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Genetics

Allosteric changes in the cAMP receptor protein of *Escherichia coli*: Hinge reorientation

(protein conformation/DNA–protein interaction/gene regulation)

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ABSTRACT **The cAMP receptor protein (CRP) of *Escherichia coli* is a dimer of a two-domain subunit. It requires binding of cAMP for a conformational change in order to function as a site-specific DNA-binding protein that regulates gene activity. The hinge region connecting the cAMP-binding domain to the DNA-binding domain is involved in the cAMP-induced allosteric change. We studied the structural changes in CRP that are required for gene regulation by making a large number of single and double amino acid substitutions at four different positions in or near the hinge. To achieve cAMP-independent transcription by CRP, amino acid residues 138 (located within the hinge region) and 141 (located in the D α-helix adjacent to the hinge) must be polar. This need for polar residues at positions 138 and 141 suggests an interaction that causes the C and D α-helices to come together. As a consequence, the F α-helix is released from the D α-helix and can interact with DNA. At position 144 in the D α-helix and within interacting distances of the F α-helix, replacement of alanine by an amino acid with a larger side chain, regardless of its nature, allows cAMP independence. This result indicates that pushing against the F α-helix may be a way of making the helix available for DNA binding. We believe that the cAMP-induced allosteric change involves similar hinge reorientation to adjust the C and D α-helices, allowing outward movement of the F α-helix.**

The cAMP receptor protein (CRP), when bound to cAMP, regulates expression of many genes in *Escherichia coli* (1–3). Free CRP binds only nonspecifically to DNA at much lower efficiency. From a variety of biochemical and biophysical studies such as limited proteolysis (4–6), chemical crosslinking (7), small-angle x-ray scattering (8), and fluorescence studies (9, 10), it appears that cAMP binding to CRP alters CRP conformation allosterically. The altered conformation binds to DNA with higher affinity and sequence specificity. Steitz and coworkers (11, 12) have determined the structure of the dimeric CRP·cAMP complex (Fig. 1). Each subunit is composed of two domains: the large amino-terminal domain binds a molecule of cAMP and participates in the dimerization. The small carboxyl-terminal domain contains the DNA-binding segment, which has the helix–turn–helix DNA-recognizing structure, similar to other DNA-binding proteins (12, 13). The two domains are connected by a hinge polypeptide (residues 135–138). Recently, Schultz *et al.* (14) have also solved the structure of a CRP·cAMP DNA cocrystal, and so it is known precisely how CRP binds to DNA. No one has solved the structure of CRP without a cAMP molecule bound, making it difficult to predict what structural effect cAMP binding to one domain would have on the other DNA-binding portion of the protein.

Taking a genetic approach to understand the structural changes that ensue following cAMP binding, we and others (15–17) have isolated and mapped mutations in the *crp* gene that allow cAMP independence. One of these mutations caused substitution of a serine for a glycine at position 141 and another of a threonine for an alanine at position 144. Glycine at position 141 in the D α-helix is oriented toward the interior of the hinge. Alanine in position 144, also in the D α-helix, faces the F α-helix, which is the recognition helix of the helix–turn–helix motif (see Fig. 1). Based on these results and the structure of the CRP·cAMP complex, we previously suggested the following model (15, 18). The mutations cause a change in CRP conformation similar to that caused by cAMP. The substituted amino acid in both the 141 and the 144 position increases the distance between the F α-helix and the D α-helix. This movement is achieved by, among other changes, alteration in the relative position between the D α-helix and C α-helix (mutation at 141) or pushing against the F α-helix (mutation at 144). The new position of the F α-helix in each subunit allows CRP to make specific contacts with base edges in two adjacent DNA major grooves.

We have genetically tested the concept that cAMP independence of CRP is caused by changes in the distance between specific helices of the protein by replacing the amino acid at position 137, 138, 141, or 144, in and around the hinge, by a variety of amino acids. In doing so, we have also studied the effect of double amino acid substitutions in the hinge region to investigate possible interaction between amino acids 141 and 137 or 138. Our work follows the principle of Murgola and Yanofsky (19), who implicated interaction between two amino acids in the α-subunit of tryptophan synthetase from *E. coli* by second-site mutational studies. Their interpretation has been verified by structural studies (20, 21). In this work, we discuss the implication of the observed properties of CRP induced by the amino acid alterations in relation to the mechanism of allosteric changes in the protein.

