Biochem. J. (1992) 287, 627–632 (Printed in Great Britain)

^{19}F n.m.r. studies of conformational changes accompanying cyclic AMP binding to 3-fluorophenylalanine-containing cyclic AMP receptor protein from *Escherichia coli*

Mark G. HINDS, Rodney W. KING and James FEENEY*
Laboratory of Molecular Structure, National Institute for Medical Research, Mill Hill, London NW7 1AA, U.K.

A fluorine-containing analogue of the cyclic AMP (cAMP) receptor protein (CRP) from *Escherichia coli* was prepared by biosynthetic incorporation of 3-fluorophenylalanine (3-F-Phe). ^{19}F n.m.r. studies on this protein have provided direct evidence for cAMP-induced conformational changes not only within the cAMP-binding domain but also within the hinge region connecting the cAMP-binding domain to the DNA-binding headpiece. At 313 K, the ^{19}F n.m.r. spectrum of [3-F-Phe]CRP showed five signals corresponding to the five phenylalanine residues as expected for a symmetrical dimer. Proteolysis of [3-F-Phe]CRP with subtilisin produced a fragment (the α-fragment) containing the cAMP-binding domain. The α-fragment contains all the phenylalanines except for Phe-136, a residue located in the hinge region. By comparing the ^{19}F spectra of [3-F-Phe]CRP and its α-fragment, the signal for Phe-136 was assigned. The chemical shifts of the corresponding signals in the two spectra are similar, indicating that the α-fragment retains the structure it has in the intact protein. The largest cAMP-induced shift was observed for the signal from Phe-136 providing direct evidence for a conformational change in the hinge region. However, whereas binding of a single cAMP molecule to a CRP dimer is known to be sufficient to activate the DNA binding, the n.m.r. data indicate that the hinge region does not have the same conformation in both subunits when only one cAMP molecule is bound.

INTRODUCTION

In bacteria, activation of several catabolite-sensitive genes is controlled by the concentration of cyclic AMP (cAMP) in the cell, a process mediated by the cAMP receptor protein (CRP). In the presence of cAMP this protein binds specifically to particular DNA sites which regulate the expression of a number of genes [1,2]. Examples of genes controlled in this way are those encoding enzymes involved in the catabolism of several sugars including lactose, arabinose and maltose [3,4]. CRP is a 47 kDa homodimer, with each of its subunits capable of binding a cAMP molecule [5,6]. The DNA-regulation site comprises the sequence 5′-TGTGA-3′ separated by a 6 bp block (with little sequence conservation) from an inverted repeat of the 5′-TGTGA-3′ or a closely related structure [1,2]. Data from X-ray crystallography [7–10] and site-directed mutagenesis [11] indicate that these two regions of the DNA sequence bind to the two subunits of the CRP dimer using a helix–turn–helix motif located in the headpiece of each monomer. For the protein from *Escherichia coli*, it has been shown [12,13] that the binding of one cAMP molecule to the dimer is sufficient to confer DNA-binding specificity. Thus binding of a single cAMP molecule to the CRP dimer causes a conformational change which allows at least one of the helices (helix F, residues Arg-180–Gln-193) to become correctly positioned for specific binding to the DNA. Contact residues between helix F and CRP have been defined [10,11] and extensive bending of the DNA on binding to CRP has also been observed [10]. At present, details of the nature of the cAMP-induced conformational change in CRP required for specific DNA binding are not known. Although crystal-structure data have been obtained for the CRP–cAMP and CRP–cAMP–DNA complexes [7,10], no published information is available for the structure of the free CRP dimer.

Two general models for the cAMP-induced conformational change have been discussed by Steitz and co-workers [7,9], and these involve either an induced change in the relative orientation of the two DNA-binding domains or a change in the overall relative orientation of the two subunits of the dimer. In the first model the conformational effects are considered to be transmitted largely through the monomer units from the cAMP-binding site to the headpiece containing distal helix F. Garges & Adhya [14] have studied mutant CRP proteins which function in the absence of cAMP and their findings implicate specific residues in helix D (located in the hinge region of the DNA-binding domain) in the allosteric conformational change. These workers postulated that cAMP binding to residues in helix C (as shown by Steitz and co-workers [7,9]) transmits conformational changes via other residues in helix C to interacting residues in the neighbouring helix D and ultimately on to helix F. The altenative model for the allosteric conformational changes requires that cAMP binding alters the overall relative positions of the cAMP-binding subunits, which in turn move the DNA-binding F-helices into the appropriate conformation for DNA binding. The crystallographic work of Steitz and co-workers [7,9] has shown that, although each bound cAMP molecule has most of its binding interactions localized in one of the monomer units, the bound cAMP also makes some interactions with residues on helix C of the other monomer unit. This clearly opens up the possibility that the relative positions of the two subunits could be repositioned by the binding of a single cAMP molecule. The fact that activation of CRP can be caused by binding only one cAMP molecule [12] could also be more directly explained using the latter model.

