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Biochemistry

Pivotal role of amino acid at position 138 in the allosteric hinge reorientation of cAMP receptor protein

SANGRYEOL RYU, JIN KIM, SANKAR ADHYA, AND SUSAN GARGES†

Laboratory of Molecular Biology, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892

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ABSTRACT The cAMP receptor protein (CRP) of *Escherichia coli* needs cAMP for an allosteric change to regulate gene expression by binding to specific DNA sites. The hinge region connecting the DNA-binding domain to the cAMP-binding domain has been proposed to participate in the cAMP-induced allosteric change necessary to adjust C and D α-helices for movement of the DNA-binding F α-helix away from the protein surface. The role of the hinge region for a conformation change in CRP was tested by studying the effects of single amino acid substitutions at residue 138 located within the hinge. Physiological studies of wild-type and mutant cells and biochemical analysis of purified wild-type and mutant CRP revealed at least three groups of altered CRPs: (*i*) CRP that behaves like wild type (CRP$^+$); (*ii*) CRP that binds cAMP but does not complete the structural changes required for specific DNA binding, proteolytic cleavage, and transcription activation (CRP^{allo}); and (*iii*) CRP that shows some or all of these conformational changes without cAMP (CRP*). These results show a pivotal role of position 138 from which change emanates and provide further evidence that a hinge reorientation involving residue 138 is involved in the interhelical adjustments.

The cAMP receptor protein (CRP) when complexed with cAMP regulates expression of genes in *Escherichia coli* by binding to specific DNA sites at or near promoters (1–5). The three-dimensional structures of the CRP–cAMP complex and of DNA–CRP–cAMP have been determined by Steitz and coworkers (6, 7). CRP is a homodimer of a 209-amino acid monomer that is composed of two domains. The small carboxyl-terminal domain contains a helix–turn–helix DNA binding motif. The large amino-terminal domain is responsible for subunit–subunit contact for dimerization and binds cAMP (Fig. 1). The two domains are connected by a hinge region (residues 135–138).

Biochemical and biophysical evidence such as changes in protease sensitivity (9, 10), chemical crosslinking (11), fluorescence studies (12–14), neutron scattering (15), and Raman spectra (16) showed that cAMP binding to CRP alters CRP conformation allosterically and that the altered conformation functions by binding to specific DNA sequences with higher affinity. Several residues that participate in the allosteric shift have been identified previously by isolating and mapping mutations in CRP genes that allow cAMP independence (10, 17–19) and mutations that suppress the cAMP-independent phenotype (20). Some of these mutations were found to be located in the D α-helix of the carboxyl-terminal domain near the hinge region of the protein. By studying the effect of a systematic substitution of amino acids at positions 137, 138, 141, or 144 of CRP (21), we have shown that cAMP binding induces the amino acid at position 138 at the hinge region to interact with at least amino acid 141 in D α-helix for hinge reorientation. An interaction between amino acids at 138 and 141 was also suggested by their inwardness and interacting distance in the structure (6, 7).

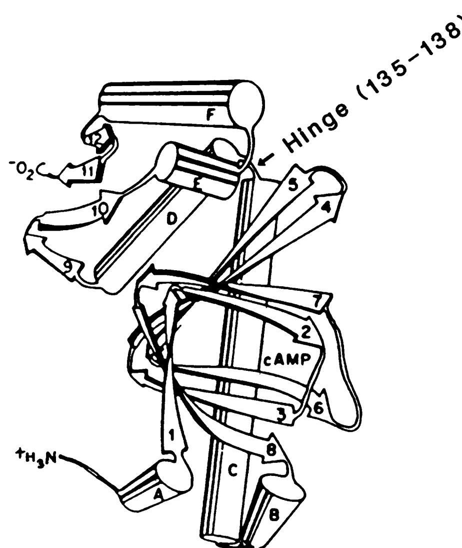

FIG. 1. Schematic diagram of a CRP monomer (taken from ref. 8). The regions that are α-helices are represented as cylinders lettered A through F. The regions in β conformation are represented as arrows 1 through 12. The larger amino-terminal domain consists of α-helices A through C and β-sheets 1 through 8. The smaller carboxyl-terminal domain consists of the D through F α-helices and β-sheets 9 through 12. The two domains are connected covalently by a tetrapeptide segment (hinge, residues 135–138) between the C and D α-helices.

