Adenosine Thiamine Triphosphate (AThTP) Inhibits Poly(ADP-Ribose) Polymerase-1 (PARP-1) Activity

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Summary Overactivation of poly(ADP-ribose) polymerase-1 (PARP-1) has been demonstrated to result in various stress-related diseases, including diabetes mellitus. Deficiency of cellular nicotinamide adenine dinucleotide (NAD+) content, consumed by PARP-1 to add ADP-ribose moieties onto target proteins, contributes to pathophysiological conditions. Adenosine thiamine triphosphate (AThTP) exists in small amounts in mammals; however, the function(s) of this metabolite remains unresolved. The structure of AThTP resembles NAD+. Recent experimental studies demonstrate beneficial impacts of high-dose thiamine treatment of diabetic complications. These findings have led us to hypothesize that AThTP may modulate the activity of PARP-1. We have chemically synthesized AThTP and evaluated the effect of AThTP on recombinant PARP-1 enzyme activity. AThTP inhibited the PARP-1 activity at 10 μM, and a structural model of the PARP-1–AThTP complex highlighted the AThTP binding site. The results provide new insights into the pharmacological importance of AThTP as an inhibitor of PARP-1.

Key Words adenosine thiamine triphosphate, poly(ADP-ribose) polymerase-1

Substantial recent experimental studies demonstrate beneficial impacts of high-dose thiamine on diabetic complications, such as diabetic retinopathy, diabetic nephropathy, diabetic neuropathy and diabetic cardiomyopathy (1–4). However, the pharmacological relevance of high-dose thiamine treatments remains unknown.

Chronic hyperglycemia results in diabetic complications in target organs. The pathogenic effect of high glucose is, at least partially, mediated to a significant extent through increased production of reactive oxygen species and reactive nitrogen species and subsequent oxidative stress (reviewed in Evans et al. (5)). Increased oxidative stress activates the nuclear enzyme, poly(ADP-ribose) polymerase-1 (PARP-1). PARP-1 activation depletes its substrate, nicotinamide adenine dinucleotide (NAD+), and also covalently attaches branched nucleic acid-like polymers of poly(ADP-ribose) to various acceptor proteins (reviewed in Kiss and Szabó (6)). A covalently attached ADP-ribose polymer, poly(ADP-riboseylation), affects the function of target proteins. The involvement of PARP-1 activation in the pathogenesis of diabetes and its complications has recently been emphasized by both in vivo and in vitro studies (reviewed in Pacher and Szabó (7), and Szabó (8)).

NAD+, used as substrate for PARP-1, consists of two nucleotides joined through their phosphate groups, with one nucleotide containing an adenine base and the other containing nicotinamide.

Adenosine thiamine triphosphate (AThTP), a new thiamine derivative, was recently identified in Escherichia coli (9), followed by the identification in small amounts in mouse brain, heart, skeletal muscle, liver and kidneys (10). AThTP is composed of two molecules, an adenine base and thiamine, which are joined through phosphate groups. The structure of AThTP appears to closely resemble NAD+.

Although the biological role of AThTP is unknown, the existence of noncoenzyme functions of thiamine derivatives has been speculated (11–14).

In the context of 1) structural resemblance of AThTP to NAD+, 2) the experimental evidence implicating PARP-1 as a causative factor in the pathogenesis of diabetes and diabetic complications in vitro and in vivo (reviewed in Szabó (8)), and 3) beneficial effects of high-dose thiamine on diabetic complications, we hypothesized that AThTP could interact with PARP-1 and mod-
ulate PARP-1 activity.

Methods

Chemical synthesis and purification of AThTP. AThTP was synthesized according to the method described by Bettendorff et al. (9) The compound was initially purified by solid phase extraction on a MEGA Bond Elut® C_{18} (Varian Inc., Harbor City, CA, USA) cartridge. After passage of the water solution of the crude compound, the cartridge was washed with water. The compound was eluted with 10 v/v% methanol. The eluted fraction was lyophilized, redissolved in water and then purified by size-exclusion chromatography on a Bio-Gel® P2 column (2.0×60 cm) equilibrated with water. The elution profile was followed by reading the absorbance at 280 nm to detect the presence of AThTP. The AThTP fraction was lyophilized and redissolved in 50 mM ammonium acetate buffer (pH 7.0). The pure AThTP was obtained using an HPLC system equipped with a semi-preparative COSMOSIL® C_{18}-MS-II column (Nacalai Tesque, Inc., Kyoto, Japan) and eluted under isocratic conditions using methanol: 50 mM ammonium acetate buffer (pH 7.0) (5:95) at a flow rate of 3 mL/min. The purity of the preparations was checked by HPLC, MS analysis and NMR.

