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Crystal structure of actin specific ADP-ribosyltransferase Ia with βTAD and actin

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1 Abstract

ADP-ribosylating toxins (ADPRTs) facilitate scission of the N-glycosyl bond between nicotinamide and the N-ribose of NAD and transfer the ADP-ribose moiety to target proteins. ADPRTs are classified into four families based on their respective targets. Type I ADPRTs target heteromeric GTP- binding proteins. They include cholera toxin (CT). Type II ADPRT diphtheria toxin (DT) modify elongation factor 2 (EF2). Type III ADPRTs (Clostridium botulinum C3 exoenzyme) ADP-ribosylate small GTP-binding proteins. Type IV ADPRTs ADP-ribosylate actin. These actin-specific ADPRTs include a family of binary toxins. C. perfringens iota-toxin consists of an enzymatic component (Ia) and a cell- membrane-binding component (Ib). Ia ADP- ribosylate G-actin, but not F-actin, at Arg-177; this activity severely reduces the ability of actin to undergo polymerization, leading to disruption of the cytoskeletal architecture and cell death.

During the last few years, it has been revealed much about the structure and mechanism of actin ADPRTs. Up to now, many actin ADPRT structures are available including Ia [ref.1]. In this study, we first revealed the complex structure of actin specific ADPRT with substrate actin.

2 Experiment

To obtain good diffraction-quality crystals of G-actin with Ia, we used ATP and latrunculin A, which is an inhibitor of actin polymerization. Moreover, to obtain stable complex crystals, we used the non- hydrolyzable NAD analog βTAD. Using these crystals, we collected diffraction data for the actin (with ATP and the polymerization-inhibiting drug latrunculin A)–Ia–βTAD complex at a resolution of 2.8 Å. The crystal space group was determined to be $P2_12_12_1$ (a =57.0, b=126.3, c=147.1Å) and to contain one actin and one Ia in an asymmetric unit. Crystals are highly nonisomorphous, and their structure was determined by molecular replacement by using MOLREP. Using the structure of Ia (1GIQ), we identified and fixed the Ia position, after which we searched for actin by using the structure of actin (1IJJ). The βTAD, ATP, and latrunculin A densities were clear, so these cofactors were built in. The model was then iteratively rebuilt and refined at a 2.8-Å resolution by using REFMAC. The final model was then refined to Rwork=22.3% (Rfree=29.6%) and consisted of Ia, actin, βTAD, ATP, latrunculin A, calcium, and 79 water molecules.

3 Results and Discussion

The structure indicates that Ia recognizes actin via five loops around βTAD: loop I (Tyr-60 – Tyr-62 in the N domain), loop II (active-site loop), loop III, loop IV (PN loop), and loop V (ADP-ribosylating turn–turn loop). We used site-directed mutagenesis to confirm that loop I on the N domain and loop II are essential for the ADP-ribosyltransferase activity. Furthermore, we revealed that Glu-378 on the EXE loop is in close proximity to Arg-177 in actin, and we proposed that the ADP- ribosylation of Arg-177 proceeds by an SN1 reaction via first an oxocarbenium ion intermediate and second a cationic intermediate by alleviating the strained conformation of the first oxocarbenium ion [ref.2].

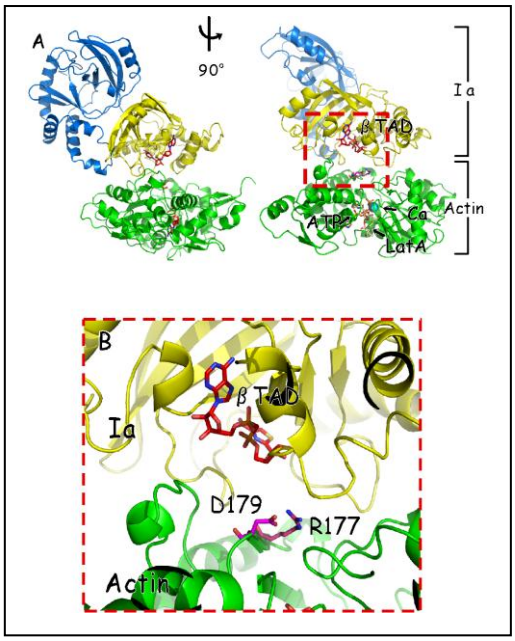

Fig. 1: (A)Ia consists of N-terminal(cyan) and C-terminal domain (yellow). Actin was drawn in green.(B)Close up of Arg177 and βTAD.

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References

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