Crystallography

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STRUCTURAL STUDIES OF HUMAN ADP-RIBOSE PYROPHOSPHATASE

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Introduction

Free ADPR is a highly active metabolite intermediate, and high concentration of free ADPR could result in non-enzymatic ADP-ribosylation of proteins[1]. Thus, precise control of the ADPR level is important in cell maintenance. Catabolism of free ADPR occurs by its hydrolysis to AMP and ribose 5'-phosphate, a process catalyzed by the ADP-ribose pyrophosphatase (ADPRase) subfamily in the presence of divalent metal ions. To better understand the underlying mechanism of hydrolysis and substrate specificity of different ADPRases, we report here the crystal structures of the full-length hNUTD5 in apo form and a truncated form of the enzyme in complexes with ADPR and AMP with bound Mg^{2+}. Our results indicate that hNUDT5 has a structure similar to those of bacterial enzymes, but different from that of hNUDT9. Structural analysis of hNUDT5 reveals insights into the molecular basis of the substrate specificity[2].

Method and Results

Expression and purification of human NUDT5 were carried out as described previously[2]. Sparse-matrix crystallization screening with the Crystal Screen and Crystal Screen II kits (Hampton Research) was performed using the hanging-drop vapor diffusion method at 4°C. Crystals of NUDT5 in complex with ligand were mounted on a cryo-loop and flash-frozen in liquid nitrogen. Data collection was carried out using the ADSC CCD detector of BL6A or 5A at PF. Data processing and scaling were performed using the HKL2000 suite. The diffraction data collection statistics are summarized in Table I. Crystal structures revealed that hNUDT5 forms a homodimer with substantial domain swapping and assumes a structure more similar to *E. coli* ADPRase ORF209 than human ADPRase NUDT9 (Figure 1). Structure information provided the molecular basis for the high selectivity of hNUDT5 for ADP-sugars over other sugar nucleotides.

Table I Statistics of diffraction data and structure refinement

Data collection	hNUDT5	ΔhNUDT5 complex	
Data sets	Apo form	ADPR	AMP
Space group	$P2_1$	$C2$	$C2$
a (Å)	81.4	112.5	112.1
b (Å)	70.1	40.6	40.2
c (Å)	86.8	99.7	100.1
(°)	100.6	121.4	121.7
Resolution (Å)	50.0-2.50 (2.59-2.50)	50.0-2.00 (2.07-2.00)	50.0-2.60 (2.69-2.60)
Mosaicity	0.78	0.53	1.31
Redundancy	3.5 (2.6)	3.3 (2.1)	5.2 (4.3)
I/σ(I)	20.1 (1.8)	27.0 (2.4)	18.7 (1.7)
Completeness (%)	96.3 (81.2)	92.8 (62.2)	97.5 (87.0)
R_{merge} (%)	7.0 (41.9)	5.4 (26.7)	11.9 (46.9)
Refinement statistics			
R_{cryst} / R_{free}	21.4/26.2	19.4 / 22.8	21.5 / 27.5
Average B factor			
protein atoms	68.8	37.5	60.2
metal ions		34.0	60.9
ligands		45.3	77.4
Ramachandran plot			
Most favored (%)	89.8	90.2	89.3
Allowed (%)	10.2	9.8	10.7

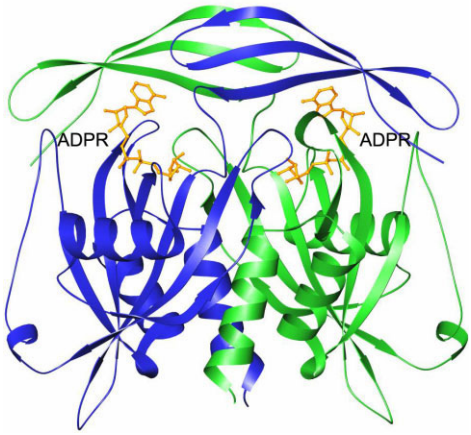

Fig. 1. Structure of the ΔhNUDT5-ADPR complex

References

[1] Jacobson, E. L. *et al. Glycation of proteins by ADP-ribose*. ***Mol Cell Biochem*** 138, 207-12 (1994).
[2] Zha, M. *et al.* Crystal structures of human NUDT5 reveal insights into the structural basis of the substrate specificity. ***J Mol Biol*** 364, 1021-33 (2006).

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