MATERIALS AND METHODS

Strains. The *E. coli* strains used are listed in the table legends. We introduced Δ*crp* and Δ*cya* markers into the hosts carrying the various tRNA suppressors by phage P1 transduction. The replacement of the chromosomal *crp* gene by the chloramphenicol resistance gene was made by replacing the *crp* gene and flanking regions (*Bss*HII fragment) in pHA5 (22) with an *Fnu*DII fragment from pBR325 (23) containing the gene encoding chloramphenicol resistance. In a similar manner to that described previously (24), we crossed the *crp* substitution first onto λcI857*crp*$^+$ and then into the chromosome. Plasmid pJK40, encoding the *crp*$^+$ gene and a nearby kanamycin-resistance marker, was derived from pHA5 (22). The kanamycin-resistance marker, a GenBlock cassette purchased from Pharmacia, was inserted at the *Cla* I site

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Abbreviation: CRP, cAMP receptor protein.
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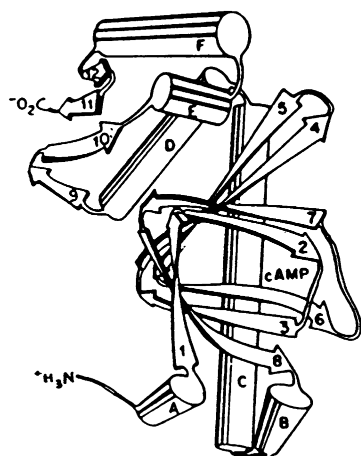

FIG. 1. Structure of one subunit of a CRP dimer. The α-helices are lettered A–F, and β-sheets are numbered. The hinge containing residues 135–138 is between the C and D α-helices. α-Helices E and F constitute the helix–turn–helix DNA-binding motif of CRP, with the specific contacts occurring via helix F. [Reprinted with permission from ref. 11 (copyright, American Society for Biochemistry and Molecular Biology).]

upstream from the *crp* gene in pHA5. The *bla* gene, encoding β-lactamase, was inactivated by removing the *Sca* I–*Mlu* I segment. *crp* mutations were introduced into pJK40 by substitution of the *Mlu* I–*Eco*RV segment of the crp^+ gene from double-stranded replicative-form DNA of bacteriophage M13 *crp* mutant clones. The M13 *crp* mutant clones were obtained by site-directed mutagenesis as described below. The mutant plasmids were confirmed by DNA sequencing and characterized by genetic and physiological studies.

Site-Directed Mutagenesis. We followed the method of Kunkel *et al.* (25) for site-directed mutagenesis of the *crp* gene in M13 *crp*. For introducing new amino acids in CRP, we used the following codons: alanine, GCA; arginine, CGT; cysteine, TGT; glutamine, CAG; isoleucine, ATT; lysine, AAA; phenylalanine, TTT; and valine, GTG.

Enzyme Assay. β-Galactosidase assays (15) were performed at least three times. Activities relative to wild type varied <10% from assay to assay.

Amber Suppression Studies. Amber mutations in *crp* were suppressed by tRNA suppressor mutations (Table 1). We introduced two deletion mutations (Δ*crp* and Δ*cya*) into the suppressor strains and then transformed them with plasmids carrying an amber mutation in *crp*, using kanamycin resistance as a selective marker. The transformed strains were tested for Crp phenotype.

RESULTS

Crp Phenotypes. The expression of the *lac* operon of *E. coli* is dependent upon an active CRP. Thus, we routinely scored the gene-activation phenotypes of CRP by color characteristics on MacConkey lactose agar plates. We define Crp phenotypes as follows. If a mutation in *crp* produces a Lac^+ phenotype, if and only if cAMP is present, the mutant is assigned a Crp^+ phenotype. If an amino acid substitution shows any Lac^+ phenotype on MacConkey lactose agar plates in the absence of endogenous cAMP, the mutant is scored Crp*. We have also included in our Crp* group mutants that are Lac^- in the absence of cAMP but are Lac^+ in the presence of exogenous cGMP. cGMP binds to CRP but, unlike cAMP, does not cause CRP to recognize specific DNA sequences (26). This finding suggests that cGMP binding does not elicit the full range of changes in CRP that cAMP does. Since a Crp* phenotype is enhanced by cGMP, the Crp* mutations must have facilitated allosteric change(s) in CRP that complete a partial transition induced by cGMP. Thus a response to cGMP reflects a change in allosteric transition. We have assigned a Crp^- phenotype for any amino acid change in the protein that produces a Lac^- phenotype in the presence of cAMP. We quantified the Crp phenotypes further by measuring β-galactosidase activity of the corresponding strains in the absence and presence of cAMP or cGMP.