We tested the importance of hinge reorientation for a conformational change in CRP by studying the *in vivo* phenotypes and biochemical characteristics of single amino acid substitutions at position 138 of CRP.

MATERIALS AND METHODS

Plasmid Strains. pJK40 is a pBR322-based plasmid carrying the wild-type *crp* gene (21). Other *crp* plasmids used are derivatives of pJK40 carrying various mutations in the amino acid at 138 of the *crp* gene (ref. 21 and as described below). Plasmid pSA601, used as the template for *in vitro* transcription, contained the *bla* gene and the wild-type *lac* promoter region followed by a transcription terminator (a gift of H. Choy, National Institutes of Health).

Site-Directed Mutagenesis. Aspartic acid at position 138 of CRP was replaced by alanine, asparagine, glutamic acid, glutamine, glycine, leucine, lysine, phenylalanine, or valine by site-directed mutagenesis as described (21). The individual mutant plasmids are designated by position 138 followed by

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Abbreviation: CRP, cAMP receptor protein.
†To whom reprint requests should be addressed.

the three-letter code of the amino acid substituted for aspartic acid, such as 138Ala, 138Asn, etc.

CRP Preparation. Plasmid encoding the wild-type *crp* gene or the mutant *crp* genes carrying various amino acid substitutions in position 138 of CRP was introduced into the Δ*cya* Δ*crp E. coli* strain G839 (21). Cells were lysed in a lysis buffer (50 mM Tris·HCl, pH 8.0/50 mM KCl/0.1 mM EDTA/0.5 mM dithiothreitol/0.5 mM phenylmethylsulfonyl fluoride/10% sucrose) containing 0.2 mg of lysozyme per ml and then were treated with DNase. CRP was recovered by ammonium sulfate fractionation between 30% and 60% saturation and was purified by fast protein liquid chromatography (FPLC; Pharmacia) using a Mono S column and a linear gradient of 0.1–0.3 M of KCl. CRP was eluted at about 0.15 M KCl and was at least 95% pure as judged by Coomassie blue staining after SDS/PAGE.

DNA-Binding Assay. DNA-binding ability of CRP was measured by using nitrocellulose filter paper (0.22 μm × 25 mm) (22). A 40-base-pair synthetic DNA fragment bearing the *E. coli lac* CRP binding site was used. CRPs (0.001 nM–100 nM) and ^{32}P-5′-labeled DNA (20 pM) were incubated at room temperature for 45 min in a 0.5-ml reaction mixture containing 10 mM Mops adjusted to pH 7.4 with NAOH, 100 mM NaCl, 100 μM cAMP, 0.1 mM dithiothreitol, and 50 μg of bovine serum albumin per ml. The reaction mixtures were filtered through presoaked filters under suction, and Cerenkov radiation of the dried filters was assayed in a Beckman LS8000 scintillation counter. Nonspecific DNA binding was monitored by using a 32-base-pair synthetic *lacZ* structural gene sequence to which wild-type CRP does not bind at the concentration range tested.

cAMP-Binding Assay. cAMP binding was measured by using tritiated cAMP (23).

