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Synthesis of 2' functionalised nucleosides via salvage pathway enzymes

AUTHORS
Matthew WILLMOTT / UNIVERSITY OF MANCHESTER, MANCHESTER INSTITUTE OF BIOLOGY, PRINCESS STREET, MANCHESTER

PURPOSE OF THE ABSTRACT
Recent work on the synthesis of islatravir has highlighted the possibility to use aldolases and other enzymes from the nucleoside salvage pathway to generate nucleoside analogues. However this approach has currently been limited to 2' deoxy nucleotides. 2' functionalised nucleosides show broad use in therapeutic oligonucleotides due to their ability to increase binding to DNA and increase resistance of the oligonucleotides to endonucleases. If the enzymes in this pathway could be expanded to improve their substrate scope at the 2' position this pathway could be used to generate these 2' functionalised nucleosides biocatalytically

The first step to this process will be improving the donor substrate scope of the aldolase enzyme. While aldolases are able to accept a broad range of acceptor (electrophiles) substrates they are generally considered to be quite restricted when it comes to the donor (nucleophile) substrate. Using bioinformatics several active site residues were identified as targets for mutagenesis with the potential to broaden the donor substrate scope of the enzyme. Single point mutations at several of these positions generated aldolases with a greatly increased activity to a range of more complex donor molecules towards which the WT enzyme showed no activity. This was further expanded into a two-step cascade include the synthesis of the aldol acceptor (D-glyceraldehyde-3-phophate) in situ via a kinase allowing a diverse range of 2' functionalised D-Ribose-5-Phosphates sugars to be synthesised stereoselectively. As demonstrated by previous work these pentose-5-phosphate sugars are key intermediates in the biocatalytic synthesis of nucleosides.

To demonstrate the potential of the nucleoside salvage pathway enzymes are able to synthesise 2' functionalised nucleosides in addition to 2' deoxynucleosides the two step aldolase/kinase cascade was combined with phosphopentomutase (PPM) and nucleoside phosphorylases (NPs) to synthesis 2' OH , 2' Me and 2' F adenosine nucleotides from simple starting materials. Work to expand the substrate scope of these remaining two enzymes to include a broader range of functional groups is ongoing in our lab. This will hopefully allow the generation of a much broader range of 2' functionalised nucleosides.

FIGURES

FIGURE 1

FIGURE 2

KEYWORDS

Biocatalysis | Biocatalytic Cascades | Nucleosides | Nucleoside Analogues

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