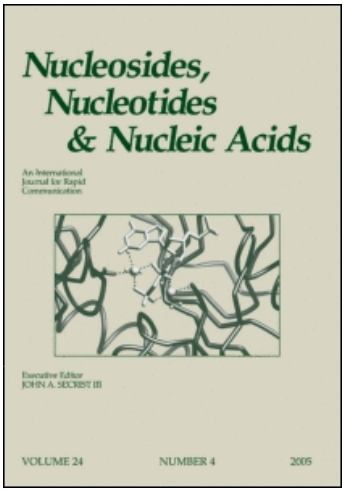

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Dinucleoside Polyphosphate NAD Analogs as Potential NMN Adenylyltransferase Inhibitors. Synthesis and Biological Evaluation

P. Franchetti[ab]; L. Cappellacci[a]; M. Pasqualini[a]; M. Grifantini[a]; T. Lorenzi[c]; N. Raffaelli[d]; G. Magni[c]
[a] Department of Chemical Sciences, University of Camerino, Camerino, Italy [b] Dipartimento di Scienze Chimiche, Università degli Studi di Camerino, Camerino (MC), Italy [c] Institute of Biochemistry, University of Ancona, Ancona, Italy [d] DIBIAGA, University of Ancona, Ancona, Italy

Online publication date: 09 August 2003

To cite this Article Franchetti, P. , Cappellacci, L. , Pasqualini, M. , Grifantini, M. , Lorenzi, T. , Raffaelli, N. and Magni, G.(2003) 'Dinucleoside Polyphosphate NAD Analogs as Potential NMN Adenylyltransferase Inhibitors. Synthesis and Biological Evaluation', Nucleosides, Nucleotides and Nucleic Acids, 22: 5, 865 — 868
To link to this Article: DOI: 10.1081/NCN-120022673
URL: http://dx.doi.org/10.1081/NCN-120022673

NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS
Vol. 22, Nos. 5–8, pp. 865–868, 2003

Dinucleoside Polyphosphate NAD Analogs as Potential NMN Adenylyltransferase Inhibitors. Synthesis and Biological Evaluation

P. Franchetti,[1,*] L. Cappellacci,[1] M. Pasqualini,[1] M. Grifantini,[1] T. Lorenzi,[2] N. Raffaelli,[3] and G. Magni[2]

[1]Department of Chemical Sciences, University of Camerino, Camerino, Italy
[2]Institute of Biochemistry and
[3]DIBIAGA, University of Ancona, Ancona, Italy

ABSTRACT

Two dinucleoside polyphosphate NAD analogs, P^1-(adenosine-5′)-P^3-(nicotinamide riboside-5′)triphosphate (Np_3A, **1**) and P^1-(adenosine-5′)-P^4-(nicotinamide riboside-5′)tetraphosphate (Np_4A, **2**), were synthesized and tested as inhibitors of both microbial and human recombinant NMN adenylyltransferase. Compounds **1** and **2** proved to be selective inhibitors of microbial enzymes.

Key Words: NMN adenylyltransferase inhibitors; NAD analogs; Dinucleoside polyphosphates.

The development of new strategies for treatment of infectious diseases involves the identification of targets mostly represented by enzymes catalyzing metabolic key-reactions. NMNAT, a member of the nucleotidyltransferase α/β phosphodiesterases superfamily, catalyzes the nucleophilic attack by the nicotinamide riboside

*Correspondence: P. Franchetti, Dipartimento di Scienze Chimiche, Università degli Studi di Camerino, Via S. Agostino 1, I-62032 Camerino (MC), Italy; Fax: +39 0737 637345; E-mail: palmarisa.franchetti@unicam.it.

DOI: 10.1081/NCN-120022673
1525-7770 (Print); 1532-2335 (Online)

5′-phosphate (NMN) or deaminated nucleotide NaMN on the α-phosphoryl of ATP, releasing PP_i and NAD or NaAD ($NMN + ATP = NAD + PP_i$). While the eukaryotic enzyme forms NAD or NaAD at similar rates, its prokaryotic counterpart prefers the deaminated substrate (NaMN). Among the enzymes involved in NAD biosynthesis, NMNAT is considered the most promising new antibacterial drug target: it is essential for bacterial cell survival and possesses catalytic and structural properties significantly different from those of the human counterpart. Therefore great effort is being devoted to the identification of molecular ligands acting as selective inhibitors of the bacterial enzyme.

In this respect, the recently found solution of the three-dimensional structure of the enzyme from both bacterial[1] and human[2] sources represents an invaluable tool for the rational design of highly selective microbial enzyme inhibitors. In fact, human NMNAT share limited sequence identity (< 20%) with those from bacteria and archaea and differ in quaternary structure, and biochemical and enzymatic properties.

With this purpose in view, dinucleoside polyphosphate NAD analogs, consisting of two nucleoside moieties, nicotinamide riboside and adenosine, joined through their 5′-position by a linear polyphosphate chain (p_n), were designed. The rationale underlying the production of these compounds arises from the hypothesis that they should mimic the structure of the hypothetical transition state of the reaction catalyzed by NMNAT and hence be good inhibitors. We report here the synthesis of two nucleoside polyphosphates, P^1-(adenosine-5′)-P^3-(nicotinamide riboside-5′)triphosphate (Np_3A, **1**) and P^1-(adenosine-5′)-P^4-(nicotinamide riboside-5′)tetraphosphate (Np_4A, **2**) and their effect on the enzymatic activity of NMNATs from different sources.