Protease Sensitivity. The conformation change in CRP mutants was monitored by the change in protease sensitivity when using subtilisin and chymotrypsin. CRPs (1.5–2.0 μg) in 40 mM Tris·HCl, pH 8.0/100 mM KCl/10 mM $MgCl_2$/0.1 mM EDTA/0.1 mM dithiothreitol were treated with either enzyme (24). For subtilisin treatment, the ratio of CRP to subtilisin was 20 to 1 by weight. The reactions were done with or without 20 μM cAMP or cGMP for 10 min at 37°C. For chymotrypsin, the ratio of CRP to chymotrypsin was 10 to 1, and the reaction was also done with or without 20 μM cAMP or cGMP at room temperature for 15 min. Both reactions were stopped by adding phenylmethylsulfonyl fluoride to a final concentration of 1 mM. Products were analyzed by electrophoresis on a SDS/8% polyacrylamide gel followed by staining with Coomassie blue.

lac Operon Expression *in Vivo*. The effects of amino acid substitutions at position 138 of CRP on *lac* operon expression of *E. coli in vivo* were monitored by using strain G839 (Δ*cya* Δ*crp*) transformed with plasmid pJK40 that encoded various amino acid substitutions in position 138 of CRP and noting the colony color on MacConkey lactose agar plates. Cyclic nucleotides were added to media to 1 mM concentration where appropriate. β-Galactosidase activity was also measured as described (17). The cells were grown in M56-fructose-B1-Casamino acids minimal medium (17). Three independent experiments were done for each mutant CRP.

***In Vitro* Transcription.** Reactions were done in a 50-μl volume containing 20 mM Tris acetate (pH 8.0); 3 mM magnesium acetate; 100 mM potassium glutamate; 1 mM dithiothreitol; 0.1 mM each of ATP, GTP, and CTP; 0.01 mM UTP; 10 μCi (370 kBq) of [^{32}P]UTP (800 Ci/mmol); 2 nM supercoiled DNA template; 40 nM CRP; 20 nM RNA polymerase holoenzyme; and 5% (vol/vol) glycerol. Either 100 μM cAMP or 1 mM cGMP was added to the reaction mixture where indicated. All components except nucleotides were incubated at 37°C for 10 min. Transcriptions were started by the addition of nucleotides and terminated after 10 min by addition of 50 μl of formamide loading buffer [80% formamide/1× TBE (89 mM Tris/89 mM boric acid/2 mM EDTA)/0.05% bromophenol blue/0.05% xylene cyanol]. RNA was resolved by electrophoresis on an 8.0% polyacrylamide/8 M urea gel. The amount of transcripts was measured by using an AMBIS Systems β scanner (San Diego).

RESULTS

lac Promoter Activity by Mutant CRPs *in Vivo*. The expression of the *lac* operon in *E. coli* is dependent upon an active CRP protein complexed with cAMP. The ability of mutant

Table 1. Properties of CRP with substitutions at position 138

			Relative constant		Protease sensitivity‡		
Amino acid-138	Phenotype group	CRP activity *in vivo*†	K_a with cAMP§	K_d for DNA¶ binding	With cAMP	Without cAMP	*In vitro*‖ transcription
Asp (wt)		1824	1.0	1.0	R	S	++
Gly	A (Crp+)	2514	2.8	4.2	R	S	++
Gln		3097	1.6	4.8	R	S	++
Leu		2758	2.1	14	S	S	++
Ala	B (Crp+)	355	1.2	175	R	R	+
Phe		891	4.7	115	S	S	+
Glu		438	0.8	175	R	S	+
Val	C (Crp−)	13	3.2	125	R	R	−
Asn	D (Crp*)	2479††	2.3	0.025	R	S	*
Lys		740††	1.2	0.2	S	S	*

†β-Galactosidase activity [Miller units (17)] was measured from a Δ*cya* Δ*crp* strain containing multicopy *crp* plasmid in the presence of cAMP unless otherwise noted.
‡R, resistant; S, at least partially sensitive.
§The association constants for each of the proteins were determined from the Scatchard analysis of the data collected by the ammonium sulfate precipitation procedure as described in text. The numbers indicate relative values compared with that of wild type (wt) carrying Asp-138 (4×10^5 M).
‖++, Same activity as wild type; +, activity lower than wild type; −, no activity; *, active in the presence of cGMP as well as cAMP.
¶The equilibrium dissociation constant is calculated from the concentration of protein required for half-saturation of DNA. The numbers indicate relative values compared with that of wild type carrying Asp-138 (2×10^{-10} M).
††β-Galactosidase activity was measured in the presence of 1 mM cGMP. There was no activity without cyclic nucleotide, and cAMP was strongly inhibitory to growth.

