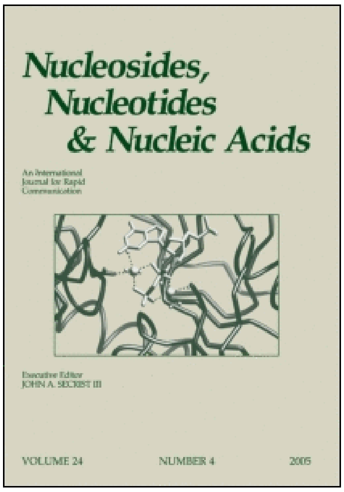

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

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To cite this Article Guranowski, Andrzej , Biochemii, Katedra and Rolnicza, Akademia(1987) 'Specific Enzymes Degrading Diadenosine Tetraphosphate (Ap_4A). Promising Targets for Selective Drugs to be Designed', Nucleosides, Nucleotides and Nucleic Acids, 6: 1, 307 — 310

To link to this Article: DOI: 10.1080/07328318708056210
URL: http://dx.doi.org/10.1080/07328318708056210

NUCLEOSIDES & NUCLEOTIDES, 6(1&2), 307-310 (1987)

SPECIFIC ENZYMES DEGRADING DIADENOSINE TETRAPHOSPHATE (Ap_4A). PROMISING TARGETS FOR SELECTIVE DRUGS TO BE DESIGNED.

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Abstract. Three types of specific Ap_4A-degrading enzymes are known: asymmetrical Ap_4A hydrolase, symmetrical Ap_4A hydrolase and Ap_4A α,β-phosphorylase(ADP-forming). Since each of the enzymes is specific for different kingdom of the organisms and differs both in the mode of Ap_4A degradation, interaction with metal ions and in substrate specificity, one can anticipate that new family of drugs could be designed which would selectively inhibit the microbial (bacterial, fungal or protozoan) enzymes without affecting the mammalian or plant counterparts.

There occur in nature several dinucleoside 5′,5‴-P^1,P^3-(or P^1,P^4-)oligophosphates (DNOPs) but their biological role is poorly understood. Ap_4A is the mostly studied DNOP and it was demonstrated in all organisms investigated. Basing on the *in vitro* studies[1-5] it is assumed that some aminoacyl-tRNA synthetases are responsible for the synthesis of Ap_4A and other adenylylated DNOPs *in vivo*. So far, Ap_4A was shown to be involved in DNA replication[6,7], activation of some enzymes[8,9], and inhibition of others[10,11], functioning of platelets[12] and regulation of the heat shock response [13]. Dramatic increase in the level of different DNOPs, both in procaryotic[14] and eucaryotic[15] cells, has been correlated with stresses. Among the factors which can affect the cellular concentration of DNOPs one has to consider, in addition to the mentioned aminoacyl-tRNA synthetases, the activities of the DNOP-degrading enzymes; these are two unspecific ones, phosphodiesterase I [16,17] and nucleotide pyrophosphatase[18] and the four specific enzymes, Ap_3A hydrolase[16,19], asymmetrical Ap_4A hydrolase[16,20-22], symmetrical Ap_4A hydrolase [23-25] and Ap_4A α,β-phosphorylase[26-27]. Properties of the latter three enzymes for which Ap_4A was proved to be a preferable substrate are summarized below.

Occurrence of specific Ap_4A-degrading enzymes. Asymmetrical Ap_4A hydrolase seems to be typical for higher eucaryotes. It was originally found in *Artemia salina*[20], then in several mammalian tissues[21,22], as well as in higher plant

Lupinus luteus[16]. *Symmetrical* Ap_4A hydrolase was first reported in slime mold *Physarum polycephalum*[23] and independently in *E. coli* and some other bacteria[24,25]. Finally, Ap_4A phosphorylase was found in yeast[26] and recently in Euglena[27].

