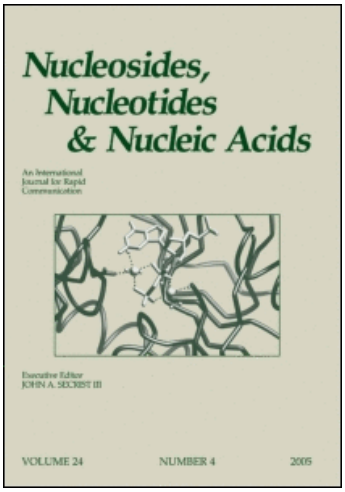

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

On The Catalytic Mechanism of Adenosylhomocysteine/Methylthioadenosine Nucleosidase From *E. coli.*

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To cite this Article Allart, Brigitte , Guillerm, Danielle and Guillerm, Georges(1999) 'On The Catalytic Mechanism of Adenosylhomocysteine/Methylthioadenosine Nucleosidase From *E. coli.*', Nucleosides, Nucleotides and Nucleic Acids, 18: 4, 861 — 862
To link to this Article: DOI: 10.1080/15257779908041582
URL: http://dx.doi.org/10.1080/15257779908041582

NUCLEOSIDES & NUCLEOTIDES, 18(4&5), 861–862 (1999)

ON THE CATALYTIC MECHANISM OF ADENOSYLHOMOCYSTEINE/ METHYLTHIOADENOSINE NUCLEOSIDASE FROM *E. COLI.*

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ABSTRACT : AdoHcy/MTA nucleosidase has been under scrutiny in a series of studies to explore its catalytic mechanism.

Adenosylhomocysteine/methylthioadenosine (AdoHcy/MTA) nucleosidase (EC 3.2.2.9)[1] is involved in a variety of important biological processes. In particular it is required for the regeneration of free homocysteine from AdoHcy in various prokaryotes[2,3] and plays a significant role in the metabolism of these microorganisms via the regulation of MTA concentration (a potent inhibitor of spermine and spermidine synthase).[4,5,6] In microorganisms where MTA phosphorylase is missing AdoHcy/MTA nucleosidase is also essential for methionine salvage.[7,8]

The knowledge of the mechanism by which this nucleosidase performs the splitting of the C-N nucleosidic bond is a prerequisite for the desing of specific inhibitors of this enzyme such as transition-state analogs.

The substrate and inhibitory specificity of AdoHcy/MTA nucleosidase has been first explored with several new 5'-*S*-methylthioadenosine analogs such as: 2'-deoxy MTA, 3'-deoxy MTA, 2',3'-deoxy MTA, anhydro MTA, 1'-eno MTA, (5'*R*)-cyclo MTA and 2'-fluoro MTA. Alteration at C-3' and both C-2' and C-3' positions in MTA abolished substrate activity. However, the 2'-deoxy analog of MTA is effective as a substrate; this result provides evidence against a possible general base catalysis involving the anchimeric assistance of the 2'-α-hydroxy group and the formation of a 1,2-epoxide as an intermediate in the catalytic process. The study of the interaction of an (8,5')-cyclo analog of MTA with the enzyme underlines the importance of the glycosidic conformation of the substrate to bind to the active site. The enzyme

discriminates against methanol attack from solvent during catalysis. This implies the participation of an enzyme-directed water nucleophile.

A poor solvent kinetic deuterium isotope effect was measured ($\leq$ 1.08) on the Vm parameter. Plots of log Vm and log (Vm/Km) for methylthioadenosine as a function of pH values from 5.0 to 8.5 are similar with two presumably essential ionisable groups for catalysis with respective apparent pKa values of 5.6 and 8.2, whereas Km is independent of pH. When the 2'-α-hydroxy group of MTA is substituted by a fluorine, a significant decrease (28500 fold) in the Vm parameter for enzyme catalysed hydrolysis of the modified substrate is observed. This result points to a transition state with a substantial oxocarbenium character. All this data supported the proposed scheme (Fig.1) for bioconversions catalysed by AdoHcy/MTA nucleosidase.

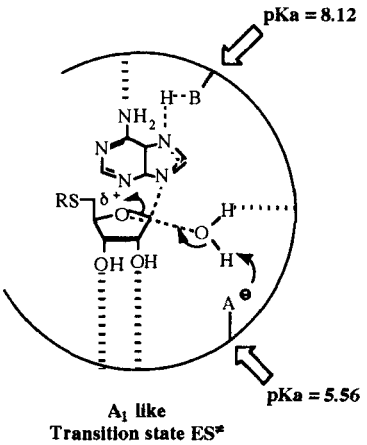

FIG. 1

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