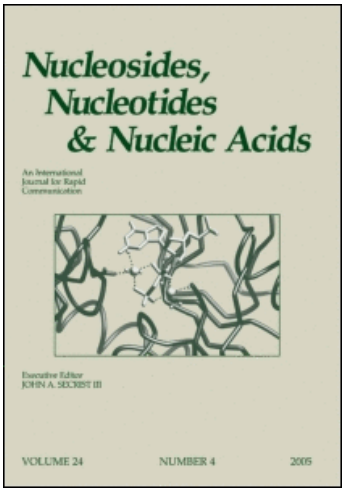

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Molecular Modelling Studies on the Binding of Some Protides to the Putative Human Phosphoramidase Hint1

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MOLECULAR MODELLING STUDIES ON THE BINDING OF SOME PROTIDES TO THE PUTATIVE HUMAN PHOSPHORAMIDASE HINT1

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□ *The aim of the present work is to investigate through molecular modelling the possible role of the human enzyme Hint1 in the final P-N bond cleavage of phosphoramidate ProTides, which would lead to the intracellular delivery of unmasked nucleoside analogue monophosphates. Herein, we report our preliminary analysis based on docking studies of (E)-5-(2-bromovinyl)-2′-deoxyuridine (BVdU) related aminoacyl phosphates with Hint1 and the effect of the amino acid moiety on the enzyme-substrate binding affinity.*

Keywords Phosphoramidate; protide; Hint1

The phosphoramidate approach was conceived as a means to improve cellular penetration of nucleotides and to bypass the first step of kinase-mediated activation of nucleoside analogues. The successful delivery of charged nucleoside monophosphates into cells and the consequential boost in activity for both anti-cancer and antiviral nucleoside analogues are widely described in the literature.[1] The intracellular activation of phosphoramidates is considered to be based on two enzymatic cleavages: the hydrolysis of the amino acid ester moiety as the trigger of the process, and the P-N bond cleavage as the final step that would release the corresponding nucleoside analogue monophosphate (Scheme 1). The correlation between the carboxyl esterase lability of our compounds and their biological activity[2] would indicate an esterase-type enzyme to be involved with the first step of the postulated activation pathway.

The aim of the present work was to investigate the final P-N enzymatic cleavage of phosphoramidates, taking into consideration the phospho-

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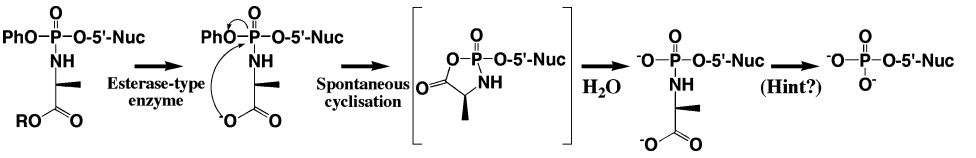

SCHEME 1 Postulated activation pathway of phosphoramidate ProTides.

ramidase activity of Hints (histidine triad nucleotide-binding proteins).[3] Notably, AMP-N-alanine methyl ester was reported as a substrate for Hints,[4] which would support their role in the activation of our phosphoramidate ProTides.

Our docking studies of AMP (Figure 1), dAMP, GMP, and dGMP predicted a similar pattern to that observed in the co-crystallised structure of AMP with human Hint1,[5] which was taken into consideration to define the catalytic site of the enzyme. In all these cases, the phosphate moiety was placed in close proximity of a histidine triad, which represents the fundamental catalytic core of Hints. As reported for the postulated mechanism of action of Hints,[6] a serine residue would play a key role in Hint active site. Moreover, an aspartic residue appeared to enhance the stability of the enzyme-substrate interactions by hydrogen bonding with the sugar hydroxyls. On these bases, the lower enzyme-substrate interactions for 2′,3′-dideoxy and 2′,3′-dideoxy-2′,3′-didehydro-nucleoside analogue monophosphates in comparison to the corresponding ribo- and 2′-deoxyribo-counterparts might be expected. Furthermore, the purine ring fitted in a hydrophobic pocket generated by the side chains of several active site residues (four isoleucines, two phenylalanines and one alanine).

Significantly, also adenosine L-alaninyl phosphate (Figure 1), a postulated substrate for Hint, showed a conformation able to adequately fit with the key residues of the active site.

On the other hand, pyrimidine based nucleoside monophosphates (UMP (Figure 1), CMP, dCMP, and dTMP) displayed a lower affinity for the active site pocket as their bases were always placed outside the hydrophobic pocket and pointing outside the enzymatic core. These data would confirm

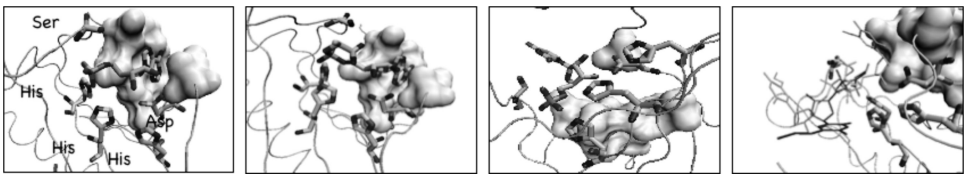

FIGURE 1 (From left) Docking of AMP, adenosine L-alanine phosphate, UMP, BVdU aminoacyl phosphates (L-alanine, L-valine, and L-proline).

the notion that Hint1 may show a higher selectivity toward purine based derivatives.[5] However, pyrimidine based derivatives appeared to be able to locate their phosphate moieties in the proper site for the P-N catalysed cleavage rendering it difficult to predict whether they could act as substrates or not.

Therefore, a series of BVdU aminoacyl phosphates (Figure 1) were considered to evaluate how the nature of the amino acid moiety could affect the binding of the phosphate in the Hint catalytic site. Indeed, our computational data showed that the nature of the amino acid might be crucial for the substrate binding affinity. L-alanine, dimethylglycine, and L-methionine emerged as able to orientate the phosphate in a suitable position for the enzymatic catalysis. For branched or bulky side chain amino acids such as L-valine, L-isoleucine, and L-phenylalanine none of the conformations were shown to occupy an adequate position in the enzymatic pocket. D-alanine and L-proline were responsible for an improper torsion of the molecular structure, which would render unlikely a proper fitting in the Hint catalytic core.

In conclusion, docking of natural nucleotides and some nucleoside analogue monophosphates appeared to agree with the general knowledge of Hint/substrate binding affinity. For activated ProTide derivatives (aminoacyl phosphates), the nature of the amino acid seemed to affect their binding and most of the data follow the general biological structure-activity relationships of known phosphoramidates.[1] In the specific case of BVdU ProTides, deviation from the corresponding SARs[7,8] may be due to the purine-specificity of human Hint1 and to the possible role played by other Hint isoforms, which may display different binding properties particularly towards pyrimidines.

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