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The α-thio and/or β-γ-hypophosphate analogs of ATP as cofactors of T4 DNA ligase

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ABSTRACT

T4 DNA ligase is one of the most commonly used enzymes for *in vitro* molecular research and a useful model for testing the ligation mechanism of ATP-dependent DNA ligation. To better understand the influence of phosphate group modifications in the ligation process, a series of ATP analogs were tested as cofactors. P-diastereomers of newly developed β,γ-hypo-ATPαS (thio) and β,γ-hypo-ATP (oxo) were synthesized and their activity was compared to ATPαS and their natural precursors. The evaluation of presented ATP analogs revealed the importance of the α-phosphate stereogenic center in ATPαS for the T4 DNA ligase activity and sheds new light on the interaction between ATP-dependent DNA ligases and cofactors.

1. Introduction

DNA ligases are enzymes present in almost all forms of life. Besides bacteria, eukaryotes and archaea, a number of viruses carry genes encoding for their functional ligases [1–3]. To date, various DNA ligases have been discovered and characterized in respect to their molecular size and type of required cofactors. Based on cofactor preferences, the ligase family is divided into ATP- and NAD^+-dependent enzymes. The bacteriophage, archaea and most of the eukaryotic DNA ligases belong to the first group of enzymes, whereas the NAD^+-dependent group includes mainly bacterial enzymes [2].

The biological significance of DNA ligases is attributed to their function in DNA replication, recombination and/or DNA repair [1,2]. In vertebrates, three different DNA ligases I, III and IV are recognized. DNA ligase I is essential for the Okazaki fragments' ligation during lagging-strand DNA synthesis as well as in several DNA-repair pathways. In budding yeast DNA ligase I is involved in mitochondrial DNA replication and repair, while in higher eukaryotes this function is shifted to DNA ligase III. In vertebrates, the isoforms of DNA ligase III are active in mitochondria as well as in the nucleus and play an important role in the replication and repair of mitochondrial DNA. Another enzyme belonging to this family is DNA ligase IV, known to participate in the DNA non-homologous end-joining (NHEJ) pathway. Orthologs of DNA ligase IV have been identified in yeast, higher plants and vertebrates [2].

All the eukaryotic ATP-dependent DNA ligases are related by sequence and structure [2]. Despite structural differences, the mechanism of their enzymatic activity is similar. These enzymes catalyze the joining of two single DNA strands on a complementary template. The covalent phosphodiester bond is formed in a three-step process (Fig. 1), with the participation of the 5′-phosphoryl group of the 3′-terminal DNA strand (a donor strand) and the 3′-hydroxyl group of the 5′-terminal DNA strand (acceptor strand).

In the first step, the ATP cofactor is locked into the enzyme's active pocket where the ε-amino group of the lysine residue (e.g. Lys-238 in *Enterobacteria phage T7*) attacks the α-phosphorus atom of ATP to form, in an S_N2P substitution reaction [4], the AMP-NH_2-ligase intermediate, with simultaneous release of a pyrophosphate anion (PPi). In the second step, the adenylyl residue from the AMP-NH_2-ligase intermediate is transferred to the 5′-phosphate of the DNA donor strand, forming the AMP-activated 5′-phosphate at the nick site. Finally, the 3′-OH group of the acceptor DNA fragment acts as a nucleophile and attacks the phosphorus atom of the AMP-activated 5′-phosphate group and the new phosphodiester bond in the break site is created while the AMP molecule is released [5]. The rate-limiting step in the DNA ligase turnover is probably the second step of the reaction [3,6]. Importantly, all steps of the DNA ligation are assisted by the DNA ligase [7] while exogenous ATP is not required for the last step of the reaction [3].

Due to the importance of biological function, DNA ligases are intensively studied in terms of their therapeutic potential [8]. Many compounds have been tested for their inhibitory activity

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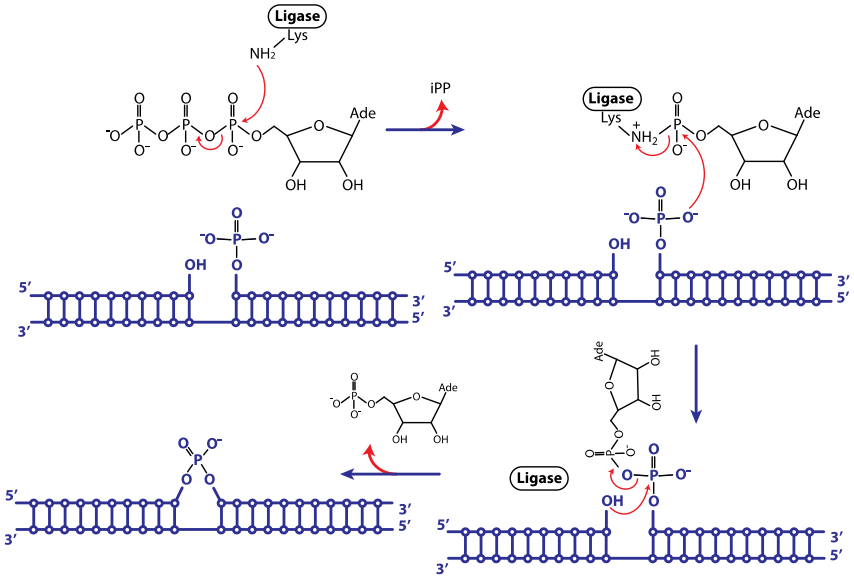