Altered CRP Made by Suppression of Amber Mutations. We introduced an amber codon at position 137, 138, 141, or 144 by oligonucleotide-directed mutagenesis. A nonsense codon in a protein can be suppressed with a variety of nonsense suppressors to produce a series of mutant proteins with different amino acid substitutions (27–29). These amber mutations in *crp* were suppressed with chromosomal suppressors *supD*, *supE*, *supF*, or *supP*, which insert the amino acids serine, glutamine, tyrosine, or leucine, respectively (29).

Table 1 shows the results of the suppression studies of single amber mutations at positions 141 and 144. In the presence of wild-type CRP and cAMP, the level of β-galactosidase synthesis varied from 220 to 1960 units, depending on the type of suppressor we used. This variation arises

Table 1. Phenotype of strain containing *crp* amber mutations, suppressed by chromosomal tRNA suppressors

crp genotype on plasmid†	Suppressor amino acid	β-Galactosidase activity,‡§ Miller units			Crp phenotype¶
		–	+ cAMP	+ cGMP	
crp^+	Serine	6	221	61	+
	Glutamine	12	620	77	+
	Tyrosine	11	873	56	+
	Leucine	36	1960	316	+
crp_{am141}	Serine	41 (6.8)	R^i	600 (9.8)	*
	Glutamine	41 (3.4)	R^i	962 (12.4)	*
	Tyrosine	10 (0.9)	328 (0.4)	15 (0.3)	+
	Leucine	31 (0.9)	3598 (1.8)	87 (0.3)	+
crp_{am144}	Serine	13 (2.2)	R^i	545 (8.9)	*
	Glutamine	118 (9.8)	R^i	1024 (13.3)	*
	Tyrosine	20 (1.8)	R^i	822 (14.7)	*
	Leucine	419 (11.6)	R^i	1750 (5.5)	*

†Host strains used G883 (Ser), G887 (Gln), G889 (Tyr), and G891 (Leu). They are all Δ*crp* Δ*cya* $lacZ_{u118am}$ and have an amber suppressor tRNA encoded in the chromosome.

‡Numbers in parentheses represent the β-galactosidase specific activity measured under the given conditions divided by β-galactosidase specific activity measured in the same host strain containing multicopy crp^+ under the same conditions.

§R^i denotes that the cells would not grow in liquid culture under these conditions. They would grow slightly on MacConkey lactose indicator plates, producing a deep red color. We assume a very high level of β-galactosidase expression in these cases.

¶*, Crp* (cAMP-independent); +, wild type.

because of difference in suppression efficiency of the $lacZ_{u118}$ amber mutation present in the *lacZ* gene. cGMP has very little effect on wild-type CRP *in vitro* and *in vivo*, when *crp* is present in single copy (13, 26). However, we consistently found a small but reproducible increase in *lacZ* expression, even in a lac^+ background, when cGMP was added to a Δcya strain containing crp^+ on a multicopy plasmid. The addition of 2.5 mM cAMP inhibited the growth of cells harboring serine- or glutamine-suppressed amber mutations at amino acid 141. This phenomenon of cAMP sensitivity is characteristic of many *crp** mutants (15–17). CRPs with serine or glutamine suppression at 141 showed a weak Lac^+ phenotype in the absence of cyclic nucleotide and were activated by cGMP. Based on our definition, we scored them as Crp*. The substitution of tyrosine or leucine at position 141 allowed CRP to respond to cAMP, but not to cGMP. The latter two behaved basically as Crp^+. An amber codon at position 144 of CRP, suppressed by the four amino acids studied, produced a Crp* phenotype. In the cases of glutamine or leucine substitution, the level of β-galactosidase was about 10-fold greater than that with wild-type CRP in the absence of any cyclic nucleotides. cGMP further activated the serine, glutamine, tyrosine, or leucine mutants. cAMP inhibited the growth of the cells in all of the cases.

