The Crystal Structures of Human S100A6 in the Ca^{2+}-Free and Ca^{2+}-Bound States

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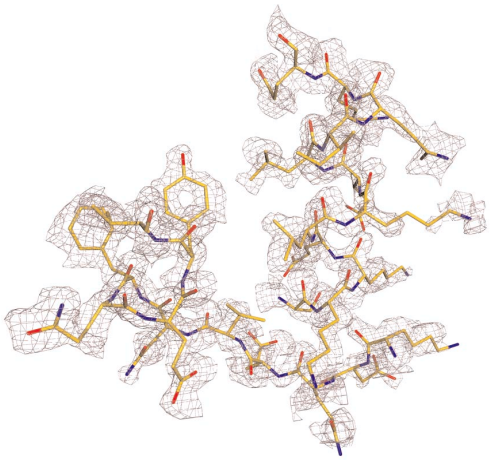
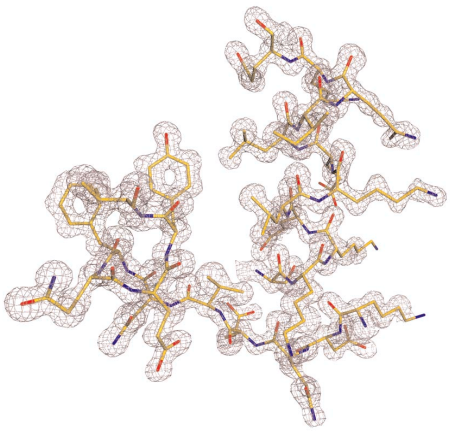


FIG. 1. (left) X-ray crystal structure of human S100A6 (S100A6) in the Ca^{2+}-free state. Experimental electron density map calculated at 1.8-Å resolution with phases refined with SHARP-SOLOMON from a four-wavelength seleno-methionine derivative experiment collected at BioCARS beamline 14-BM-D. (right0 2Fo-Fc electron density map corresponding to the final refined model of S100A6 calculated at 1.15 Å resolution with data collected at BioCARS beamline 14-BM-C.



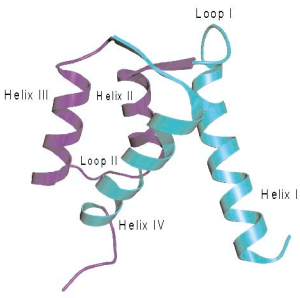
Introduction

S100A6 (Calcyclin) is a member of the S100 subfamily of EF-hand Ca^{2+}-binding proteins. Members of this group have been implicated in the Ca^{2+}-dependent regulation of a variety of cellular processes, including cell growth and differentiation, the regulation of protein phosphorylation by various kinases, and the dynamic reshaping of the cytoskeleton. S100A6, like most members of the subfamily, forms a stable homodimer in solution both in the Ca^{2+}-free and Ca^{2+}-bound states. Each S100A6 monomer contains 90 amino acids with two EF-hand Ca^{2+}-binding motifs. The biological function of S100A6 remains unclear, but several targets have been identified. S100A6 has been shown to interact *in vitro* in a Ca^{2+}-dependent manner with caldesmon and annexins II, VI, and XI.

We have determined the x-ray crystal structures of human S100A6 in the Ca^{2+}-free and Ca^{2+}-bound states to a resolution of 1.15 Å and 1.44 Å, respectively. Our Ca^{2+}-free (Apo) structure represents the first x-ray determination for a S100 protein in the Apo state.

Methods and Materials

Recombinant human S100A6 mutant C3M (containing two methionines in 89 amino acids) was expressed in *Escherichia coli* strain BL21 DE3 pLys. Bacteria were growth in minimal media supplemented with seleno-methionine. The protein was purified using phenyl sepharose and DE52 affinity columns. Crystals were grown using the hanging-drop vapor diffusion method. All the data were collected at the Advanced Photon Source, Argonne National Laboratory. A four-wavelength (λ_1 = 0.9778, λ_2 = 0.9407, λ_3 = 0.9781, λ_4 = 1.0332) multiple wavelength anomolous dispersion (MAD) experiment was performed on a seleno-methionine Ca^{2+}-free S100A6 crystal at BioCARS beamline 14-BM-D. The same crystal was then collected at higher resolution at BioCARS beamline 14-BM-C (λ=$_1$). Ca^{2+}-bound S100A6 was collected at IMCA-CAT beamline 17-ID (λ=$_1$). All the data sets were processed using the programs DENZO and SCALEPACK.

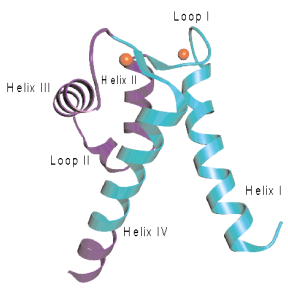


Results

The MAD-phasing experiment conducted at BioCARS beamline 14-BM-D allowed for the location of the two Se-Met sites in the asymmetric unit. The positions, occupancies, and B-factors of the Se atoms were refined with the program SHARP, resulting in a first experimental set of phases that were further refined by solvent flipping with the program SOLOMON. The model was automatically built with the program wARP based on the experimental electron density map calculated to 1.8 Å resolution with the SOLOMON-refined phases (Fig. 1, left). The final model was refined to 1.15 Å resolution using the data collected at BioCARS beamline 14-BM-C (Fig. 1, right).

The structure of Ca^{2+}-bound S100A6 was solved by molecular replacement and refined to 1.44 Å resolution using data collected at IMCA-CAT beamline 17-ID. While the Ca^{2+}-bound structure is very similar to that of other S100 proteins reported previously,[1] our Ca^{2+}-free structure represents the first successful x-ray determination for an S100 protein in the Apo state and is significantly different from two previous NMR determinations.[2,3] Comparison of the two S100A6 structures (Ca^{2+}-free and Ca^{2+}-bound)

FIG. 2. Ribbon representation of the x-ray crystal structures of human S100A6 (S100A6) in the Ca^{2+}-free (left) and Ca^{2+}-bound (right) states, determined to a resolution of 1.15 Å and 1.44 Å, respectively. Represented in purple are the regions that undergo the major conformational changes upon Ca^{2+} binding.



reveals that major conformational changes in helix II, III, the C-terminus of helix IV, and loop II occur as the result of Ca^{2+} binding, which exposes a mainly hydrophobic target-binding site (Fig. 2).

A manuscript describing the two crystal structures of human S100A6 is now in preparation.

Acknowledgments

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