In order to obtain more detailed information about cAMP binding to CRP in solution, we have used high-resolution n.m.r. spectroscopy to examine complexes in which cAMP is bound to isotopically labelled CRP molecules. We have previously examined samples of CRP containing 3-fluorotyrosine ([3-F-Tyr]CRP) and 5-fluorotryptophan ([5-F-Trp]CRP) [15]. Studies

Abbreviations used: cAMP, cyclic AMP; CRP, cAMP receptor protein; [3-F-Phe]CRP, 3-fluorophenylalanine-containing CRP; [3-F-Tyr]CRP, 3-fluorotyrosine-containing CRP; [5-F-Trp]CRP, 5-fluorotryptophan-containing CRP.

* To whom correspondence should be addressed.

with these proteins indicated that some conformational changes accompany the cAMP binding [15] and furthermore showed that the two cAMP-binding sites on CRP interact with each other [16]. In this present study we have extended the ^{19}F n.m.r. measurements to include a CRP molecule containing 3-fluorophenylalanine ([3-F-Phe]CRP).

Fluorine-19 has proved to be a useful nucleus for n.m.r. studies of large proteins. It has good natural sensitivity (second only to that of the proton among naturally occurring isotopes) and gives spectra free of unwanted background signals. Although the signals are broad, useful information can usually be obtained from the n.m.r. spectra for two reasons. Firstly, the sensitivity to the shielding of the ^{19}F nucleus to changes in its environment can result in appreciable chemical-shift differences which can potentially give rise to simple ^{19}F spectra containing well-resolved resonances even when these are broad. Secondly, the factors controlling large ^{19}F chemical-shift differences in proteins can be attributed to differences in the electric fields [17–20] experienced by the ^{19}F nuclei and in some cases these can be interpreted in terms of conformational differences between complexes.

Second-order electric-field effects appear to dominate the shielding [19,20] and these give rise to deshielding contributions (that is downfield shifts). Hull & Sykes [20] have estimated that the van der Waal's second-order term gives large deshielding contributions when the interacting group is close to the ^{19}F nucleus (greater than 10 p.p.m. at 0.25 nm falling to less than 1 p.p.m. at 0.5 nm). To a first approximation, the ^{19}F deshielding effects can be correlated with steric interactions where groups in close proximity to the fluorine nucleus cause a decrease in the shielding of the nucleus (that is a chemical shift to lower field values). If a downfield shift of the fluorine nucleus is observed on binding the ligand, then this can arise from either the ligand being in close spatial proximity to the fluorine nucleus or the ligand inducing a conformational change which brings some other group in the protein near to the fluorine nucleus in question. If, on the other hand, the binding of a ligand causes the fluorine signal to move to higher field values, this can only be attributed to the removal of a steric interaction and this is only possible as a result of an induced conformational change.

An ^{19}F nucleus close to the bound ligand can also experience 'direct' shielding effects (for example from interactions involving charged groups). An approximate estimate of the shielding effects arising from the presence of a neighbouring charged group can be obtained by measuring the pH-dependence of ^{19}F nuclei in appropriate molecules containing ionizable groups. For example, for 6-F-tryptophan, deprotonation of the carboxylic acid group causes an upfield shift of 0.16 p.p.m. (J. Feeney & R. W. King, unpublished work); most of this contribution will arise from the direct electric-field shielding effect from the charged carboxylate since inductive effects would be expected to be very small. The distances between the 6-fluoro substituent and the carboxylate oxygens vary in the range 0.42–0.8 nm in the various conformations. These findings indicate that for distances greater than 1 nm the direct shielding effects from a charged group will probably be less than 0.1 p.p.m.