When suppressor tRNA are used to recognize amber codons at different positions in different proteins, suppression can be affected by the surrounding sequence (29). Therefore, we made direct substitutions at positions 141 and 144 by using the cognate codon for one of the suppressed amino acids, glutamine. Using direct codon substitutions, we also strove for a greater diversity of amino acids at both positions 141 and 144. Table 2 shows the results of direct codon substitutions that were constructed by site-directed mutagenesis. At position 141, arginine-, glutamine-, or lysine-substituted mutants displayed a Crp* phenotype; i.e., they exhibited a high level of β-galactosidase in the absence of exogenous cyclic nucleotides. The case of glutamine confirmed that at least for this amino acid both direct substitution and suppression gave qualitatively the same results. Adding cAMP as well as cGMP inhibited the growth of cells carrying these mutations. Alanine, isoleucine, or valine substitution at 141 resulted in Crp^+ phenotypes. These mutants responded well to cAMP but, unlike wild-type CRP, did not show the minimal response to cGMP. At position 144, phenylalanine- or valine-substituted mutants were active in the absence of cyclic nucleotides. Addition of cAMP inhibited the growth of the cells carrying phenylalanine, valine, or cysteine substitution. cGMP also inhibited the growth of cells harboring the phenylalanine-substituted mutant. Although the cysteine mutant had no activity in the absence of cyclic nucleotide, it responded strongly to cGMP.

The numbers in Table 2 cannot be compared directly with those in Table 1 because the strain background in Table 2 is lac^+ rather than the lac_{am} of Table 1. However, within each table, each *crp* mutant can be expressed relative to wild type, allowing comparisons between the two groups. We summarize Tables 1 and 2 as follows. The polar amino acids serine, glutamine, arginine, and lysine at position 141 show a Crp* phenotype. Substitution by hydrophobic leucine, alanine, isoleucine, valine, or tyrosine causes a phenotype that is basically wild type, but with a difference. The cGMP stimulation of wild-type CRP that occurs when crp^+ is multicopy (Table 2, line 1) is absent or decreased in these mutants. This finding implies that these mutants with a hydrophobic amino acid 141 must have a difference in their conformation, compared with wild type, that prevents the low-level cGMP stimulation. Perhaps these hydrophobic amino acids "glue" the C and D helices into an inactive conformation. At position 144, any amino acid that was substituted in this set showed a Crp* property, regardless of its nature. However, they all have larger side chains than does alanine.

Double Mutation. Amino acid 141 of the D α-helix of the carboxyl domain appears to be oriented into the hinge toward the C α-helix in the three-dimensional structure of CRP (30). There is potential for interaction of these amino acids with amino acids 133 and 134 of the C α-helix (not shown in the figure) and 137 and 138 of the hinge. Since our results show that polar amino acids at position 141 allow a Crp* phenotype, we studied the nature of interaction, if any, between amino acid 141 and amino acid 137 (leucine in wild type) or 138 (aspartic acid in wild type) across the hinge. We varied the amino acid at position 141 by site-directed mutagenesis and introduced an amber codon at position 137 or 138 for each variant at 141. Suppression of the amber codon with different tRNA suppressors created various combinations of double mutants whose phenotypes we tested.

We have studied in this way 20 different combinations of amino acids at positions 141 and 137: glycine (wild type), valine, isoleucine, serine, or arginine at 141 and leucine (wild type), serine, glutamine, or tyrosine at 137. In almost all of these combinations, the Crp^+ or Crp* phenotype corresponding to the amino acid at 141 more or less persisted regardless of the amino acid at position 137. The presence of glycine, valine, or isoleucine at position 141 gave the protein a wild-type phenotype for all of the changes at position 137. Similarly, serine or arginine at 141 maintained a Crp* phenotype with the four different amino acids at position 137, the only exception being the Ser^{137}–Arg^{141} combination, which caused a Crp^- phenotype (data not shown).

