1 M sodium phosphate buffer (pH 8), 0.1 M NaCl, and 6×10^{-5} M DTNB with either 100 μg of CRP (○) or 55 μg of αCRP (●). The absorbance at 412 nm was continuously recorded against a blank containing identical concentrations of sodium phosphate, NaCl, and DTNB. After 5 min, 20 μL of 10% $NaDodSO_4$ was added to each cuvette.

$DodSO_4$–polyacrylamide gel patterns obtained following digestion of CRP in the presence and absence of cAMP by subtilisin, trypsin, chymotrypsin, and the *Staph. aureus* V8 protease. In each case, CRP was relatively resistant to proteolysis in the absence of cAMP, while digestion in the presence of cAMP resulted in a resistant core. The molecular weights for the core polypeptides obtained after digestion by subtilisin and trypsin digestion were ~12 500 and after chymotrypsin digestion ~13 000. The core polypeptide formed after incubation with the *Staph. aureus* V8 protease was much larger with a molecular weight of ~18 000. This protease cleaves at the carboxyl-terminal side of glutamic and aspartic acid residues (Houmard and Drapeau, 1972). The resistant core fragments produced by digestion with each of the proteases presumably reflect the hydrolysis of the protease-specific bond closest to the resistant core region.

The sedimentation patterns of CRP and αCRP are shown in Figure 4. Anderson et al. (1971) have reported a sedimentation coefficient of 3.5 S for the native CRP protomer of 45 000 molecular weight consisting of two identical subunits of 22 500 daltons. Using CRP and lysozyme as standards, αCRP was shown to have a sedimentation coefficient of 2.7 S, corresponding to a molecular weight of 25 000. This would indicate the association of two polypeptide chains of 12 500 molecular weight in the αCRP protomer. No effect of 10^{-4} M cAMP, 10^{-4} M cGMP, or 0.5 M KCl on the sedimentation of αCRP could be detected (not shown).

The Stokes radius of αCRP was determined using the gel-filtration method of Siegel and Monty (1966) who showed a linear correlation of the parameter $(-\log K_{av})^{1/2}$ with Stokes radius. Using this relationship, αCRP was shown to have a Stokes radius of 25 Å. With this value and that of 0.725 cm^3/g as the partial specific volume ($\bar{v}$) representative of most proteins (Martin and Ames, 1961), a frictional ratio (f/f_0) of 1.24 was calculated for αCRP. If it were assumed instead that αCRP retains the $\bar{v}$ of CRP measured at 0.752 cm^3/g (Anderson et al., 1971), the f/f_0 of αCRP is calculated to be 1.23. A Stokes radius of 28 Å for CRP was calculated using the reported centrifugal parameters (Anderson et al., 1971).

CRP and αCRP can be resolved on Bio-Rex 70 using a 0–0.6 M linear KCl gradient for elution. The αCRP elutes at 0.25 M KCl followed by CRP at 0.35 M KCl. Since αCRP is released from the cation-exchange resin at a lower salt concentration than that required for CRP, αCRP appears to be somewhat less positively charged than is CRP at pH 7. These data suggest that αCRP has a lower isoelectric point than CRP.

Spectrophotometric titrations to determine the sulfhydryl content of CRP and αCRP were performed using DTNB (Figure 5). Based on the molar extinction coefficient of the TNB anion at 412 nm (Ellman, 1959), CRP contains 1.9 mol of available SH/mol of protomer with an additional 2.0 mol of "buried" cysteine residues titratable only in the presence of $NaDodSO_4$. In contrast to this, αCRP does not appear to contain SH groups in the native conformation that are able to react with DTNB. Denaturation of the αCRP with $NaDodSO_4$ resulted in the titration of 2.2 mol of SH/mol of αCRP protomer.

