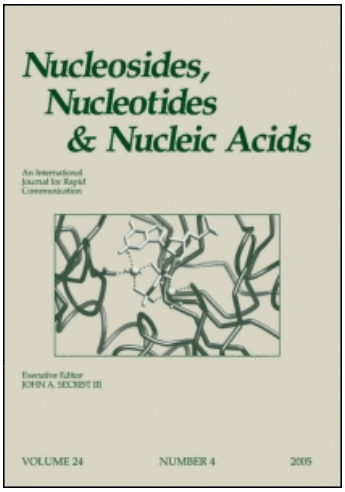

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Functionally Competent Analogs and Their Use for the Determination of Nucleotide Conformation in the Productive Enzyme-Substrate Complexes

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To cite this Article Mikhailov, S. N.(1998) 'Functionally Competent Analogs and Their Use for the Determination of Nucleotide Conformation in the Productive Enzyme-Substrate Complexes', Nucleosides, Nucleotides and Nucleic Acids, 17: 9, 1915 — 1918
To link to this Article: DOI: 10.1080/07328319808004730
URL: http://dx.doi.org/10.1080/07328319808004730

NUCLEOSIDES & NUCLEOTIDES, 17(9-11), 1915-1918 (1998)

FUNCTIONALLY COMPETENT ANALOGS AND THEIR USE FOR THE DETERMINATION OF NUCLEOTIDE CONFORMATION IN THE PRODUCTIVE ENZYME-SUBSTRATE COMPLEXES

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Abstract. Substrate properties of C'-methylnucleotides were investigated in enzymatic reactions. The obtained results allowed to specify the conformation of a substrate in the course of its enzymatic transformation.

Study of the molecular mechanisms of enzyme action includes the design of a model of the productive enzyme-substrate complex and a description of the subsequent events during the product formation. The modelling of a productive enzyme-substrate complex is presently based mainly on the results of X-ray and NMR investigations of the enzyme complexes with inhibitors. The question arises as to the relationship between the structures of the enzyme-inhibitor complex and the enzyme-substrate complex. Several examples may be found in the literature demonstrating their principal difference. So the determination of the nucleotide conformation in the productive enzyme-substrate complex is the important step in development of the molecular model of action of enzymes operating with nucleic acids. With this aim we performed the comparative study of the enzymatic transformation of natural substrates and their derivatives having the restricted glycosidic conformations due to substitution of ribose protons by methyl groups.

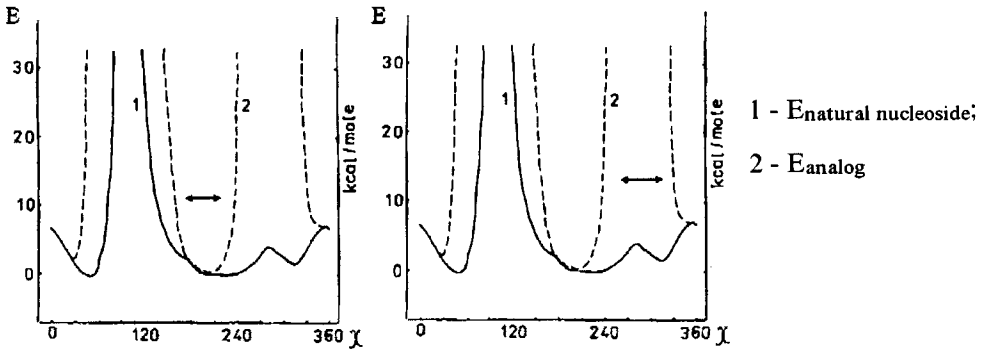
The preparation[1] of C'-methylnucleosides and their physico-chemical properties[2] have been reviewed. Close values of chemical shifts of 5'-C-methyl-nucleosides (5'MeN) and natural nucleoside proton signals indicate the near identical conformational situation in these compounds[2]. The S <-> N equilibrium in the case of 5'MeN is slightly shifted to the S-family. As follows from the CD and

PMR spectra, a more pronounced shift to S-conformers is observed with 3'-C-methylnucleosides (3'MeN)[2]. Close values for chemical shifts of 1'- and 2'-protons in 3'MeUrd and Urd indicate that they exist in solution mainly in anti-conformation. The big values of $J_{3',4'}$ are typical for 2'-C-methylnucleosides (2'MeN)[2] thus indicating the prevalence of N-conformers. The amplitude of the long wavelength Cotton effect for 2'MeUrd is much higher than for Urd which is also in agreement with anti-conformation of 2'MeUrd in solution. It should be noted that in a crystal C'-methylnucleosides exist in anti- and gauche-gauche conformations[2].

