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Letter to the Editor: Sequence-specific ^{1}H, ^{13}C and ^{15}N assignments of a calcium binding protein from *Entamoeba histolytica*

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Biological context

Entamoeba histolytica, a protozoan parasite, is the causative agent of amoebiasis and amoebic dysentry worldwide. It infects nearly 50 million people worldwide resulting in 50 000 deaths every year (Walsh, 1986). Though ubiquitous in distribution, this parasite is more prevalent in tropical and subtropical regions of the world. It can also invade extraintestinal tissues such as liver and brain, leading to formation of abscesses. The mechanisms governing pathogenesis is not yet clear. Calcium is thought to be involved in the pathogenetic mechanisms of amoebiasis (Munoz et al., 1992). In order to understand the mechanisms by which calcium affects virulence, a gene encoding a novel calcium binding protein from *E. histolytica* (*Eh* CaBP) was isolated and characterized (Prasad et al., 1992). Though the *Eh* CaBP has four EF domains, similar to the calcium signal transducing molecule calmodulin, detailed analysis showed that it is functionally and structurally distinct from calmodulin and possibly involved in a novel signal transduction pathway (Yadav et al., 1997). Analysis of different species of *Entamoeba* clearly indicates that this protein may not be present in nonpathogenic species *E. invadens* and *E. moshkovskii*, suggesting that it may be involved in pathogenesis (Prasad et al., 1993). *Eh* CaBP is a 15 kDa monomeric protein and shows homology with many calcium binding proteins only in the calcium binding loops and not in the interloop regions which are suspected to be the sites that interact with other proteins. The 3D structure of this protein is not yet known. In order to characterize the structure–function relationship of this protein we have initiated its 3D solution structure determination, in Ca^{2+} bound state, using NMR.

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Methods and results

Eh CaBP was expressed in *E. coli* BL21(DE3) strain containing the PET-3c expression system. The protocol as described earlier (Prasad et al., 1993) was modified so that the expression could be carried out in the minimal (M9) media. Isotopically ^{15}N labeled and doubly labeled (both ^{13}C and ^{15}N) *Eh* CaBP were produced using M9 medium containing 1 g $^{15}NH_4Cl$ and 2.5 g ^{13}C-glucose per litre of culture as sole sources of ^{15}N and ^{13}C, respectively. Mid-log phase cells (O.D. = 0.60) were treated with IPTG for 4 h. *Eh* CaBP is expressed to the extent of 30% of the total cell proteins. The purity of the protein was checked by SDS-PAGE. The yield of uniformly labeled *Eh* CaBP was 50 mg of purified protein per litre of culture.

NMR experiments were carried out on a Varian Unity+ 600 MHz NMR spectrometer equipped with a pulsed-field-gradient unit and triple resonance probe with actively shielded Z-gradients, operating at an ^{1}H frequency of 600.051 MHz. NMR measurements were performed with a sample of 0.6 ml of 3 mM *Eh* CaBP in 30 mM $CaCl_2$ and 50 mM deuterated TRIS buffer, pH = 6.0 at 35 °C, either in 99.9% D_2O or in a mixed solvent of 90% H_2O and 10% D_2O. The parameters used in recording and processing various multi-dimensional NMR experiments with [u-^{15}N] and [u-$^{15}N/^{13}C$] labeled *Eh* CaBP are compiled

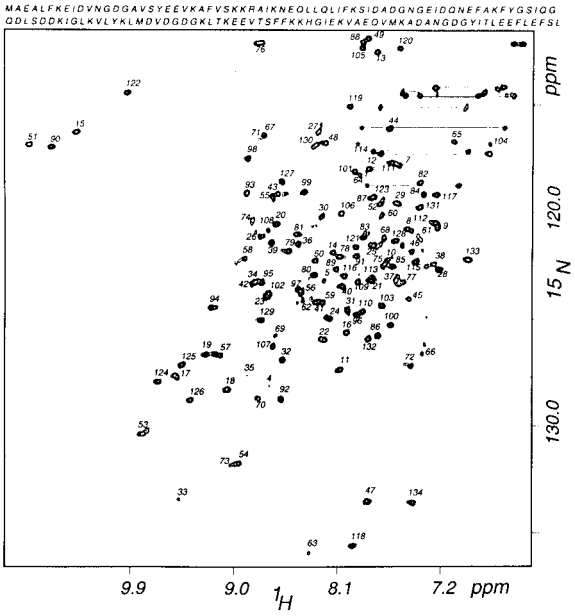

Figure 1. 2D ^{15}N-^{1}H HSQC spectrum of 3.0 mM [u-^{15}N] Ca^{2+}-bound *Eh* CaBP recorded in a mixed solvent of 90% H_2O and 10% D_2O with assignments. Experimental parameters were as follows: τ = 5 ms, recycle delay 1 s, 8 scans/t_1 increment, time domain data points were 128 and 2048 along t_1 and t_2, respectively. The ^{1}H carrier frequency was kept at the water resonance (4.68 ppm) and the ^{15}N carrier frequency at the center of the amide nitrogen region (123.8 ppm). The data were multiplied with a sine bell window function shifted by $\pi/3$ and a Gaussian resolution enhancement window function along the t_1 and t_2 axes, respectively and zero-filled to 512 and 4096 real data points along t_1 and t_2, respectively, prior to 2D-FT. The digital resolution along the ω_1 and ω_2 axes corresponds to 3.32 and 2.08 Hz/pt, respectively. The amino acid sequence of *Eh* CaBP is shown at the top.

in a supplementary table. Data transformation and processing were done on a Silicon Graphics workstation (R10000 based Indigo II Solid Impact Graphics) using the FELIX95.0 software (Biosym Technologies, San Diego, CA, U.S.A.).

Extent of assignments and data deposition

Eh CaBP is free of Pro, Cys and Trp residues. Sequence-specific resonance assignments (Wüthrich, 1986) for nearly all $^{1}H^{N}$, ^{15}N, $^{13}C^{\alpha}$, $^{13}C^{\beta}$, $^{13}C'$, $^{1}H^{\alpha}$ and $^{1}H^{\beta}$ spins have been carried out with the concerted use of ^{15}N-NOESY-HSQC, ^{15}N-TOCSY-HSQC, HNCA (Grzesiek et al., 1992), HN(CO)CA (Grzesiek et al., 1992), HNCO (Kay et al., 1990), CBCANH (Wittekind et al., 1993) and CBCA(CO)NH (Kay et al., 1993) spectra (Figure 1). The resonances for M1, A2 and the ^{15}N spin of E3 at the N-terminal end of the polypeptide chain could not be assigned. The chemical shifts of all resonances thus obtained have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under accession number 4271. The chemical shifts of side-chain resonances beyond $^{1}H^{\beta}$ protons have not been reported. Preliminary investigations of NMR data throw valuable light on the secondary structure of *Eh* CaBP. The ^{15}N edited 3D NOESY-HSQC spectral planes show a large number of sequential NH-NH NOE connectivities, indicating a predominantly α-helical structure. Possibly, the molecule is locked into helix-loop-helix motif domains as is the case with other calcium binding proteins whose 3D structures are known. Detailed spectral analysis and 3D structure calculations for *Eh* CaBP are in progress.

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