This report features the work of Yu-Yuan Hsiao and his collaborators published in Nat Commun. ***12****, 601 (2021).*

TLS 13B1 Protein Crystallography
TLS 15A1 Biopharmaceuticals Protein Crystallography
TPS 05A Protein Microcrystallography

- Protein Crystallography
- Biological Macromolecules, Protein Structures, Life Science

References

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Poa1p Macro Domain Structure: Deciphering a Non-Canonical 3"-*O*AADPR Deacetylase

In this report, the undefined enzymatic activity and substrate specificity of the Poa1p macro domain were revealed on deciphering the crystal structures in a combination of biochemical approaches and guided a study of biological effects of the OAADPR metabolism in epigenetics.

O-acetyl-ADP-ribose (*O*AADPR) is a fascinating signaling molecule that has been implicated in the regulation of numerous cellular processes, including the formation of silent information regulator complexes and gene silencing. This molecule was first identified from the conserved NAD-dependent histone/protein deacetylase reaction catalyzed by sirtuins. In cells, the ratio of 2"- and 3"-*O*AADPR exists in equilibrium as 48:52 at slightly alkaline pH through a non-enzymatic intermolecular transesterification.[1]

There is accumulating evidence that a group of macro domain proteins efficiently catalyzes the deacetylation of 2"-*O*AADPR, including MacroD-like proteins (human MacroD1, human MacroD2, *E. coli* Ymdb, *O. iheyensis* MacroD), the sirtuin-linked macro domain SAV0325 from *S. aureus*, and the human TARG1-like macro domain C6orf130.[2] The catalysis ability of macro domains stems from their physical or genetic link with sirtuins, thus revealing the functional connections with sirtuins and a novel aspect of *O*AADPR metabolism.[3] To date, only 2"-*O*AADPR deacetylases but no 3"-*O*AADPR deacetylase was reported.

S. cerevisiae contains sirtuins as a major NAD-consuming family but lacks any poly(ADP-ribose) polymerase (PARP) homologues, which indicates that sirtuin reaction product *O*AADPR might hold the most potential physiological roles in yeast. Qualitative analysis of yeast cell extracts revealed at least three distinct activities contributing to the metabolism of *O*AADPR *in vivo*, but only Nudix hydrolase Ysa1 was reported to hydrolyze *O*AADPR/ADPR to AMP and ribose phosphate or acetyl-ribose phosphate, thereby lowering the cellular *O*AADPR/ADPR levels.[4] Protein identities of the unknown deacetylase and acetyl transferase remain obscure.[2,4] Poa1p is a unique fungal-type macro domain protein that shares little sequence homology with other macro domains. To explore the function of the uncharacterized macro domain, a research team led by Chun-Hua Hsu (National Taiwan University) determined the structures of *apo*, ADPR-bound Poa1p, and its mutants. The diffraction data were collected at **TPS 05A**, **TLS 15A1** and **TLS 13B1**.[5]

Our biochemical data reveals that, within two macro domains from *S. cerevisiae*, only Poa1p carried a robust catalytic activity toward deacetylation of *O*AADPR, yielding a significant reaction product, ADPR. To elucidate enzymatic properties of Poa1p at a molecular level, the crystal structure of ADPR-bound Poa1p was determined (**Fig. 1(a)**). The structure of Poa1p-ADPR complex, at resolution 1.78 Å, adopts a three-layered α/β/α sandwich fold with the ADPR molecule accommodated within the central crevice of Poa1p. This condition strongly indicates a typical macro domain fold for ADPR binding. Having structurally characterized the Poa1p macro domain, the authors then investigated the substrate specificity of Poa1p for *O*AADPR deacetylation. The RP-HPLC and ^{18}O-labeling results revealed that Poa1p can hydrolyze both 3"- and 1"-*O*AADPR isomers, and preferentially facilitate 3"-*O*AADPR cleavage at neutral pH. To decipher the molecular mechanism of this finding, interactions between Poa1p and ADPR were examined (**Fig. 1(b)**, see next page).

The interaction network of the ADPR binding groove consists of four binding surfaces (S1-S4) and thus provides effective hydrophobic interaction and hydrogen-bond networks with ADPR. Of note, the steric position of water molecule W7 appears to be the corresponding occupant of a conceivable nucleophilic water site by the acetyl group of 2″-*O*AADPR. Establishment of the steric hindrance (Gly87, Gly88, and Phe152) in the vicinity of the ribose 3″-hydroxyl group seems to retain the proper orientation of 3″-*O*AADPR for catalysis. The side chain of Tyr50 from the α2-helix neighboring Asn26 evidently points toward the 3″-hydroxyl groups of the distal ribose moiety, which seems reasonable to concede a nearby nucleophilic water for catalysis in stereochemistry.