CRPs introduced by a multicopy plasmid into a Δ*crp* Δ*cya* bacterial strain to activate the *lac* promoter *in vivo* with or without external cAMP or cGMP was monitored by colony color on MacConkey lactose agar plates. Each mutant CRP was assigned a phenotype according to the definition described below. If an amino acid substitution at position 138 of CRP shows a Lac$^+$ phenotype (red colonies on MacConkey lactose plate) only in the presence of cAMP, it was assigned a Crp$^+$ phenotype. If an amino acid substitution shows Lac$^+$ phenotype in the absence of cAMP, it was scored to have a Crp* phenotype. An amino acid substitution that is Lac$^-$ in the absence of cAMP but is Lac$^+$ in the presence of exogenous cGMP was also included in the Crp* group for reasons discussed before (17, 21). Any amino acid change that showed a Lac$^-$ phenotype in the presence of cAMP was given a Crp$^-$ phenotype. The Crp phenotypes were quantified by measuring the levels of β-galactosidase made from the *lac* promoter in cells carrying various *crp* mutants (Table 1). Wild-type CRP has an aspartic acid at position 138. We have found that the mutants designated 138Gly, 138Gln, 138Leu, 138Ala, 138Glu, and 138Phe show a Crp$^+$ phenotype. However, there were differences in colony color on MacConkey lactose plates among the various Crp$^+$ mutants, which indicated differences in their activities. Such differences were quantified by measurement of β-galactosidase synthesis stimulated by CRP in the corresponding strains in the absence and presence of cyclic nucleotides. Mutants 138Gly, 138Gln, and 138Leu showed slightly higher activity than wild-type CRP, whereas mutants 138Ala, 138Glu, and 138Phe had lower activity than wild-type CRP, showing a weak Crp$^+$ phenotype. Mutants 138Asn and 138Lys showed Crp* phenotype. Both of these mutants were inactive in the absence of cAMP but were active in the presence of cGMP. Measurement of β-galactosidase activity was not possible in these two mutants in the presence of cAMP because the cells containing either mutation in the *crp* plasmid could not grow in the presence of cAMP. The cAMP sensitivity in CRP* mutants is an associated property of many CRP* mutants characterized previously (17, 19–21). β-Galactosidase activities in the presence of cGMP in cells containing mutants 138Asn and 138Lys were high (2479 and 740 units, respectively). β-Galactosidase activity obtained by wild-type CRP in the presence of cAMP was 1824 units. The mutant that had a strong Crp$^-$ phenotype was 138Val. Wild-type and each mutant CRP were purified, and their cAMP-binding and DNA-binding activities, sensitivity to proteases, and ability to stimulate transcription from *lac* promoter *in vitro* were further studied.

cAMP Association. The cAMP-binding ability of the mutant CRPs studied did not change much even though some of the amino acid substitutions strongly influenced CRP activity *in vivo* (Table 1, column 4). The association constant for each mutant protein was within 4-fold of the wild-type value of 4 × 10^5 M. These results suggest that amino acid substitutions at position 138 of the hinge in CRP do not affect the structure required for cAMP binding in the amino-terminal domain of CRP.

