Abstract

Protein crystallography has been used for the study of the two proteins calmodulin and thrombin. Calmodulin is a dumbbell shaped Ca^{2+}-binding protein with two domains connected by a long α-helix, and each domain contains two Ca^{2+}-binding helix-loop-helix motifs. Calmodulin can be cleaved by trypsin mainly after Lys77 in the long connecting α-helix, thereby producing the fragments TR_1C and TR_2C. The crystal structures of these very similar fragments have been determined and refined. The main purpose of the experiments was to confirm that the structures of the fragments were not significantly affected by the tryptic cleavage compared to the corresponding domains of intact calmodulin.

The structure of TR_2C was refined to 1.7 Å resolution, and it was subsequently compared with the C-terminal domain of calmodulin as well as with the NMR structure of Ca^{2+}-bound TR_2C. The structure of TR_1C was refined to 1.8 Å resolution and this fragment was compared with the amino-terminal half of calmodulin. A study of the similarities between this fragment and the NMR structure of Ce^{3+}-bound TR_1C was also performed. TR_1C not only crystallized in a monomeric form, but it was also found to exist as a dimer. In the refinement of the TR_1C dimer, all available data to 1.7 Å resolution were used. The monomers of the dimer are attached to each other by hydrophobic interactions mediated by their hydrophobic clefts. In the hydrophobic cavity thus formed, the dimer was found to host a large molecule.

When the crystal structures of the fragments were compared with the other calmodulin structures it could be confirmed that the main features of the secondary structure elements were the same. Some of the angles between these elements differ, however, and especially the N- and C-terminal helices and the loops connecting the EF-hands have slightly different positions.

In collaboration with the pharmaceutical company AstraZeneca, several thrombin-inhibitor complexes have been refined, but only one of these structures has been published so far. The inhibitor involved in the published complex is a constrained PPACK (D-Phe-Pro-Arg-Chloromethyl Ketone) analogue, and the structure of this complex revealed that the analogue binds in a similar way as PPACK does.

Keywords

Calmodulin; fragments; TR_1C; TR_2C; dimer; EF-hand; calcium-binding; crystal structure; thrombin; inhibitor; PPACK analogue

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