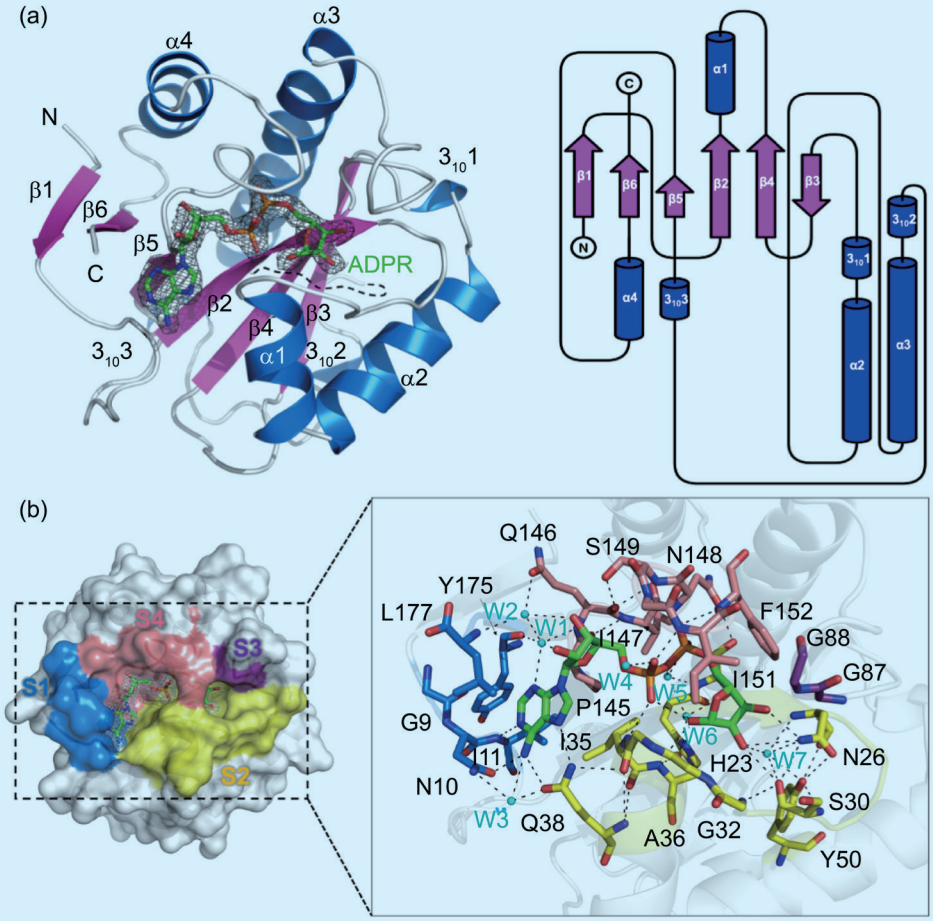

Fig. 1: (a) Overall structure and topology of Poa1p in a complex with ADP-ribose (ADPR). The disordered region is shown as dashed lines. (b) ADPR binding surfaces (S1-S4) and interaction network of ADPR with protein residues of Poa1p. Residues within S1-S4 are shown as sticks with carbon in blue, yellow, purple and salmon, respectively. [Reproduced from Ref. 5]

Since the discovery of the non-canonical substrate specificity of Poa1p, crucial residues involved in the intriguing activity remain obscure. To gain insight into the relevance of the substrate specificity and activity of Poa1p, potentially critical residues for substrate binding and catalysis were designated for mutational study. Kinetic analysis of the designed Poa1p mutants showed a decreased turnover rate (K_{cat}) to certain degrees. In addition, the Poa1pF152A-ADPR structure revealed that the phenyl group of Phe152 established a steric hindrance to stabilize the orientation of the distal ribose of *O*AADPR for catalysis. Of note, the RP-HPLC results of these mutants confirmed their catalytic roles in distinct substrate specificities of *O*AADPR isomers. His23 was engaged in catalyzing 1″-*O*AADPR hydrolysis, which indicates its potential role in protein de-mono-ADP-ribosylation, whereas Asn26 and Tyr50 were mainly contributed to 3″-*O*AADPR hydrolysis, in line with the authors' hypothesis from the structural information. To elaborate the catalytic specificity of 3″-*O*AADPR hydrolysis from RP-HPLC and kinetic assays, we present a structural comparison side by side of the active site of the Poa1p-ADPR complex (**Fig. 2**). The α2 helix in Poa1p adopts a core structure more compact than other macro domains, thus providing the best chance for the non-conserved tyrosine residue (Tyr50 in Poa1p) to engage in 3″-*O*AADPR catalysis. As compared with the isostructural residues of other macro domains, all residues are non-catalytic and far from the distal ribose of the ADPR moiety. The biological consequence of the *O*AADPR hydrolysis *via* Poa1p was further explored. A disruption of Poa1p expression in yeast showed a striking sensitivity to transcriptional stress, which implies a physiological role in response to nucleotide depletion.