MATERIALS AND METHODS

cAMP was purchased from Sigma Chemical Co. [3-F-Phe]-CRP was prepared biosynthetically using a similar method to that described previously for [3-F-Tyr]CRP and [5-F-Trp]CRP [15]. *E. coli* GCSC strain 2849t, auxotrophic for phenylalanine, tyrosine and typtophan, contains a plasmid coding for CRP. The organism was grown in M9/glucose minimal medium containing 50 mg of tyrosine and tryptophan/l, 10 mg of phenylalanine/l and 20 mg of 3-fluorophenylalanine/l. Other components of the medium were as described in Sixl *et al.* [15]. The cell contents were released by passage through a Manton–Gaulin homogenizer at low temperature in order to preserve the integrity of the protein. This was achieved by precooling the cell suspension to zero degrees and passing the output from the homogenizer directly on to a stainless-steel cooling coil at -12 °C. Two passes through the homogenizer released 90% of the available CRP. Purification of the [3-F-Phe]CRP was achieved by using a phosphocellulose column as described in Donoso-Pardo *et al.* [21]. The step involving use of a DNA–cellulose affinity column was omitted since the required decrease in pH caused irreversible denaturation of the protein. Instead, the central fractions of the peak of cAMP-binding activity eluted from the column were collected, dialysed against column starting buffer and rechromatographed on phosphocellulose. The central fractions from this peak were then pooled and the protein was precipitated using $(NH_4)_2SO_4$ at 70% saturation. The purity of the collected protein precipitate was estimated by SDS/PAGE as being greater than 95%. Samples prepared from these precipitates were then sufficiently pure for n.m.r. analysis.

[3-F-Phe]α-CRP was prepared by proteolytic digestion of [3-F-Phe]CRP with subtilisin by the procedure of Tsugita *et al.* [22]. The cleavage takes place at the Leu-116–Ser-117 linkage. Purification of the α-CRP was achieved by ion-exchange chromatography on phosphocellulose in a manner similar to that used for purifying the intact protein.

Samples for ^{19}F n.m.r. experiments were made by dialysing the protein precipitates in a small volume against the required (aqueous) buffer. The solutions for n.m.r. study were made up to 1:9 (v/v) $^2H_2O/H_2O$ by the addition of the appropriate volume of H_2O/buffer mixture, and the final CRP concentation was determined by adsorption spectroscopy. Microlitre additions of concentrated cAMP solution were made as required.

The ^{19}F n.m.r. measurements were carried out at 188.1 MHz using a Bruker WM200 spectrometer operating in the Fourier-transform mode. The spectra were typically obtained with a spectral width of 10 kHz, 8 K data points, a 90° (12 μs) pulse width and collection between 10000 and 50000 transients with a repetition time of 0.41 s and processed with a line-broadening function of 5 Hz. The ^2H in the ^2H_2O in the solution was used as a field frequency lock. No proton decoupling was employed. Free 5-fluorotryptophan (in 0.1 M-NaOH) was used as an external reference for measuring the ^{19}F chemical shifts.

RESULTS AND DISCUSSION

CRP contains five phenylalanine residues per monomer (at positions 14, 69, 76, 102 and 136). According to the crystal structure of Steitz and co-workers [7–9], each monomer consists of a cAMP-binding domain and a DNA-binding domain connected via a hinge region. Four of the phenylalanine residues are in the cAMP-binding domain, but none of these residues is expected to be in direct contact with the bound cAMP. There are no phenylalanine residues in the DNA-binding domain but Phe-136 is located in the hinge region. Fig. 1 shows the ^{19}F spectra of [3-F-Phe]CRP recorded in the absence of ligand over the temperature range 288–323 K. At 288 K the observed resonances are fairly broad and more than five signals are resolved. As the temperature is raised, the five major signals become narrower while other small broad signals decrease in intensity. These spectral changes are reversible with temperature and indicate the presence of multi-conformational states undergoing reversible interconversions. The simplest explanation of such interconverting conformational forms is that the asymmetrically substituted phenylalanine ring exists in two different rotational isomeric states involving hindered rotation about its Cβ–Cγ