A similar study of the 20 combinations of amino acids between positions 141 and 138 gave us different results (Table 3). For each of the five amino acids tried at position 141, there

Table 2. β-Galactosidase activity for mutant CRP plasmids

crp genotype on plasmid	Amino acid	β-Galactosidase activity, Miller units			Crp phenotype
		–	+ cAMP	+ cGMP	
crp^+	—	24	1451	420	+
crp^- 141	Arginine	401 (16.7)	R^i	R^i	*
(wild type is	Glutamine	1193 (49.7)	R^i	R^i	*
glycine)	Lysine	1029 (42.9)	R^i	R^i	*
	Alanine	16 (0.7)	1625 (1.1)	44 (0.1)	+
	Isoleucine	15 (0.6)	2079 (1.4)	15 (0.04)	+
	Valine	19 (0.8)	2220 (1.5)	40 (0.1)	+
crp^- 144	Phenylalanine	553 (23.0)	R^i	R^i	*
(wild type is	Valine	228 (9.5)	R^i	639 (1.5)	*
alanine)	Cysteine	20 (0.8)	R^i	1227 (2.9)	*

Host strain for the plasmids was G839. It is $\Delta crp\ \Delta cya\ lac^+$. Numbers in parentheses represent the β-galactosidase specific activity measured under the given conditions divided by β-galactosidase specific activity measured in G839 containing multicopy crp^+. For explanation of symbols, see footnote to Table 1.

Table 3. Phenotype of CRP with double amino acid substitutions (positions 141 and 138)

crp genotype	Suppressor amino acid	β-Galactosidase activity, Miller units –	+ cAMP	+ cGMP	Crp phenotype
crp_{am138} Gly[141]	Serine	7 (1.2)	487 (2.2)	10 (0.2)	+
	Glutamine	11 (0.9)	574 (1.6)	14 (0.2)	+
	Tyrosine	12 (1.1)	71 (8.713)	13 (0.2)	–
	Leucine	40 (1.1)	655 (0.3)	43 (0.1)	+
crp_{am138} Val[141]	Serine	9 (1.5)	524 (2.4)	106 (1.7)	+
	Glutamine	13 (1.1)	505 (0.8)	12 (0.2)	+
	Tyrosine	13 (1.2)	28 (0.03)	12 (0.2)	–
	Leucine	32 (0.9)	189 (0.1)	37 (0.1)	–
crp_{am138} Ser[141]	Serine	52 (8.7)	R[i]	580 (9.5)	*
	Glutamine	12 (1.0)	1250 (2.0)	54 (0.7)	+
	Tyrosine	3 (0.3)	1 (0.001)	1 (0.02)	–
	Leucine	36 (1.0)	1110 (0.6)	41 (0.1)	+
crp_{am138} Ile[141]	Serine	8 (1.3)	539	11 (0.2)	+
	Glutamine	10 (0.8)	55	11 (0.1)	–
	Tyrosine	10 (0.9)	11	11 (0.2)	–
	Leucine	31 (0.9)	36	32 (0.1)	–
crp_{am138} Arg[141]	Serine	63 (10.5)	R[i]	632 (10.4)	*
	Glutamine	12 (1.0)	1068 (1.7)	57 (0.7)	+
	Tyrosine	12 (1.1)	705 (0.8)	14 (0.3)	+
	Leucine	30 (0.8)	2786 (1.4)	85 (0.3)	+

Host strains were those in Table 1. –, Crp⁻ phenotype; for other symbols, see footnotes to Table 1.

was at least one amino acid at position 138 which retained the original Crp⁺ or Crp* phenotype due to a single change at position 141. But 12 of the 20 combinations changed the phenotype of a single substitution at 141. Noteworthy of these are the changes of the Crp* behavior of Ser[141]–Asp[138] to Crp⁺ of Ser[141]–Leu[138], the Crp⁺ of Ile[141]–Asp[138] to Crp⁻ of Ile[141]–Gln[138]/Leu[138] and Crp* of Arg[141]–Asp[138] to Crp⁺ of Arg[141]–Gln[138]/Leu[138]. Unlike the 141–137 combinations, some of the 141–138 pairs showed a strong Crp⁻ phenotype. The amino acid at position 138 also seems to affect the cGMP stimulation of multicopy wild-type CRP. For example, when we substituted serine, glutamine, tyrosine, or leucine for wild-type aspartate at position 138, there was no cGMP stimulation (compare lines 1–4 of Tables 1 and 3).