DNA Binding. In our model of allosteric change in CRP, the conformational shift of the DNA-binding Fα-helix involves its protrusion from the surface of the protein, allowing specific DNA recognition through major groove contact (17, 20, 21). We have proposed that an interaction between amino acids at positions 138 and 141 of Dα-helix effects this movement of the Fα-helix. Therefore, the nature of the amino acid at 138 would greatly affect the DNA-binding ability of CRP. Mutants 138Gly, 138Gln, and 138Leu showed DNA-binding affinity similar to that of wild type. The DNA-binding affinities of mutants 138Lys and 138Asn were higher by factors of 5 and 40, respectively, whereas those of mutants 138Phe, 138Glu, 138Val, and 138Ala were lower by a factor of about 100 than that of wild-type CRP carrying Asp-138 (Table 1, column 5). None of these mutants showed any change in nonspecific DNA-binding activity (data not shown). When compared with the *in vivo* gene regulatory activity of CRP, as judged by the level of β-galactosidase synthesis, there was a direct relationship between specific DNA-binding affinity of CRP and its *in vivo* activity. In other words, weak Crp$^+$ phenotypes *in vivo* of several CRP mutants are associated with their low DNA-binding activity.

Protease Sensitivity. The sensitivity to proteases such as chymotrypsin or subtilisin is one of the diagnostics of the cAMP-induced conformational change in CRP. Wild-type CRP is sensitive to proteases only in the presence of cAMP, whereas the CRP* phenotype reflects sensitivity even in the absence of cAMP (9, 10, 25). The conformational changes in CRP mutants were studied by using both chymotrypsin and subtilisin in the absence and presence of cAMP or cGMP. The sensitivity of each CRP mutant to chymotrypsin was the same as that to subtilisin. The results of protease sensitivity in the absence and presence of cAMP are shown in Fig. 2 and are summarized in Table 1 (columns 6 and 7).

All mutant CRPs except the 138Ala and 138Val mutants were sensitive to subtilisin and chymotrypsin in the presence of cAMP. Mutants 138Ala and 138Val were resistant to proteases even in the presence of cAMP, although both bind cAMP well. The 138Val mutant was completely Crp$^-$ *in vivo*, suggesting that in this case it could not undergo the correct