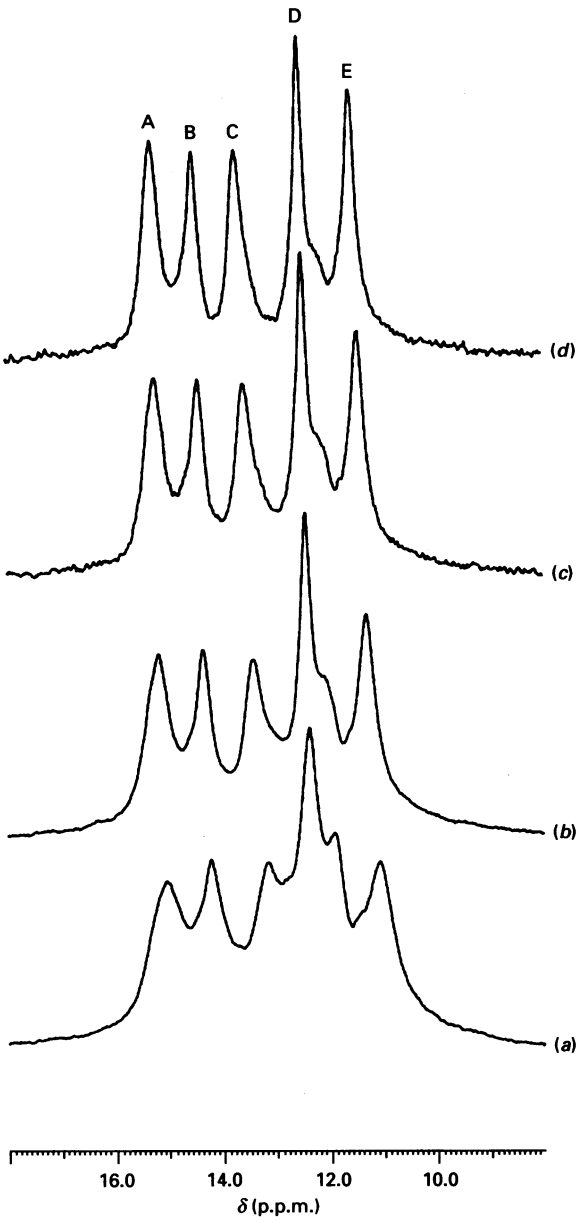

Fig. 1. ^{19}F n.m.r. spectra of [3-F-Phe]CRP at (*a*) 288, (*b*) 303, (*c*) 313 and (*d*) 323 K

Spectra were recorded at 188.1 MHz in 500 mM-KCl/10 mM-potassium phosphate, pH 7.2, in $^2H_2O/H_2O$ (1:9, v/v). The spectra are referenced to the ^{19}F signal of a free 5-fluorotryptophan (in 0.1 M-NaOH) external reference.