DISCUSSION

CRP makes contact with DNA through amino acid–base interactions in which amino acid side chains of the recognition α-helix enter the major groove to make specific contacts with base edges (11–14). We have proposed that the recognition helix of CRP is unable to make such major-groove contacts unless its solvent-exposed amino acid side chains protrude out of the protein bulk (15, 18). We believe that the protrusion is a result of the cAMP-induced allosteric transition of CRP. This conformation can also be achieved without cAMP, at least partially, by mutational changes in the protein (15–17, 30–32). Previous genetic studies combined with structural information have suggested specific amino acid interactions to achieve a hinge bending-initiated protrusion of the F α-helix (11, 12, 14, 15). One such potential interaction is between Gly[141] of the D α-helix and Leu[137] or Asp[138] at the C α-helix end of the hinge region. We propose that this interaction(s) causes or facilitates hinge bending and repositioning, resulting in susceptibility of the carboxyl-terminal portion of the C α-helix to proteases. Reorientation or swiveling of the D α-helix changes the side of the D α-helix that faces the F α-helix. The amino acid residues at the D–F α-helices interface push the F α-helix away from the body of protein, very likely because of van der Waals repulsion. This protrusion of the F α-helix makes DNA contacts more favorable (Fig. 2). Of course, concomitant to this process around the hinge, cAMP also induces alterations in the alignment between domains (11, 12, 18). The domain–domain interaction is probably a result of cAMP interactions with the β-roll portion of the cAMP binding site; e.g., there may be hydrogen bond formation between Tyr[63] in β-sheet 5 and Glu[171] in the E α-helix. Interactions such as 138–141 close to the hinge and distal interactions such as 63–171 undoubtedly contribute to stability. It seems that the 138–141 interaction may be necessary, but not sufficient, for optimal property.

In this study, we have tested the proposed interactions near the hinge by substituting a number of other amino acids for Gly[141] and Ala[144]. We have also made double amino acid substitutions to investigate probable interaction between 141 and 137 or 138 as suggested above.

Position 141. If the wild-type Gly[141] is replaced by a hydrophobic amino acid (Ala, Val, Leu, or Ile), the protein retains a Crp⁺ phenotype. However, replacement of Gly[141] by a polar amino acid (Ser, Gln, Lys, Glu, or Asp) results in

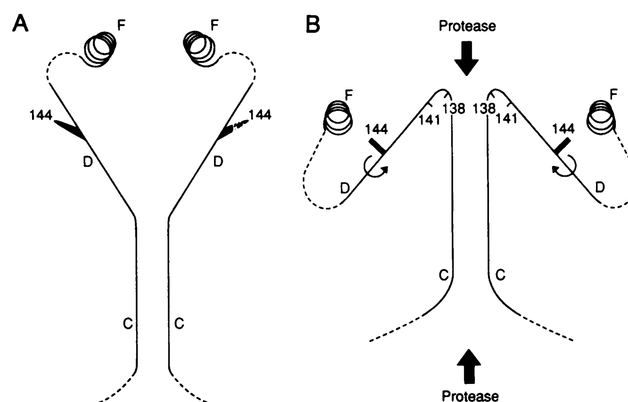

FIG. 2. Model of CRP activation. The proposed change in CRP caused by cAMP addition or by CRP* mutation is shown in exaggerated form. When the hinge of the protein is in an open conformation (*A*), the protein is resistant to protease. In the active form of CRP (*B*), the hinge angle has decreased: there is interaction between residues 138 and 141. A concomitant swiveling of the D α-helix allows an interaction between amino acids on the 144 side of the helix and the F α-helix. The protein is sensitive to various proteases (shown by large arrows), which could attack from the top or bottom.

at least partial independence of CRP from cAMP and stimulation by cGMP—i.e., imparts a Crp* phenotype. We correlated the relative β-galactosidase activity of the various 141-substitution mutants with the hydration potential (33) and bulkiness of the side chain (34). Substitutions assayed in the lac_{am} strain were compared among themselves, as well as those assayed in a genotypically lac^+ background. There was no correlation between bulkiness of the 141 side chain and activity in the absence of cAMP ($r = -0.29$ for the lac_{am} set and $r = -0.01$ for the lac^+ set). However, there was a strong correlation between negative hydration potential and cyclic nucleotide-independent activity ($r = -0.824$ for the lac_{am} set and $r = -0.966$ for the lac^+ set). These results show that a critical requirement for cAMP independence was the presence of a polar residue at 141. Double substitution experiments involving positions 141 and 137 or 138 gave an insight into how polar amino acids at position 141 may invoke an active CRP structure without cAMP.