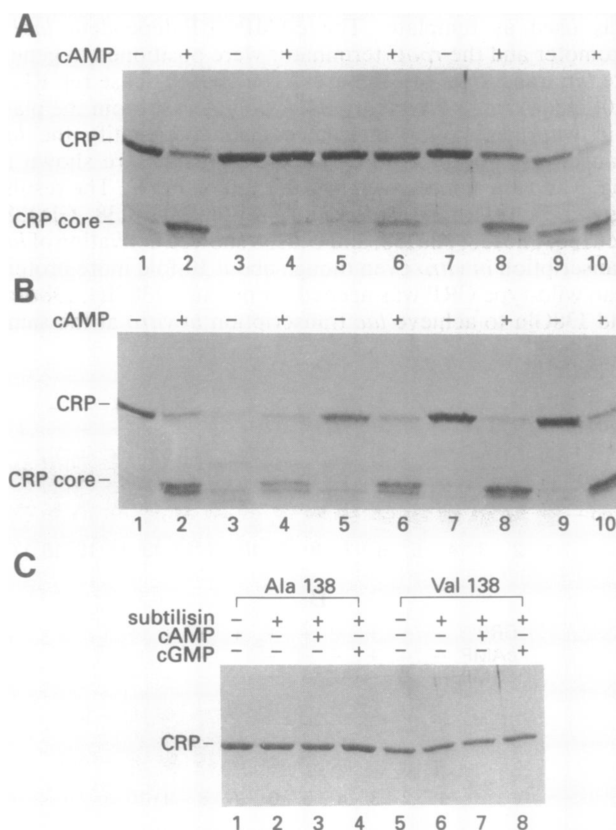

FIG. 2. (*A*) Sensitivity of CRPs to chymotrypsin. CRPs (14 μg) with or without 20 μM cAMP were treated with 0.14 μg of chymotrypsin at room temperature for 15 min as described in text. Lanes: 1 and 2, wild-type CRP; 3 and 4, 138Ala; lanes 5 and 6, 138Val; lane 7, 138Glu; lanes 9 and 10, 138Phe. (*B*) Same as in *A*. Lanes: 1 and 2, 138Asn; 3 and 4, 138Leu; 5 and 6, 138Lys; 7 and 8, 138Gly; 9 and 10, 138Gln. The molecular weight of the chymotryptic core in *A* and *B* is 13 kDa (26). (*C*) Sensitivity of mutant CRPs 138Ala (lanes 1–4) and 138Val (lanes 5–8) to subtilisin. CRPs (2 μg) without or with 20 μM cAMP or cGMP were treated with 0.12 μg of subtilisin at 37°C for 10 min as described in text.

conformational change upon cAMP binding. Since mutant 138Ala had a weak Crp^+ phenotype, we believe there was some degree of cAMP-dependent conformational change in the 138Ala protein, which, however, could not be detected by the protease assay. In the absence of cAMP, mutants 138Leu, 138Phe, and 138Lys were, at least partially, sensitive to proteases, which is one of the properties of the Crp* phenotype. But among them, only 138Lys was actually Crp* (active in the presence of cGMP) *in vivo*. These results suggest that the Asp-138 → Lys change brought about structural alterations similar to that which cAMP normally induces for sensitivity to proteases, with cGMP carrying the change further for DNA-binding and gene-activation characteristics, whereas the Asp-138 → Leu and Asp-138 → Phe changes brought about enough conformational change to make the 138Leu and 138Phe mutants sensitive to proteases but could not achieve correct conformational change necessary for DNA binding (138Phe) and transcription activation (for 138Leu). These results suggest that the protease sensitivity test is not a diagnostic tool for the full degree of conformational changes in CRP induced by cAMP. Both mutants 138Lys and 138Asn showed protease sensitivity in the presence of cGMP (data not shown).

***In Vitro* Transcription.** The activity of CRP mutants on activation of *lac* transcription was studied by following RNA synthesis from the wild-type *lac* promoter in a purified system. Supercoiled plasmid pSA601 containing the $lacP^+$ promoter followed by a transcription terminator, *rpoC* (27), was used as template. The cAMP·CRP-dependent *lacP1* promoter and the *rpoC* terminator were positioned to generate *lac* transcripts of 117 bases. The 106/107-base rep RNA (10) made constitutively by RNA polymerase from the plasmid was used as an internal control to quantify the *lac* transcripts. The *in vitro* transcription results are shown in Fig. 3 and are summarized in Table 1 (column 8). The results agreed well with *in vivo* results. Mutants Gly138, Gln138, Leu138, Phe138, Ala138, and Glu138 showed activation of *lac* transcription *in vitro* even though about 10-fold more protein than wild-type CRP was needed for mutants 138Phe, 138Ala, and 138Glu to achieve *lac* transcription *in vitro* at the same