Evaluation of PARP-1 enzyme activity in a cell-free assay. The effect of AThTP on PARP-1 enzyme activity was evaluated by a HT Universal Colorimetric PARP Assay Kit with Histone-Coated Strip Wells purchased from Trevigen (Gaithersburg, MD, USA), following the manufacturer’s instructions. This assay kit measures the incorporation of biotinylated poly(ADP-ribose) onto histone proteins in a 96-well plate.

Molecular modeling of the PARP-1–AThTP complex. For modeling of the human PARP-1 molecule complexed with AThTP, a model of the PARP-1 catalytic domain was prepared by taking the coordinate set, 1A26 including an ADP molecule (15), from the Protein Data Bank (PDB http://www.rcsb.org/pdb/). Adenosine and the triphosphate of AThTP were modeled from this ADP molecule. The PARP-1 binding structure of the AThTP thiamin moiety was constructed by referring to the ABT-888 binding to PARP2 (PDB code: 3KJD) (16). The thiazole and pyrrolidine rings of the thiamin moiety were positioned in a similar location to the imidazole and pyrrolidine rings of ABT-888. Water molecules were randomly distributed in a 10 Å shell around the complex. After energy minimization using the MMFF94x force field (17), 200-picosecond (ps) molecular dynamics simulations were performed at 300 K using a 0.002 ps time step and the NVT method (18, 19). Finally, the most stable structure during the last 50 ps simulations was optimized by energy minimization. The potential energy of molecular system after final optimization was $-4.99 \times 10^4$ kcal/mol including the PARP-1 catalytic domain (from Lys662 to Ser1012), AThTP and 2810 water molecules. All operations were performed using the package for molecular structure analyses, MOE (Molecular Operating Environment, Chemical Computing Group Inc., Québec, CA http://www.chemcomp.com/).

Results and Discussion

To test our hypothesis, we chemically synthesized AThTP and the effect of AThTP on PARP-1 enzyme activity was evaluated using recombinant PARP-1 in a cell-free assay. As expected, PARP-1 activity was markedly reduced by 10 mM 3-aminobenzamide (approximately 80% inhibition, data not shown). AThTP showed a dose-dependent effect on PARP-1 activity, producing almost complete inhibition at 10 μM (Fig. 1). Adenosine thiamine diphosphate (AThDP), thiamine, thiamine diphosphate, and thiamine triphosphate did not inhibit PARP-1 activity at 20 μM (data not shown). It is interesting to examine a synergistic inhibition effect of these materials with ATP and/or ADP, which are not inhibited alone, in detail. A difference of the PARP-1 inhibitory activity between AThTP and AThDP, structurally more close to NAD⁺, is thought to be a difference of interaction with the phosphate to PARP-1 enzyme and a structural flexibility of the phosphate moiety in AThTP.

Although the inhibitory concentration of AThTP (10 μM) is much higher than the concentration in mouse tissues (10), it may be possible that AThTP, synthesized by enzyme, is increased by a mass effect when a tissue concentration of thiamine increases. The hepatic concentration of total thiamine is increased by high-dose thiamine and this phenomenon is robust in streptozotocine-induced diabetic rats (unpublished data). Therefore, AThTP is thought to have pharmacological significance as PARP-1 inhibitor.