Fig. 1. The mechanism of templated DNA ligation catalyzed by the T4 DNA ligase.

towards DNA ligases to examine whether this could affect cellular replication and DNA repair processes [9,10].

Besides the significant impact on important cellular pathways, another aspect, which makes these enzymes particularly interesting, is the application of DNA ligases in genetic engineering manipulations. Among them, T4 DNA ligase is the most commonly used for *in vitro* molecular research. Compared to other ligases the advantageous features of this enzyme include the ability to ligate substrates with base pair mismatches, a relatively fast turnover rate and a large kcat/K_M ratio. Furthermore, the ligation reaction catalyzed by T4 DNA ligase usually does not require any ligation enhancer such as polyethylene glycol [3]. Despite the widespread use of T4 DNA ligase, stereopreference towards modified cofactors has not been fully examined.

In the present study, ATP cofactor analogs, possessing an α-phosphate thio-modified group with β,γ-phosphate or β,γ-hypophosphate moieties were synthesized and examined with respect of cofactor activity towards T4 DNA ligase.

The series of different ATP analogs have been previously tested as substrates, cofactors or inhibitors of variety of enzymes [11,12] including T4 DNA ligase [13–15]. Considering its enantioselective properties, T4 DNA ligase was tested with L-ATP and results suggest that the enzyme is adenylated by L-ATP but this enantiomer is not an effective cofactor in the end-joining reaction compared to natural D-ATP [13]. With respect to this class of compound, α-thio-analogs of ATP seem to be especially interesting. ATPαS has been tested previously in the context of its activity as a cofactor for T4 DNA ligase [15], however not in the form of pure diastereoisomer. In the present work, not only the importance of the α-phosphorus configuration in α-thio-ATP, but also β,γ-bond were estimated. Using compounds with both a modified α-phosphate moiety and β,γ-phosphate linkage enabled this enhanced study on the T4 DNA ligase: cofactor interaction. In this report the synthesis and biological activity of hypophosphate analogs (thio and oxo) of ATP, along with ATP and pure α-thio-ATP diastereoisomers have been shown for the first time.

2. Results and discussion

In the present study, we used a set of compounds (Fig. 2) consisting of ATP (a), dATP (b), an ATP analog containing β,γ-nonhydrolyzable hypophosphate P-P bond (c), and three pairs

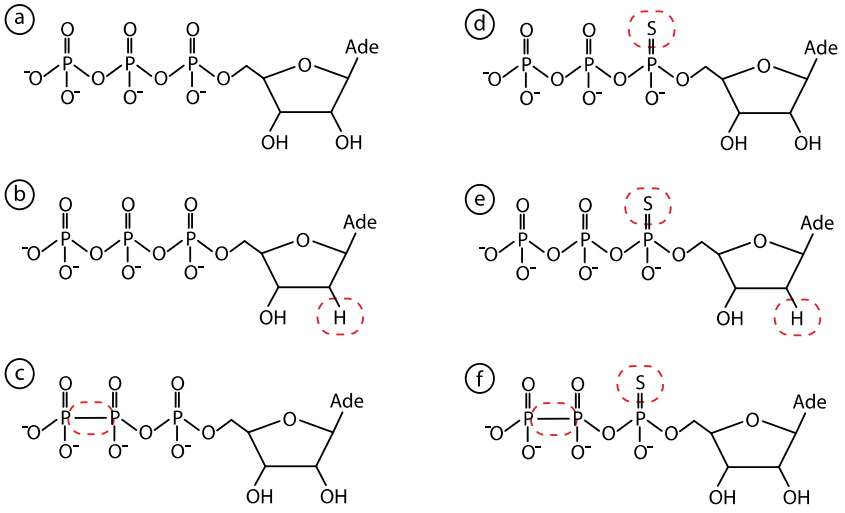

Fig. 2. ATP analogs used in these studies as cofactors and inhibitors of the T4 DNA ligase. (a) ATP, (b) dATP, (c) β,γ-hypo-ATP, (d) ATPαS (R_P and S_P diastereomers), (e) dATPαS (R_P and S_P), (f) β,γ-hypo-ATPαS (fractions "fast" and "slow").