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Crystal Structure Analysis of ADP-ribosylating Enzyme and Substrate Protein Complex

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1 Introduction

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 IV ADPRTs (Ia, C2I, and SpvB) ADP-ribosylates actin and this activity severely reduces the ability of actin to undergo polymerization, leading to disruption of the cytoskeletal architecture and cell death. Recently we resolved the first crystal structure of Ia in complex with actin and the non-hydrolyzable NAD^+ analog βTAD [1]; however, the structures of the NAD^+ bound form (NAD^+-Ia-actin) and the ADP-ribosylated form (Ia-ADP-ribosylated (ADPR)-actin) remain uncertain. Here we report high-resolution structures of NAD^+-Ia-actin and Ia-ADPR-actin obtained by soaking apo-Ia-actin crystal with NAD^+ under different conditions.

2 Experiment

We were able to produce small apo-Ia-actin crystals, after which we refined the crystallization conditions to grow larger crystals. Using these crystals, we applied the soaking with NAD^+ under different conditions, and we obtained each data set of NAD^+-Ia-actin and Ia-ADPR-actin at 1.75 Å and 2.2 Å resolution, respectively [2]. Specifically, soaking apo-Ia(wt)-actin crystals with 10 mM NAD^+ in cryo-protectant containing 30% ethylene glycol for 30 min at room temperature yielded NAD^+-Ia-actin, while soaking the complex with 10 mM NAD^+ in mother liquor for 30 min at room temperature yielded Ia-ADPR-actin.

3 Results and Discussion

With NAD^+-Ia-actin, the difference map showed a clear NAD^+ electron density (**Fig 1**). With Ia-ADPR-actin, the 2Fo-Fc maps showed obvious differences from NAD^+-Ia-actin, including the presence of an ADP-ribosylated arginine density instead of NAD^+ (**Fig 2**). In NAD^+-Ia-actin, the NAD^+ conformation is highly folded, as is seen in all ARTs. That is, the ADP moiety was gripped by Ia via Asn335, Gln300, Arg295 and Arg352, and the folded nicotinamide makes a hydrogen bond with the Arg296 main chain carbonyl and nitrogen. In Ia-ADPR-actin, the ADP moiety is gripped by the same residues as in NAD^+-Ia-actin, but N-ribose was largely moved to Arg177 of actin after nicotinamide cleavage. It is important to note that there are no water molecules close to NC1 of N-ribose, which suggests that actin prevents unfavorable reactions from proceeding (NADase) by making water molecules unavailable. Thus we revealed the structure of the NAD^+ -Ia-actin and Ia-ADPR-actin complexes, providing insight into the reaction mechanism of ADP ribosylation.

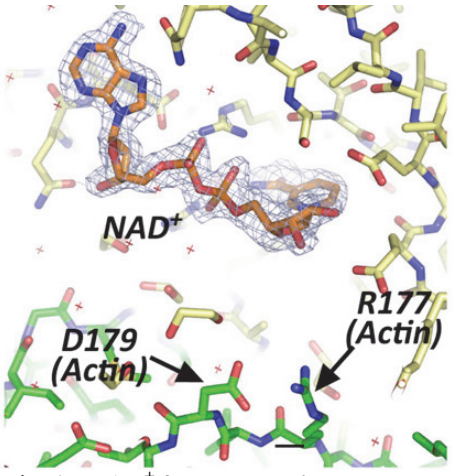

Fig. 1: **NAD^+ 2Fo-Fc density**

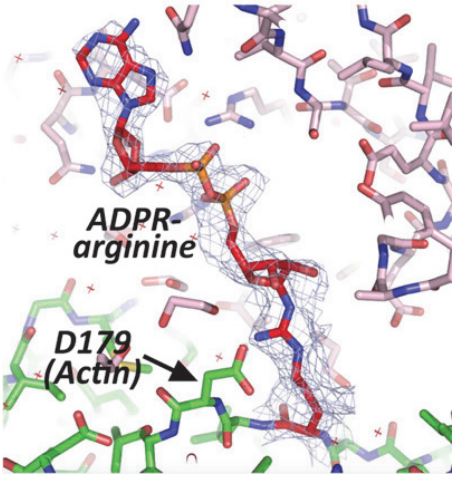

Fig. 2: **ADP -ribosylated arginine 2Fo-Fc density**

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References

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