In this study, we evaluated the PARP-1 enzyme activity in the commericial available PARP-1 enzyme activity kit, which is used for the screening of PARP-1 inhibitors and for measuring the activity of PARP-1 in cell extracts. Unfortunately the precise inhibitory mechanism could not be evaluated by this kit. Accordingly, to gain further insight, we constructed an initial structural model of the PARP-1–AThTP complex by considering the X-ray structures of the PARP-1–ADP analogue complex (15) and the PARP2–PARP inhibitor (ABT-888) complex (16), deposited in the Protein Data Bank (http://www.rcsb.org/pdb/) as 1A26 and 3KJD. The energy-minimized structure after 200 ps of molecular dynamics simulations is shown in Fig. 2A as a ster-
Fig. 2. Predicted interaction between human PARP-1 and AThTP. A simulated complex structure of human PARP-1 with AThTP is shown in (A) without hydrogen atoms. AThTP is presented by a light-blue ball-and-stick model, and PARP-1 residues adjacent to AThTP are shown by white stick models. Nitrogen, oxygen, sulfur and phosphorus atoms are colored blue, red, yellow and purple, respectively. Each hydrogen bond between PARP-1 and AThTP is indicated by a thin green line with its distance (Å), and the related atoms in PARP-1 are represented by balls. The Ring-stacking interaction between the AThTP thiazole ring and the Tyr907 side-chain is indicated by green circles and dotted lines. An interaction scheme between human PARP-1 and AThTP is shown in (B) with related amino acid residues. AThTP is colored light-blue. Hydrogen bonds, hydrophobic interactions and ring-stacking interactions are illustrated as purple dotted lines, yellow circles and a green circle, respectively.
eo view, and the predicted interaction between PARP-1 and AThTP is summarized in Fig. 2B. The initial structure of AThTP was not provided, but the structure of this simulation resulted in a U-shaped conformation of AThTP. In this structure, the adenine moiety of AThTP is positioned by hydrophobic interactions with the Met890 side-chain. Two hydroxyl groups of the AThTP ribose moiety are fixed by hydrogen bonds with the Gln988 side-chain, and the triphosphate is stabilized by hydrogen bonds and electrostatic interactions with a positive charged pocket (His826, Lys903 and the main-chains of Tyr985 and Leu986). These interactions are similar to those observed in the ADP analogue complex (15). Ring-stacking interactions were observed between the AThTP thiazole ring and the Tyr907 phenyl ring. Furthermore, this phenyl group contributes in fixing a phosphate group of AThTP via a hydrogen bond. The AThTP methyl-pyrimidine enters into a hydrophobic hole (His862, Leu877, Ile895 and Tyr896), and the amino group of the pyrimidine moiety appears to interact with Glu763 and Asp766 through hydrogen bonds. AThTP methyl-pyrimidine enters into a hydrophobic pocket (His862, Leu877, Ile895 and Tyr896), and the amino group of the pyrimidine moiety appears to interact with Glu763 and Asp766 through hydrogen bonds. Ring-stacking interactions of the thiazole moiety with this enzyme were also similar to the X-ray structure of the PARP2–ABT-888 complex (16) and the PARP-1–PARP-1 inhibitor complexes (20–22).

The ADP and ABT-888 binding sites, described above, were reported as “acceptor” and “donor” binding sites for ADP-ribose elongation by PARP-1 (15). The poly (ADP-ribose) chain (acceptor) and an NAD+ molecule (donor) would bind to PARP-1 via the ADP and ABT-888 binding sites, respectively. The NAD+ nicotinamide moiety is thought to be stabilized by the AThTP binding site, especially Tyr907 via ring-stacking interactions (16), and many PARP-1 inhibitors have been created mainly for binding to this “donor” binding site. By comparing these binding modes, it appears that AThTP in the structure of the PARP-1 complex could interact with both binding sites at the same time. Furthermore, the AThTP pyrimidine moiety in the above model could interact with an inner hydrophobic hole near the binding site of the phenoxypropyl group of a quinazoline derivative (PARP-1 inhibitor) (21). However, it is also probable that this pyrimidine ring cannot enter into the hydrophobic hole but covers this hole in a similar manner to other inhibitors (22).

The results herein have reported that AThTP inhibited PARP-1 activity, providing new insights into the pharmacological relevance of AThTP as a regulator of PARP-1.

AThTP is reported to be detectable in the mouse brain, skeletal muscle, heart, kidney, and liver (10) and thiamine diphosphate adenyl transferase, synthesizing adenosine thiamine triphosphate, has been characterized in E. coli (23). We believe that the beneficial impacts of high-dose thiamine on diabetic complications could result from not only the coenzymatic function of thiamine but also, at least partly, from a non-cofactor role for thiamine derivatives in living cells, i.e., the inhibitory function of AThTP against PARP-1 activity.

The involvement of PARP-1 overactivation has been demonstrated in numerous stress-related diseases (reviewed in Pacher and Szabó (7) and Szabó (8)). Accordingly, high-dose thiamine intervention could be beneficial in treating not only diabetic complications but also in the treatment of various stress-related diseases. Using an obese rat model, we recently found that high-dose thiamine prevented the metabolic syndrome (24) and mitigated the development of hypertension in spontaneous hypertensive rats (SHR) (25), even though the relevance of AThTP still remains to be verified.

REFERENCES