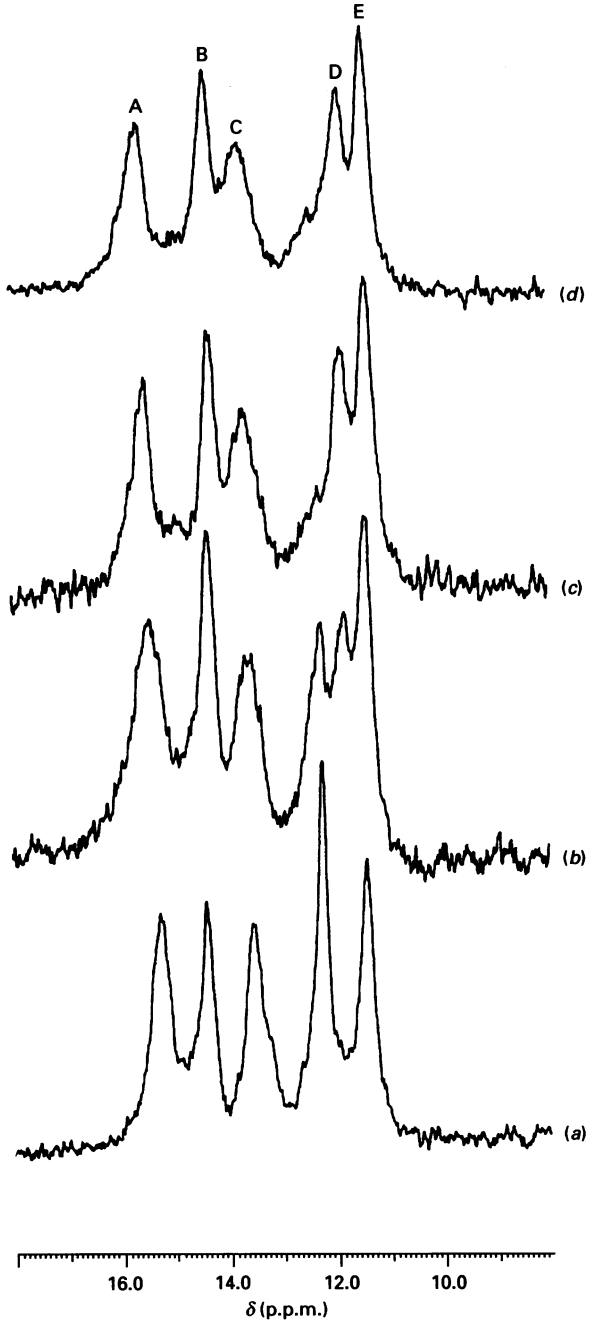

Fig. 2. ^{19}F n.m.r. spectra of [3-F-Phe]CRP (*a*) alone, (*b*) in the presence of 1 equivalent of cAMP, (*c*) in the presence of 2 equivalents of cAMP and (*d*) in the presence of 10 equivalents of cAMP (with respect to dimer)

Spectra were recorded at 188.1 MHz in 500 mM-KCl/10 mM-potassium phosphate, pH 7.2, in $^2H_2O/H_2O$ (1:9, v/v) at 313 K. The spectra are referenced to the ^{19}F signal in free 5-fluorotryptophan (in 0.1 M-NaOH).

bond. Interconversion between these two rotational isomeric states could be brought about by a 180° ring flip of the aromatic ring. Such rotational isomerism has been seen before for non-labelled phenylalanine and tyrosine residues in proteins [23–25] and for aromatic rings of bound ligands including the asymmetrically fluorine-substituted aromatic rings in aromatic sulphonamide drugs bound to carbonic anhydrase [26]. The temperature chosen for making comparisons of spectra of complexes of [3-F-Phe]CRP was 313 K where the protein gives an ^{19}F spectrum containing five major signals.

Detection of a cAMP-induced conformational change in the hinge region

In the spectrum of [3-F-Phe]CRP examined in the presence of excess cAMP at 313 K, five ^{19}F signals are observed (Fig. 2*d*). When the ^{19}F chemical shifts are compared with those of free [3-F-Phe]CRP at 313 K, signal D is found to have an upfield chemical shift (0.36 p.p.m.), whereas signals A, B, C and E show ligand-induced chemical shifts of 0.1–0.3 p.p.m. (see Tables 1 and 2). The X-ray data [7,10] indicate that none of the phenylalanine residues is in direct contact with the bound cAMP (the fluorine substituents in all the phenylalanine residues are more than 1 nm from the cAMP phosphate group). Direct ^{19}F shielding effects from interactions with the charged phosphate

Table 1. ^{19}F chemical shifts of [3-F-Phe]CRP and [3-F-Phe]αCRP examined alone and in complexes with excess cAMP (10 equivalents with respect to dimer) at 313 K

Errors are ±0.05 p.p.m. except where stated otherwise.

	^{19}F chemical shifts (p.p.m.)				
	F_A	F_B	F_C	F_D*	F_E
[3-F-Phe]CRP (alone)	15.21	14.39	13.51†	12.22	11.40
[3-F-Phe]CRP+cAMP	15.50	14.26	13.68†	11.88	11.32
[3-F-Phe]α-CRP (alone)	15.23	14.36	13.50†	–	11.43
[3-F-Phe]α-CRP+cAMP	15.57	14.32	13.87†	–	11.41

* Signal F_D is assigned to Phe-136.
† Errors ±0.1 p.p.m.

will thus be less than 0.1 p.p.m and the observed cAMP-induced chemical shifts on signals F_A, F_B, F_C and F_D must arise from conformational changes accompanying cAMP binding.

The titration against cAMP shown in Fig. 2 indicates that the cAMP binds essentially under slow-exchange conditions for signal D, although there is some evidence for exchange broadening.

In order to assign the ^{19}F signal from Phe-136, we have also examined the ^{19}F spectrum of the α-fragment of [3-F-Phe]CRP which does not contain this residue (see Fig. 3*d*). The ^{19}F spectrum of this fragment shows only four signals and these have very similar chemical shifts to four of the signals in the spectrum of the intact protein, with the signal at 12.22 p.p.m. no longer being present (see Table 1). The chemical-shift changes induced by the addition of cAMP to the α-fragment are seen to be similar to but not identical with those of the corresponding signals in the intact protein. By comparing the spectra of the complexes of the intact protein and the α-fragment formed with excess cAMP (Figs. 3*b* and 3*c*), one can again clearly identify the missing signal in the α-fragment spectrum (signal D at 11.6 p.p.m. in the [3-F-Phe]CRP–cAMP spectrum). Thus signal D can be firmly assigned to the fluorine in Phe-136 which is absent from the α-fragment. It is interesting that the largest chemical shift induced by cAMP binding is for the fluorine in the phenylalanine residue furthest removed from the cAMP-binding site, namely Phe-136 in the hinge region between the cAMP-binding domain and the DNA-binding domain. This phenylalanine residue is more than 1.7 nm from the cAMP phosphate group [7,9].