Discussion

The fact that no net loss in the specific activity of cAMP-dependent $d(A\text{-}T)_n$ binding activity is observed when CRP is incubated with subtilisin in the absence of cAMP (Figure 1A) suggests that proteolytic cleavage is random and does not reflect any specific conformational state of the CRP. When proteolysis is performed in the presence of cAMP (Figure 1B), cAMP-dependent $d(A\text{-}T)_n$ binding activity decreases rapidly, indicating a specific cleavage or series of cleavages between the α and β regions (Eilen and Krakow, 1977b). Since Na-$DodSO_4$-polyacrylamide gel electrophoresis of these products shows only the α fragment of ~12 500 daltons (Figure 2) and since the resulting mixture shows no cAMP-dependent DNA-binding activity (Krakow and Pastan, 1973), it can be assumed that the DNA-binding region is rapidly degraded by the protease.

The results of the titration of CRP with DTNB showing 1.9 available and 3.9 total cysteines per protomer (Figure 5) are in substantial agreement with those of Anderson et al. (1971), who titrated 1.33 and 1.66 cysteines per protomer under non-denaturing conditions with DTNB and 4,4′-dithiodipyridine, respectively, with four total SH groups reacting in 6 M guanidinium hydrochloride. The αCRP protomer retains only the two buried sulfhydryls after the proteolytic digestion of CRP in the presence of cAMP. Since αCRP retains cAMP binding but has lost the DNA binding activity, two regions of CRP may be defined: the α region containing the two buried sulfhydryls and the cAMP-binding domain, and the β region containing the two available sulfhydryls and the DNA-binding domain. Since αCRP has been shown to have the same N-terminal amino acid sequence as CRP, although 40% of the N-terminal dipeptide is lost on proteolysis (Schlesinger, 1978), the β region must be located in the C-terminal region of CRP.

In light of the above, one may conclude that the initially rapid rate of proteolysis of CRP in 10^{-4} M cAMP reflects the loss of the β region containing the DNA-binding site; the resulting α fragment is more resistant to proteolysis when cAMP is present (Eilen and Krakow, 1977a).

The sedimentation velocity results shown in Figure 4 indicate that the dimeric structure of the CRP protomer is maintained in the αCRP fragment. This suggests that the contact regions for subunit–subunit interaction in CRP remain even after the proteolytic production of the αCRP core. The inability of cyclic nucleotides to affect the sedimentation behavior of either CRP or αCRP (not shown) indicates that cyclic nucleotide induced conformational shifts in these proteins are not due to changes in their aggregation patterns and that the conformational changes in CRP and αCRP occur at the level of the protomer.

The hydrodynamic behavior of αCRP is similar to that of the parent CRP. The calculation of the frictional ratio for αCRP required the determination of its Stokes radius, which was found to be 24 Å (Figure 5). Using this value, an f/f_0 of 1.23 was calculated for CRP. When this is compared with the f/f_0 of 1.17 for CRP (Anderson et al., 1971), it is evident that the globular nature of the CRP is retained in the αCRP core. It should be noted that hydrodynamic methods are relatively insensitive to changes in molecular shape. This is because pairs of hydrodynamic properties, rather than the value of a single property, are required to define a particular shape or size and that these combinations are not very sensitive to particle shape (Loeb and Scheraga, 1956). One can conclude, however, that the αCRP protomer is not elongated but clearly globular as stated above.

The isoelectric pH of CRP has been measured as 9.12 (Anderson et al., 1971). In order to estimate the change in isoelectric pH with the removal of the DNA-binding region, CRP and αCRP were eluted from Bio-Rex 70 using a linear gradient of KCl (not shown). The αCRP eluted from the resin at a lower salt concentration, indicating that the α core is left with a lower net positive charge when the CRP is digested with subtilisin in the presence of cAMP. The inferred lower p*I* of αCRP presumably reflects a preferential removal of basic groups in the degradation of the DNA-binding domain of CRP. The function of positively charged areas in the DNA-binding region may be in nonspecific binding to the phosphate groups in DNA.

In conclusion, the α core fragment of the CRP, which retains the buried sulfhydryl groups of the CRP, is relatively acidic in comparison with the native CRP. The dimeric structure of αCRP suggests that at least some portion of the contact region between the original CRP subunits is retained in αCRP.

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