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Letter to the Editor: Backbone NMR assignments of a high molecular weight protein (47 kDa), cyclic AMP receptor protein (apo-CRP)

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Biological context

The cyclic AMP receptor protein (CRP) plays a key role in the regulation of the expression of more than 100 genes. CRP is a dimeric protein composed of two chemically identical subunits. Each subunit has a molecular mass of 23 619, as deduced from the nucleotide sequence (Aiba et al., 1982), and is 209 amino acids long with two cAMP binding sites (Passner and Steitz, 1997). CRP functions by binding, in the presence of the allosteric effector cAMP, to specific DNA sites, and interacting with RNA polymerase (Adhya and Garges, 1990). The structures of CRP*cAMP and CRP*cAMP*DNA complexes were solved by X-ray crystallography (Weber and Steitz, 1987; Parkinson et al., 1996). However, a structural understanding of the allosteric conformational change of CRP by the cAMP binding, which is essential for its function, is still missing, because the structure of apo-CRP, CRP without a bound molecule, has not been solved yet. In this report, we describe the nearly complete NMR backbone assignments of apo-CRP.

Methods and results

CRP is a relatively large molecule (47 kDa molecular mass) that has been considered rather large to be assigned by NMR. Therefore, to assign the protein, we used triple-resonance spectroscopy in the constant time mode for the fully (∼90%) deuterated and the ^{13}C, ^{15}N labeled sample to enhance sensitivity and resolution (Yamazaki et al., 1994; Shan et al., 1996).

Triply (^{15}N, ^{13}C, 90% ^{2}H)-labeled CRP was prepared from the overproducing *E. coli* strain pp47 containing the plasmid pLCRP1. The cells were adapted to growth in D_2O, and a single colony from the 90% D_2O plate was grown in triple-labeled M9 media (90% D_2O, 1 g/L $^{15}NH_4Cl$, 2 g/L ^{13}C-Glucose) containing a mixture of vitamins for large-scale purification. Specific amino acid selectively labeled CRP was prepared from the overproducing *E. coli* strain BL21 containing the plasmid pT_7-CRP. The expression of CRP was induced by adding IPTG to a concentration of 0.5 mM with an isotope-labeled amino acid. In this manner, we performed selective ^{15}N-labeling for the following 12 specific amino acids: Ala, Cys, Phe, His, Ile, Lys, Leu, Met, Arg, Thr, Val, and Tyr. CRP was purified by sequential application of a cation-exchange column (Bio-Rex 70) and adsorption chromatography (HTP), and concentrated to a final concentration of 0.6 mM for dimer.

All of the NMR spectra were obtained at 40 °C with protein dissolved in 50 mM potassium phosphate buffer (pH 6.0) containing 0.5 M KCl. For the fully (90%) deuterated and the ^{13}C,^{15}N labeled sample, five triple resonance spectra (HNCA, HN(CO)CA, HNCO, HN(CA)CB, HN(COCA)CB) and a ^{15}N-edited NOESY-HSQC (150 ms mixing time) were recorded on a Bruker DRX 600 spectrometer. The ^{15}N dimensions of the triple resonance spectra were recorded in the constant time mode. The ^{1}H-^{15}N HSQC spectrum of the triple-labeled CRP was collected on a Bruker DRX 800 spectrometer. The ^{1}H-

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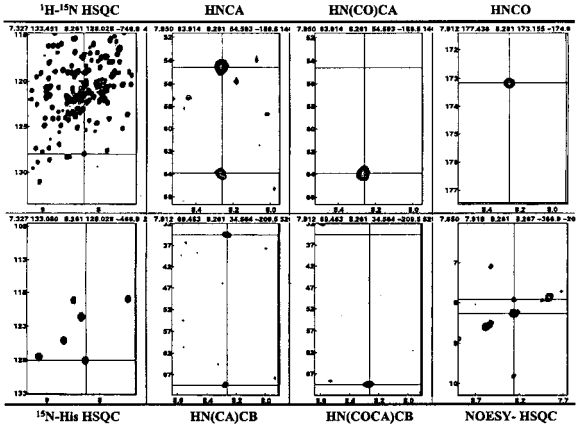

Figure 1. Verification of a peak cluster. The 12 peaks are combined into a peak cluster. The x-axis of the individual spectrum represents the H^N chemical shifts. The y-axis of the HSQC and NOESY-HSQC spectra represents ^{15}N and ^{1}H chemical shifts, respectively, whereas the y-axes of the other spectra represent the ^{13}C carbon chemical shifts of the same ^{15}N-edited slice. Each peak at the center of the crosshair in each spectrum appears with the same ^{1}H and ^{15}N chemical shifts. This drawing was made using the NMRView program.

^{15}N selective HSQC spectra of the above mentioned 12 specific amino acid labeled samples were collected on Bruker AMX 500 and DRX 500 spectrometers. All spectra were processed and analyzed using the NMRPipe/NMRDraw software (Delaglio et al., 1995) and the NMRView program (Johnson and Blevins, 1994).

We observed that the number of peaks in the HSQC spectra (^{15}N-fully and selectively labeled HSQC) is equal to the number of residues of one subunit. Thus, our data demonstrate that the two subunits of apo-CRP in solution are symmetric.

Extent of assignments and data deposition

Sequential assignment was achieved by verifying and linking of peak clusters. Because any one residue manifests the same H^N and ^{15}N chemical shift signals in each spectrum, the related peaks could be combined into a peak cluster. Each cluster provides information on the intra- and inter-residue chemical shifts {^{15}N(i), H^N(i), $^{13}C^{\alpha}$(i), $^{13}C^{\beta}$(i), $^{13}C^{\alpha}$(i−1), $^{13}C^{\beta}$(i−1), ^{13}C=O(i−1), H^N(i−1), and H^N(i+1)}. For example, each subunit of CRP has five histidines, and Figure 1 shows a peak cluster composed of 12 peaks originating from a His residue, which was finally assigned to His159. The individual amino acid type of each peak cluster was identified according to the selective HSQC, and we used an in-house program to assist in the identification of the spin system, which could not be assigned by the selective HSQC. Based on the chemical shift statistics in the BioMagResBank, this program showed several amino acid candidates for each cluster. These clusters could be sequentially linked according to the $^{13}C^{\alpha}$, $^{13}C^{\beta}$ and, in helical regions, H^N chemical shifts of the clusters.

Consequently, the backbone amide assignments ($^{1}H^N$, ^{15}N) were obtained for 192 (95%) of the 202 possible amide resonances (209 residues minus six prolines and the N-terminal residue). Nearly complete assignments were achieved for other nuclei: 97% for $^{13}C^{\alpha}$, 97% for $^{13}C^{\beta}$, and 95% for ^{13}C=O. The $^{1}H^N$, ^{15}N, $^{13}C^{\alpha}$, $^{13}C^{\beta}$, and ^{13}C=O chemical shifts have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under accession number 4388.

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