We did not find any essential interaction between the amino acids 141 and 137. With the exception of the Arg^{141}–Ser^{137} combination, changing the residue at 137 from the wild-type Leu had no effect on the CRP phenotype. Residue 141 determined the phenotype. Indeed, when total hydration potential (the hydration potential for the residue at 141 plus the hydration potential for 137) was plotted vs. relative β-galactosidase activity (data not shown), the correlation (r) of only -0.256 for the lac_{am} set. For bulkiness, there was a correlation of only -0.0997.

On the other hand, we demonstrated an interaction between 141 and 138. Regardless of the amino acid at 141, the CRP phenotype was strongly affected by residue 138. The CRP* phenotype required polar amino acids at positions 138 and 141. For the lac_{am} set, the correlation between total hydration potential for residues at 141 and 138 and relative β-galactosidase was $r = -0.649$. For the smaller lac^+ set, the correlation was even higher: $r = -0.966$. There were a few exceptions to this rule: Tyrosine never resulted in a CRP* phenotype when at either position 138 or 141. When arginine was at position 141 and glutamine at position 138, there was no CRP* phenotype, although the total hydration potential was high. Similarly, it is difficult to explain why a Ser^{141}–Glu^{138} combination is not CRP*. Clearly, having polar amino acids at these two positions is a necessary, but not sufficient, component of CRP*. There was no correlation between total bulkiness and relative β-galactosidase ($r = 0.000$ for the lac_{am} set and $r = -0.011$ for the lac^+ set).

We propose the following. (*i*) **Crp***. A polar interaction between residues 138 and 141 shapes the hinge into a conformation similar to the one induced by cAMP. An interaction between two polar amino acids at positions 141 and 138 mimics what is normally achieved by cAMP binding. (*ii*) **CRP⁺**. Occupancy of one of the two sites by a polar amino acid and the other by a hydrophobic residue causes a cAMP-dependent Crp^+ phenotype. Interestingly, this is true regardless of the relative positions of the polar and hydrophobic residues. (*iii*) **Crp^-**. If two hydrophobic amino acids are inserted at 141 and 138, CRP becomes totally or partially defective (Crp^-). For example, an Ile^{141}–Leu^{138} combination does not activate the *lac* operon even in the presence of cAMP.

Position 144. Replacement of Ala^{144} by any amino acid tried resulted in a cAMP-independent phenotype. What these replacement amino acids have in common is that they are all larger than alanine. There is a good correlation between bulkiness and relative β-galactosidase activity: for the lac_{am} set, $r = 0.579$, and for the lac^+ set, $r = 0.909$. Based on this result and the position of amino acid 144 in the crystal structure, we affirm the model that the increased length of the substituted amino acid side chain allows pushing against the DNA-binding F α-helix. This change sets the F α-helix for proper binding in the major groove of DNA. Fig. 2 describes our model.

Conformational change in protein by hinge bending has been well documented for T4 lysozyme (35) and *E. coli* Trp repressor (36). Trp repressor is a gene-regulatory allosteric protein that requires L-tryptophan for the recognition of the operator sequence. By contrasting the crystal structures of the unliganded aporepressor and the liganded repressor, Sigler and coworkers showed that the binding of L-tryptophan to the aporepressor caused a change in the orientation of the DNA-binding helix–turn–helix motif by affecting the hinge connecting those helices (reviewed in ref. 36). This finding was further supported by the isolation of mutants in the hinge connecting two helices. In this mutant the small side chain of alanine was replaced by the bulkier side chain of valine and the mutant repressor did not require L-tryptophan for operator specific binding. Thus, the presence of a bulkier group in the hinge maintained the helix–turn–helix motif in the DNA-binding form. In a similar fashion, the hinge of CRP can be reoriented by cAMP or by amino acid substitution.

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