Mode of Ap_4A cleavage and substrate specificity. *Asymmetrical* Ap_4A hydrolase splits Ap_4A to AMP plus ATP. Among Ap_4A homologs, the Ap_3A is not degraded, Ap_5A is hydrolyzed to ADP plus ATP, and Ap_6A - into 2 ATPs. DNOPs containing other nucleosides than adenosine linked by tetraphosphate chain were proved to be good substrates, too. Mixed dinucleoside tetraphosphates, like Ap_4G, were hydrolyzed randomly and ATP, GMP, GTP and AMP were identified as products[16]. Also p_4A is effectively hydrolyzed to ATP.
Symmetrical Ap_4A hydrolase degrades Ap_4A into 2 ADPs. Various analogs of Ap_4A, including Ap_3A, Ap_5A, Ap_6A and p_4A, act also as substrates and corresponding NDP appears always as one of the reaction products.
Ap_4A phosphorylase cleaves the substrates phosphorolytically at one of the two α,β-anhydride bonds. The inorganic phosphate is incorporated into the corresponding NDP formed. The yeast phosphorylase does not degrade Ap_3A whereas the *Euglena* enzyme does; into 2 ADPs. Cleavage of Ap_5A yields p_4A plus ADP. In addition, the phosphorylase supports the NDP-P_i exchange[28], so it can be used as a tool for the synthesis of NDPs labeled in the β-position. In the both reactions mentioned, phosphate can be substituted by such anions as arsenate, chromate, molybdate, tungstate or vanadate[29] and then NMP always accumulates as a product of the reactions indicating that an unstable NMP-anion is formed as an intermediate.

Metal cation requirements. *Asymmetrical* Ap_4A hydrolase absolutely requires Mg^{2+} as the reaction cofactor and among different other cations tested only Mn^{2+} supports enzymatic activity albeit to a lower extent than Mg^{2+}. Interestingly, Mg^{2+} is not effective in the reaction catalyzed by *E. coli* *symmetrical* Ap_4A hydrolase. The enzyme, however, is strongly stimulated by Co^{2+} and to some extent by Mn^{2+}, Ni^{2+} and Cd^{2+}. In the hydrolysis of Ap_3A, Mn^{2+} was proved to be 9-fold more effective than Co^{2+} [25]. *Physarum* *symmetrical* Ap_4A hydrolase can split Ap_4A and other DNOPs in the absence of metal cations[23]. Phosphorolysis of Ap_4A catalyzed by the yeast and *Euglena* Ap_4A phosphorylases proceeds only in the presence of Mn^{2+} or Mg^{2+}. Other cations, Co^{2+}, Cd^{2+} and Ca^{2+} are less effective as cofactors. However, the NDP-P_i exchange and the anionolysis of NDPs do not require the metal cations.

Recently, reports about chemical synthesis of monothiophosphate-[30] and phosphonate[31,32] analogs of Ap_4A (AppppA) were communicated. The following phosphonate analogs: $AppCH_2ppA$, $ApppCH_2pA$ and $ApCH_2ppCH_2pA$ were tested as potential substrates and inhibitors of procaryotic and eucaryotic Ap_4A-degrading enzymes[33]. None of the analogs was cleaved by the *E. coli* *symmetrical* Ap_4A hydrolase and the yeast Ap_4A phosphorylase cut only $ApppCH_2pA$ (100-fold slower than Ap_4A).

The K_i values computed in the bacterial system for the analogs are lower than the K_m value estimated for Ap_4A (25 μM). This is the first experimental evidence, important from the chemotherapeutic point of view, that specific DNOP-degrading enzymes, particularly those of microbial (bacterial, fungal or protozoan) origin, can be considered as targets for new selective drugs to be designed. Ideal drugs should impair microbial metabolism leaving the mammalian or plant ones not affected.

Acknowledgements. This work was partially supported by Polish Academy of Sciences within Project CPBR 3.13.4.4.4.

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