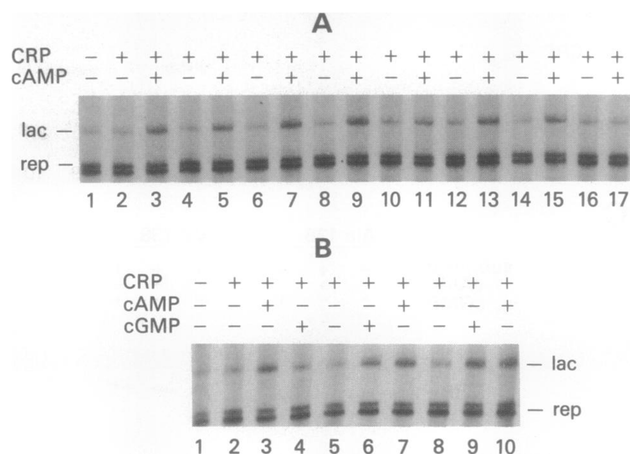

FIG. 3. (*A*) *In vitro* transcription results of wild-type and mutant Crp^+ and Crp^- mutants with supercoiled template pSA601. Transcription reactions contained 2 nM DNA, 20 nM RNA polymerase, and 40 nM (lanes 2–9) or 400 nM (lanes 10–17) CRP. cAMP (100 μM) was added to the reaction mixtures where indicated. Only the 106/107-base rep RNA and 117-base lac RNA from the cAMP·CRP-dependent *lacP1* promoter as resolved on 8% acrylamide/8 M urea gel are shown. Lanes: 1–3, wild-type CRP; 4 and 5, 138Gly; 6 and 7, 138Gln; 8 and 9, 138Leu; 10 and 11, 138Phe; 12 and 13, 138Glu; 14 and 15, 138Ala; 16 and 17, 138Val mutant CRP. (*B*) *In vitro* transcription results of CRP* mutants. Lanes: 1–4, wild-type CRP; 5–7, 138Asn; 8–10, 138Lys.

level. The low DNA-binding affinities of these mutant CRPs explain why more protein was needed for *in vitro* transcription activation. The 138Val mutant did not activate transcription under any condition *in vitro*, as *in vivo*.

The 138Asn and 138Lys mutants, which showed a Crp* phenotype *in vivo*, had activity higher than that of wild-type CRP by the *in vitro* transcription assay in the presence of cAMP. Less protein by at least a factor of three was enough to show the same activity as wild-type CRP in the presence of cAMP. Their high DNA-binding affinity in the presence of cAMP seemed to be the main reason for this property. They also could activate transcription from *lac* promoters in the presence of cGMP *in vitro*, which is in agreement with their *in vivo* behavior. The activities of these mutants in the presence of cGMP were almost the same as that of wild-type CRP in the presence of cAMP.

DISCUSSION

Pairwise amino acid substitutions in and around the hinge region involving position 141 at the hinge end of D α-helix and position 138 at the hinge have provided evidence of hinge reorientation in CRP because combinations of amino acids at these two positions result in a Crp^+, Crp*, or Crp^- phenotype, and that a phenotype generated by a given amino acid substitution at 141 can be reversed by at least one other at 138 (21). In general, if polar amino acids are at both the 138 and 141 positions, CRP becomes cAMP-independent or CRP*. If one site contains a polar amino acid and one contains a nonpolar one, the protein has a CRP^+ phenotype. If nonpolar amino acids are at both 138 and 141, the protein has a weak CRP^+ or a CRP^- phenotype. We have proposed that in the cAMP-induced allosteric change, hinge reorientation similar to hinge bending by an interaction between polar amino acids at positions 138 and 141 helps the D α-helix to move closer to the C α-helix across the hinge (17, 20, 21). This eventually allows the F α-helix to flex away from the D α-helix. We report here further studies on the effects of single amino acid substitutions at position 138 of CRP to investigate the effect of hinge reorientation for CRP conformational change. We showed, in effect, four groups of Crp mutants by different substitutions at position 138 of CRP as summarized in Table 1. Aspartic acid is the wild-type amino acid at position 138 of CRP. First, CRP mutants in Group A (138Gly, 138Gln, and 138Leu) were almost similar to wild-type CRP in cAMP binding, DNA binding, protease sensitivity, and *in vivo* and *in vitro* transcription activation. Group B, which included 138Ala, 138Phe, and 138Glu substitutions, displayed weak Crp^+ phenotypes, showing cAMP binding that was similar to that of wild-type CRP but DNA-binding activity that was less by a factor of 100 or more. They were also able to activate *lac* transcription in the presence of cAMP both *in vivo* and *in vitro* although less efficiently compared with wild-type CRP. Thus, the weaker DNA-binding affinity of these groups is the cause of their lower transcription activity. The CRP mutant in Group C (138Val) was completely Crp^- and defective in DNA binding as well as in transcription activation. It showed a DNA-binding affinity that was less by a factor of 125 than that of wild-type CRP and had no gene regulatory activity *in vivo* nor *in vitro*, showing that this mutant could not undergo any conformation change upon cAMP binding. We call this phenotype CRP^{allo}. Note that, according to our model, replacement of Asp-138 with leucine, alanine, or valine creates a nonpolar–nonpolar combination with Gly-141 and is expected to cause the Crp^- phenotype. Consistently, mutants 138Ala and 138Val were weakly Crp^+ or Crp^- but mutant 138Leu was Crp^+. The failure of mutant 138Leu to conform to the proposed rule remains to be investigated. Group D included CRP mutants 138Asn and 138Lys. Both of them showed a CRP* phenotype and high DNA-binding affinity in