In summary, five conclusions were drawn from the structural and biochemical data. (1) Poa1p is the only catalytically active macro domain in yeast toward deacetylation of *O*AADPR. (2) The first complex structure of Poa1p with ADPR was determined. (3) Poa1p exhibits the non-canonical 3″-and 1″-*O*AADPR deacetylase activities that are distinct from canonical 2″-*O*AADPR deacetylase. (4) The specialized residue Tyr50 and compact folding of the α2 helix in Poa1p contribute to the distinct substrate specificity toward 3″-*O*AADPR. (5) Phenotypic consequences of the transcriptional effect in yeast might serve as a model organism to discover further physiological functions of *O*AADPR deacetylases. Altogether, our studies provide a new picture for the diverse catalytic properties of POA1-like macro domains but decipher its biological roles

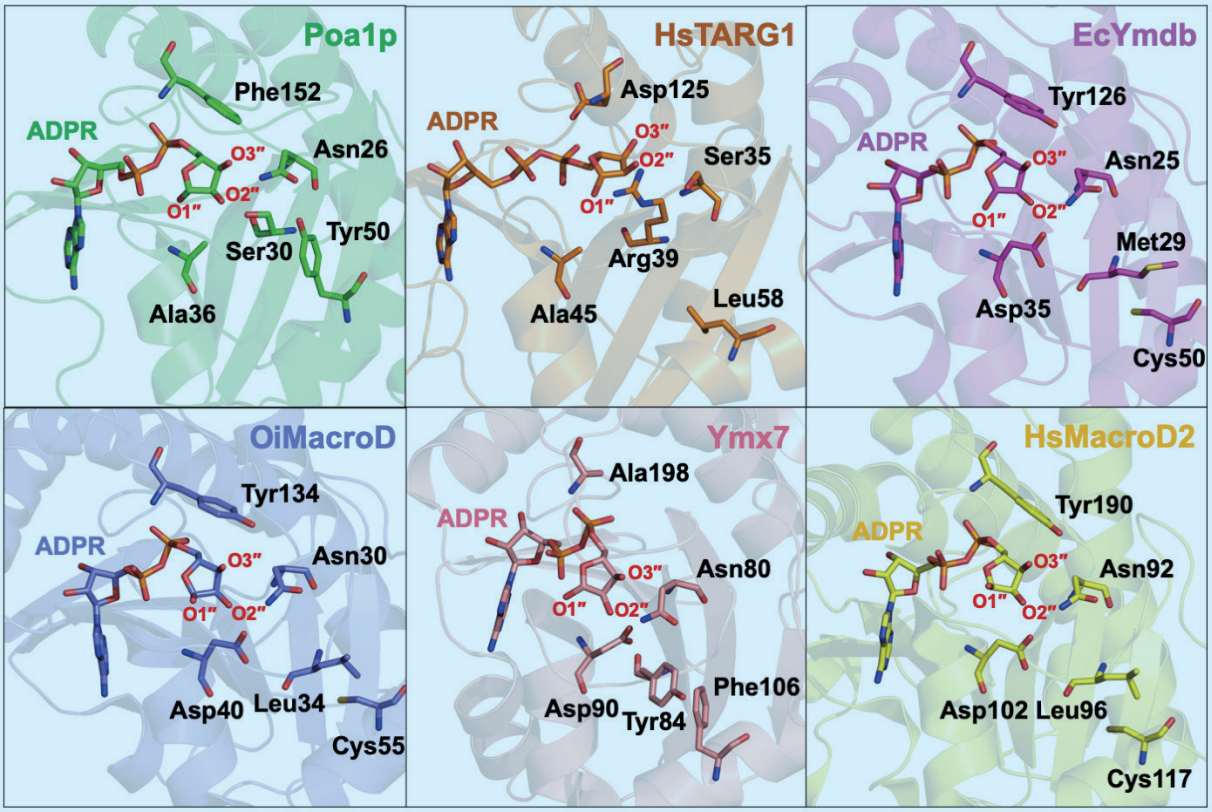

Fig. 2: Structural comparison of ADPR-bound Poa1p with other ADPR-bound macro domains, including HsTARG1 (PDB code 2L8R), EcYmdb (PDB code 5CB3), OiMacroD (PDB code 5L9K), Ymx7 (PDB code 1TXZ), and HsMacroD2 (PDB code 4IQY) colored green, orange, magenta, slate, salmon and yellow, respectively. [Reproduced from Ref. 5]

in regulating cellular *O*AADPR and ADPR. (Reported by Chun-Hua Hsu, National Taiwan University)

This report features the work of Chun-Hua Hsu and his colleagues published in ACS Catal. ***11****, 11075 (2021).*

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