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X-ray Crystal Structure Analysis of Serine / Threonine Phosphatase and Inhibitor Complex

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Background

Protein Phosphatase 1 (PP1), one of the four major enzymes that dephosphorylate serine/threonine residues of proteins in eukaryotic cells, has been shown to be specifically inhibited by a number of natural toxins such as okadaic acid, calyculin A, and microcystin-LR (MCLR). However, with the exception of binding to MCLR [1] and okadaic acid [2], the binding modes of these inhibitors with PP1 are unknown. Calyculin A strongly inhibits PP1 and is the only phosphorylated metabolite among the potent inhibitors of PP1. To elucidate the binding mode of calyculin A to PP1, it is indispensable to determine the three-dimensional structure of their complex.

Results

The crystal structure of the catalytic subunit of protein phosphatase1 (PP1), PP1γ, in complex with a marine toxin, calyculin A, was determined at 2.0 Å resolution [3]. The overall structure of PP1γ in the PP1γ-calyculin A complex is similar to PP1α in the PP1α-MCLR complex and to PP1γ in the PP1γ-okadaic acid complex, although the loop (β12 - β13 loop) region in PP1α-MCLR, which is located near the metal binding site, shows prominent differences (Figure 1). On the molecular surface, there are three grooves connected at the bifurcation point and two metals are located at the bifurcation point. Calyculin A is located in two of the three grooves, namely, in the hydrophobic groove and the acidic groove. The metal binding site contains the phosphate group of calyculin A and forms a tight network via the hydrophilic interactions between PP1 and calyculin A. This is the first observation to note that the inhibitor adopts not a pseudocyclic conformation but an extended conformation in the hydrophobic groove and acidic groove on the PP1γ surface in order to form a complex with the protein. The conformation of β12-β13 loop, which is considered to play an important role for binding inhibitors, is similar to that of PP1γ-okadaic acid complex [2], and not similar to that of PP1α-microcystin-LR complex [3]. In PP1γ-calyculin A β12-β13 loop, only the Tyr272 residue interacted with the inhibitor. The crystal structure indicates that the amino acid terminus of calyculin A contributes in a limited manner to the binding to PP1γ. This result is consistent with findings from the studies of dose-inhibition analysis, which showed that the inhibitory activity is largely retained in hemicalyculin A, a derivative which lacks the C29-C37 component. The crystal structure also shows the importance of two salt bridges formed between calyculin A and two arginine residues (Arg96 and Arg221) in the substrate recognition site.

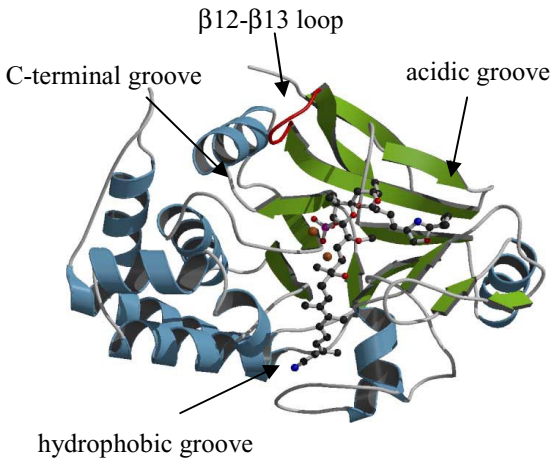

Figure 1

References

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