the presence of cAMP. The higher DNA-binding affinity of mutants 138Asn and 138Lys could indicate an extra contact with a phosphate for each subunit. Since amino acid 138, which is located at the apex of the hinge, is quite close to the DNA backbone in the recently determined CRP·DNA cocrystal structure (7), such an interaction is theoretically possible.

The protease sensitivity that is imparted upon CRP by cAMP binding may be a component of the allosteric changes associated with specific DNA binding and transcription activation by CRP. We found a correlation between altered allosteric behavior and the protease sensitivity of CRP associated with the mutation changes in amino acid at position 138. CRP mutants that were defective in a step after cAMP binding (138Ala and 138Val) were also protease resistant in the presence of cAMP. On the other hand, a CRP* mutant that mimicked the cAMP effect (138Lys) showed protease sensitivity in the absence of cAMP. Those that appeared to be exceptions were 138Leu, which was CRP^+, and 138Phe, which was defective in DNA binding. They are protease-sensitive mutants even in the absence of cAMP. Although we do not know the exact sites of proteolytic cleavage in wild-type CRP or any of the mutant CRPs, the protease sensitivity of 138Phe and 138Leu could reflect an incomplete (localized) allosteric change—i.e., a more localized and thus incomplete CRP* phenotype rather than an aberrant change(s). Although CRP* mutant 138Asn was insensitive to protease in the absence of cAMP, both 138Asn and 138Lys showed protease sensitivity in the presence of cGMP in agreement with their *in vivo* property.

The results presented in this paper show that cAMP-induced structural changes can be studied at different levels of CRP behavior. We have been able to define a critical change that is a hinge-reorienting interaction between amino acids at positions 138 (at the hinge) and 141 (at the D α-helix) (see Fig. 1). Such an interaction, which can be achieved without cAMP binding by proper mutational changes, results in conferring to the protein the ability to bind to specific DNA sequences, the susceptibility to proteolytic cleavage, and the ability to activate transcription. Our results show that one can obtain amino acid substitutions at position 138 that can block (*i*) DNA binding (138Glu), (*ii*) DNA binding and proteolytic cleavage (138Ala), or (*iii*) DNA binding, proteolytic cleavage, and activation (138Val). This gives a pattern to the nature of the allosteric changes that normally emanates from position 138. Consistent with this pattern, amino acid substitutions at 138 also make CRP mimic either partially or fully cAMP-dependent changes. Substitutions that make CRP protease sensitive without cAMP have either no effect (138Leu) or inhibitory (138Phe) effect on DNA binding. Thus, the position 138 plays a pivotal role in transmitting allosteric signals to different regions of the protein. The extent of such signal transmission and the requirement of cAMP for the process depends on the nature of the amino acid occupant at that position.

The variety of Crp phenotypes obtained in this study by single amino acid substitutions at position 138 of CRP reaffirms the involvement of hinge reorientation for CRP conformational change, which eventually affects the conformational shift of the F α-helix. The latter is believed to be a major object of the cAMP-induced allosteric change. The nature of the interactions between amino acids at position 138 and Gly-141, effecting different interhelical orientations, remains to be determined by structural determinations of the altered proteins.

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