The α-fragment retains the conformation of the intact protein

The ^{19}F chemical shifts for the cAMP complexes of [3-F-Phe]CRP and its α-fragment are given in Table 1 and the cAMP-induced chemical shifts for all the fluorine-containing CRP molecules so far examined are collected in Table 2. From comparisons of the ^{19}F chemical shifts in the intact protein, the α-fragment and their complexes with cAMP, it is possible to deduce some information about their conformation. For example, the ^{19}F chemical shifts in the α-fragment of [3-F-Phe]CRP are seen to be similar to, although not identical with, those in the intact protein, and one can thus conclude that the α-fragment essentially retains the structure it has in the intact protein. The observed cAMP-induced chemical shifts are also similar for the corresponding nuclei in the intact protein and the α-fragment, which indicates that the α-fragment undergoes similar conformational changes on cAMP binding to those observed when it is part of the intact protein. Similar findings were observed in the earlier work on [3-F-Tyr]CRP [15] (see Table 2).

Binding of one cAMP molecule to the CRP dimer does not result in the same hinge-region conformation in both subunits

Whereas the binding of one cAMP molecule to the CRP dimer is sufficient to activate the specific DNA binding, such binding does not result in similar conformational changes in both subunits of the CRP dimer. In Fig. 2(*b*), the spectrum of [3-F-Phe]CRP in the presence of one equivalent of cAMP is shown: under these conditions approx. 50% of the CRP monomer will be occupied by cAMP and approx 75% of the CRP dimers will have at least one bound cAMP. However, it is seen that for signal D from Phe-136, only approx. 50% of the intensity of this signal has shifted to the new chemical shift value (11.88 p.p.m.) corresponding to the bound species. Thus only the CRP subunits occupied by cAMP experience the conformational changes as monitored by the ^{19}F chemical-shift changes. If the binding of a single cAMP molecule to the CRP dimer causes subunit reorientation in order to achieve specific DNA binding, this does not result in the hinge region having the same conformation in both subunits when one cAMP binds. Clearly the observed conformational change in the hinge region is transmitted through the α-fragment of the subunit

Table 2. ^{19}F chemical-shift differences caused by binding cAMP to [3-F-Phe]CRP, [3-F-Tyr]CRP, [5-F-Trp]CRP and their α-fragments

Errors are ±0.1 p.p.m. except where stated otherwise.

	^{19}F chemical-shift differences (p.p.m.)					
	F_A	F_B	F_C	F_D	F_E	
[3-F-Phe]CRP (313 K)	−0.29	+0.13	−0.17	+0.36	+0.08	
[3-F-Phe]α-CRP (313 K)	−0.34	−0.04	−0.37†		+0.02	
	Y_A	Y_B	Y_C	Y_D	Y_E	Y_F§
[3-F-Tyr]CRP (293 K)*	−0.34†	+0.60‡	−0.10	−0.10	−0.16	+0.01
[3-F-Tyr]α-CRP (293 K)*	−0.39†	+0.49‡	0.00	+0.45	+0.05	
	W_A	W_B				
[5-F-Trp]CRP (293 K)*	−0.60	+0.60				

* Data taken from ref. [15].
† Errors ±0.2 p.p.m.
‡ Errors ±0.3 p.p.m.
§ Signal Y_F is assigned to Tyr-206 [15].