RESULTS AND DISCUSSION

The dinucleotides **1** and **2** were synthesized by coupling ATP as sodium salt with the electrophilic nicotinamide riboside monophosphate imidazolide, obtained by reaction of NMN with 1,1′-carbonyldiimidazole as an activating agent. The structure of Np_3A and Np_4A was determined by mass spectrometric analysis using an atmospheric pressure electrospray ionization (API-ESI) source, which confirmed the expected m/z ratio of 743.4 and 823.3, respectively. The structures were also

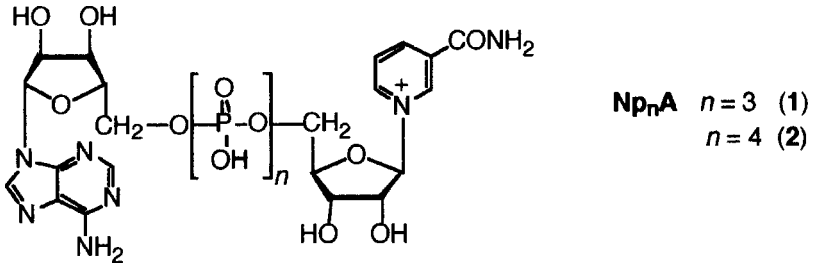

Figure 1. Chemical structures of dinucleotides NAD analogs.

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

confirmed by nuclear magnetic resonance ^{1}H-, and ^{31}P-NMR in D_2O. The ^{31}P spectrum of dinucleotide **1** showed characteristic peaks at –11.0 (br s, 2P) and −22.6 (m,1P) ppm corresponding to CH_2OP and O-P-O, respectively. For compound **2**, the peaks at −11.0 (d, 2P) and −22.8 (br s, 2P) ppm, corresponding to CH_2OP and P-O-P, respectively, were also observed.

The effect of the nucleoside polyphosphates Np_3A and Np_4A on NMNAT activity was evaluated on both microbial and human recombinant enzymes, including NMNAT from *M. jannaschii*,[3] NMNAT from yeast *S. cerevisiae*,[4] human NMNAT-1[5] and NMNAT-2.[6] The assay for the mesophilic and the archaeal enzymes was performed as previously described.[3,5]

The polyphosphates **1** and **2** proved to be inhibitors toward the enzyme isolated from different sources. Interestingly, both Np_3A and Np_4A exerted a different inhibitory effect toward the bacterial enzyme with respect to eukaryotic NMNAT. In particular, about 60% inhibition of the *M. jannaschii* enzyme was observed in the presence of 0.1 mM Np_4A, whereas, at the same concentration the compound had no effect on the two human NMNAT isoenzymes. So, compound **2** might represent a lead for the development of more potent and selective bacterial NMNAT inhibitors. Our study on the different effect of Np_3A and Np_4A on the archaeal and human enzymes will be extended to the eubacterial protein, and might allow identification of the enzyme structural determinants as the basis for the rational design of selective inhibitors. This should be facilitated by the availability of the recently solved crystal structures of archaeal, eubacterial, and human NMNATs.

ACKNOWLEDGMENT

This research was supported by a grant from MIUR (COFIN 2000), Italy.

REFERENCES

1. Zhang, H.; Zhou, T.; Kurnasov, O.; Cheek, S.; Grishin, N.V.; Osterman, A. Crystal structures of *E. coli* nicotinate mononucleotide adenylytransferase and its complex with deamido-NAD. Structure **2002**, *10*, 69–79.
2. (a) Garavaglia, S.; D'Angelo, I.; Emanuelli, M.; Carnevali, F.; Pierella, F.; Magni, G.; Rizzi, M. Structure of human NMN adenylyltransferase. A key nuclear enzyme for NAD homeostasis. J. Biol. Chem. **2002**, *277*, 8524–8530; (b) Zhou, T.; Kurnasov, O.; Tomchick, D.R.; Binns, D.D.; Grishin, N.V.; Marquez, V.E.; Osterman, A.L.; Zhang, H. Structure of human nicotinamide/nicotinic acid mononucleotide adenylyltransferase. Basis for the dual substrate specificity and activation of the oncolytic agent tiazofurin. J. Biol. Chem. **2002**, *277*, 13,148–13,154.
3. Raffaelli, N.; Lorenzi, T.; Emanuelli, M.; Amici, A.; Ruggieri, S.; Magni, G. Nicotinamide-mononucleotide adenylyltransferase from Methanococcus jannaschii. Methods Enzymol. **2001**, *331*, 292–298.

4. Emanuelli, M.; Carnevali, F.; Lorenzi, T.; Raffaelli, N.; Amici, A.; Ruggieri, S.; Magni, G. Identification and characterization of YLR328W, the Saccharomyces cerevisiae structural gene encoding NMN adenylyltransferase. Expression and characterization of the recombinant enzyme. FEBS Lett. **1999**, *455*, 13–17.
5. Emanuelli, M.; Carnevali, F.; Saccucci, F.; Pierella, F.; Amici, A.; Raffaelli, N.; Magni, G. Molecular cloning, chromosomal localization, tissue mRNA levels, bacterial expression, and enzymatic properties of human NMN adenylyltransferase. J. Biol. Chem. **2001**, *276*, 406–412.
6. Raffaelli, N.; Sorci, L.; Amici, A.; Emanuelli, M.; Mazzola, F.; Magni, G. Identification of a novel human nicotinamide mononucleotide adenylyltransferase. Biochem. Biophys. Res. Commun. **2002**, *297*, 835–840.

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016