To detect intramolecular contacts between methyl group and heterocyclic base, conformational analysis of nucleosides and their analogs using force-field method in the "rigid" bond lengths approximation was carried out. The energetically forbidden conformation was defined as a conformation fitting the following condition: $E_{analog} - E_{natural\ nucleoside} > 5$ kcal/mol. The conformational energy maps (the χ-P dependence versus the potential energy E, free rotation around C4'-C5' bond) are nearly identical for 5'MeN and natural nucleosides. Differences appear when the methyl group in an analog is arranged above the furanose cycle[2]. Only N-conformers of 3'MeN have energetically forbidden conformations, whereas no intramolecular collisions occur in S-conformers. The energy barrier of the syn-anti conversion is noticeably higher when a methyl group is introduced in the 2'-position[2]. These intramolecular contacts of the methyl group with the heterocyclic base should be considered during analysis of enzymatic transformations of C'-methylnucleosides and their phosphoric esters. The introduction of methyl groups results in the appearance of nucleoside analogs having all functional groups of natural compounds, e.g. all possible binding sites for the enzymes of nucleic acids biosynthesis. As a result, comparative binding constants for analogs and natural compounds can be expected. This was confirmed by a number of data. However in some cases a significant decrease of analog affinity to the enzyme was observed, which may be due to the intra- or intermolecular encounter of the voluminous methyl group with the heterocyclic base or the protein amino acid residues during the enzyme-substrate complex formation.

Two extreme cases of the applicability of this approach may be considered : 1) the analog is transformed and is well bound with the enzyme, so that the condition

$E_{analog} \approx E_{natural\ nucleoside}$ for substrate conformation in the enzyme-substrate complex is satisfied; 2) analog is not bound to the enzyme, then an appropriate substrate conformation should be sought in the region where $E_{analog} >> E_{natural\ nucleoside}$, provided that the introduction of the voluminous methyl group causes intramolecular, and not intermolecular steric hindrance[2,3].



Functionally competent analogs were used for studying the mechanism of action and specificity of various enzymes of nucleic acids metabolism. Substrate properties of C'-methylnucleoside 5'-triphosphates were studied in the reactions of DNA[4] and RNA[4,5] synthesis catalysed by *E.coli* DNA polymerase I (Klenow fragment), DNA polymerase α from calf thymus, avian myeloblastosis virus reverse transcriptase and *E. coli* and T7 RNA polymerase. For example, when *E. coli* RNA polymerase was used, 3'MeUTP acted as a terminating substrate and 2'MeUTP can substitute UTP in the reactions of RNA synthesis[4]. Therefore, its conformations in enzyme-substrate complexes do not acquire forbidden states.

A comparative study of kinetic parameters of the hydrolysis reactions of natural UpA and its C'-methyl derivatives catalysed by RNA-depolymerases allows to conclude about the substrate conformation in the corresponding enzyme-substrate complexes[6]. From a series of possible conformations in a solution only one productive substrate conformation is fixed in an active site of enzyme. In the case of RNAase A this is achieved by specific binding of heterocyclic base and phosphate group of the nucleotide in N-conformation of ribose residue. The kinetic parameters of the cleavage reactions of UpA and 3'MeUpA by pyrimidine specific

RNAase A and non-specific RNAase *Penicillium brevicompactum* change non significantly, but 2'MeUpA is completely resistant towards the RNAases action.

Adenosine deaminase deaminated only one 5'tMeAdo (L-talo) diastereomer as well as 2'MeAdo. The other two compounds 5'aMeAdo (D-allo) and 3'MeAdo were not deaminated[2]. This can be due to the intramolecular encounter of a bulky 3'-methyl group with the adenine base. Adenosine conformation in the enzyme-substrate complex is determined by the overlapping of energetically permitted regions in 2'MeAdo and 5'tMeAdo with the energetically forbidden ones for 3'MeAdo. Thus, in the case of the enzyme-sunstrate complex formation we may consider that adenosine is fixed in anti-conformation and 8-proton of adenine base is located near 3'-proton of the sugar moiety in N-conformation and trans-gauche orientation of the exocyclic CH_2OH group[2]. This region is energetically favourable in the case of adenosine.

The described results demonstrate that the proposed method is of general value for the determination of nucleotide conformation in the productive enzyme-substrate complexes.

Acknowledgements. This research was supported by the Russian Foundation for Basic Research.

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