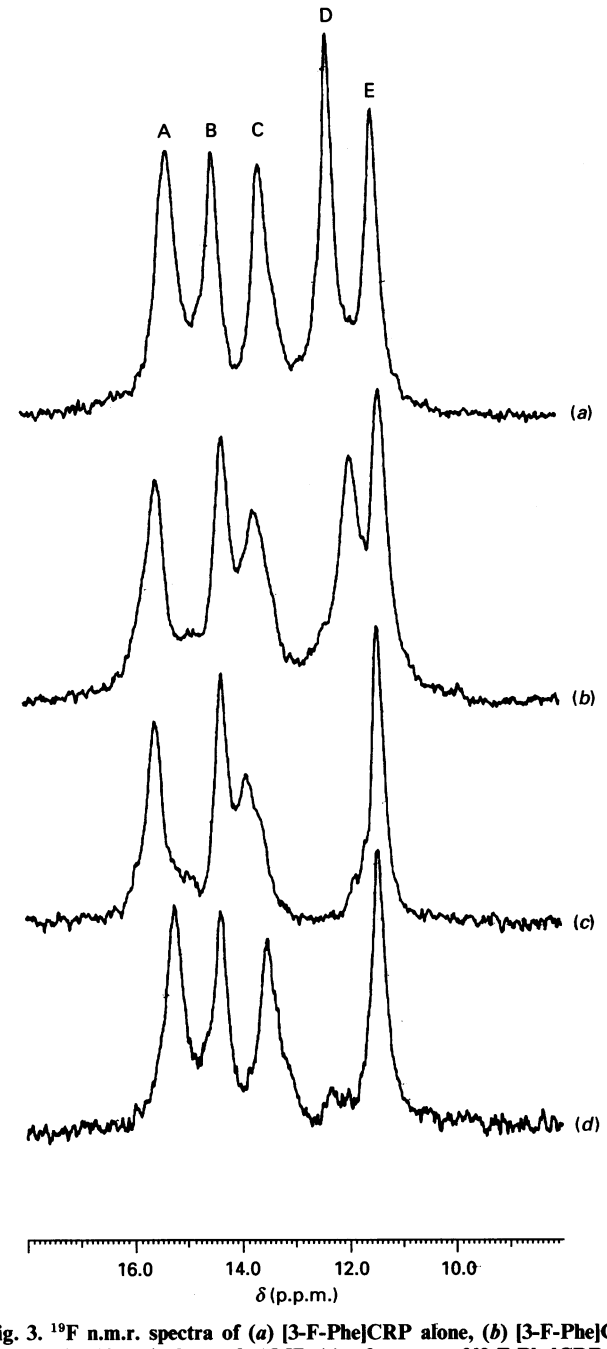

Fig. 3. ^{19}F n.m.r. spectra of (*a*) [3-F-Phe]CRP alone, (*b*) [3-F-Phe]CRP with 10 equivalents of cAMP, (*c*) α-fragment of [3-F-Phe]CRP with 10 equivalents of *c*AMP (with respect to dimer) and (*d*) α-fragment of [3-F-Phe]CRP

Spectra were recorded at 188.1 MHz in 500 mM-KCl/10 mM-potassium phosphate, pH 7.2, in $^2H_2O/H_2O$ (1:9, v/v) at 313 K. The spectra are referenced to the ^{19}F signal of free 5-fluorotryptophan (in 0.1 M-NaOH).

binding the cAMP and not simply from a general reorientation of the two subunits.

CONCLUSIONS

Six of the 13 residues monitored show cAMP-induced ^{19}F chemical-shift changes of approx 0.3 p.p.m. or greater (see Table 2). None of the aromatic residues is in direct contact with the bound cAMP and thus the observed chemical shifts must be caused by a long-range conformational change induced by the cAMP. The observed chemical-shift changes are fairly small when considered in the context of the sensitivity of ^{19}F shielding to small changes in the nuclear environment and thus the conformational changes monitored by these shift changes are probably modest. Most of the residues showing chemical-shift changes are in the α-core part of the intact protein (see Table 2). However, one of the substantial shifts induced by cAMP binding is for signal D which arises from Phe-136, a residue located in the hinge region of the intact protein. The observed increase in shielding on binding cAMP is consistent with an induced long-range conformational change resulting in lower steric interactions for the fluorine in Phe-136. It is worth noting that the only other available reporter group outside of the α-core, namely [3-F-Tyr-206] in the DNA binding headpiece, shows no perturbation of its ^{19}F chemical shift on cAMP binding [15].

Thus the data indicate that cAMP binding causes modest conformational changes within the α-core of CRP and that these are transmitted to the hinge region. The binding of a single cAMP molecule, although not optimally organizing the headpieces in both subunits for DNA interaction, could result in one of the F-helices becoming available for specific DNA interaction. The F-helix of the other subunit would then become involved in the binding during a subsequent 'zipper'-type [27,28] interaction to complete the specific interaction with the DNA. In such an interaction an initial nucleation complex can be formed by interaction of a segment of the ligand with a subsite on the protein and this is followed by conformational rearrangements of the partially bound ligand to complete the binding of the remaining segments with their appropriate subsites.

The n.m.r. measurements were made using the facilities at the MRC Biomedical NMR Centre, Mill Hill, London NW7 1AA, U.K.

REFERENCES

1. de Crombrugghe, B. & Pastan, I. (1978) in The Operon (Miller, J. & Reznikoff, W., eds.), pp. 303–324, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
2. de Crombrugghe, B., Busby, B. & Buc, H. (1984) in Biological Regulation and Development (Goldberger, R. G. & Yamamoto, K. R., eds.), vol. 3B, pp. 129–167, Plenum Publishing Corp., New York
3. de Crombrugghe, B., Chen, B., Gottesman, M., Pastan, I., Varmus, H. E., Emmer, M. & Perlman, R. L. (1971) Nature (London) New Biol. **230**, 37–40
4. Ogden, S., Haggerty, D., Stoner, D., Kolodonbetz, D. & Schleit, R. (1980) Proc. Natl. Acad. Sci. U.S.A. **77**, 3346–3350
5. Anderson, W. F., Schneider, A. B., Emmer, M., Perlman, R. L. & Pastan, I. (1971) J. Biol. Chem. **246**, 5929–5937
6. Anderson, W. F., Perlman, R. L. & Pastan, I. (1972) J. Biol. Chem. **247**, 2717–2722
7. McKay, D. B. & Steitz, T. A. (1981) Nature (London) **290**, 744–749
8. Steitz, T. A., Ohlendorf, D., Makay, D., Anderson, W. F. & Matthews, J. B. (1982) Proc. Natl. Acad. Sci. U.S.A. **79**, 3097–3100
9. Steitz, T. A., Weber, I. T. & Matthews, J. B. (1982) Cold Spring Harbor Symp. Quant. Biol. **47**, 419–426
10. Schultz, S. C., Shields, G. C. & Steitz, T. A. (1991) Science **253**, 1001–1007
11. Gent, M. E., Gronenborn, A. M., Davies, R. W. & Clore, G. M. (1987) Biochem. J. **242**, 645–653
12. Fried, M. G. & Crothers, D. M. (1984) J. Mol. Biol. **172**, 241–262
13. Heyduk, T. & Lee, J. C. (1989) Biochemistry **28**, 6914–6924
14. Garges, S. & Adhya, S. (1985) Cell **41**, 745–751
15. Sixl, F., King, R. W., Braken, M. & Feeney, J. (1990) Biochem. J. **266**, 545–552
16. Hinds, M. G., King, R. W. & Feeney, J. (1991) FEBS Lett. **283**, 127–130

17. Buckingham, A. D. (1960) Can. J. Chem. **38**, 300–307
18. Raynes, W. T., Buckingham, A. D. & Berstein, H. J. (1962) J. Chem. Phys. **36**, 3481–3488
19. Feeney, J., Sutcliffe, L. H. & Walker, S. M. (1966) Mol. Phys. **11**, 117–128
20. Hull, W. E. & Sykes, B. D. (1976) Biochemistry **15**, 1535–1546
21. Donoso-Pardo, J. L., Turner, P. C. & King, R. W. (1987) Eur. J. Biochem. **168**, 687–694
22. Tsugita, A., Blazy, B., Takahashi, M. & Baudras, A. (1982) FEBS Lett. **144**, 304–308
23. Campbell, I. D., Dobson, C. D., Moore, G. R., Perkins, S. J. & Williams, R. J. P. (1975) Proc. R. Soc. London Ser. B **189**, 503–509
24. Snyder, G. H., Rowan III, R., Karplus, S. & Sykes, B. D. (1975) Biochemistry **14**, 3765–3777
25. Wüthrich, K. & Wagner, G. (1975) FEBS Lett. **50**, 265–268
26. Dugad, L. B., Cooley, C. R. & Gerig, J. T. (1989) Biochemistry **28**, 3955–3960
27. Burgen, A. S. V., Roberts, G. C. K. & Feeney, J. (1975) Nature (London) **253**, 753–755
28. Laiken, N. & Nemethy, G. (1970) J. Phys. Chem. **74**, 4421–4441

Received 10 January 1992/14 April 1992; accepted 23 April 1992