Sialic acid-synthesising enzymes: Directed evolution and mechanistic enzymology

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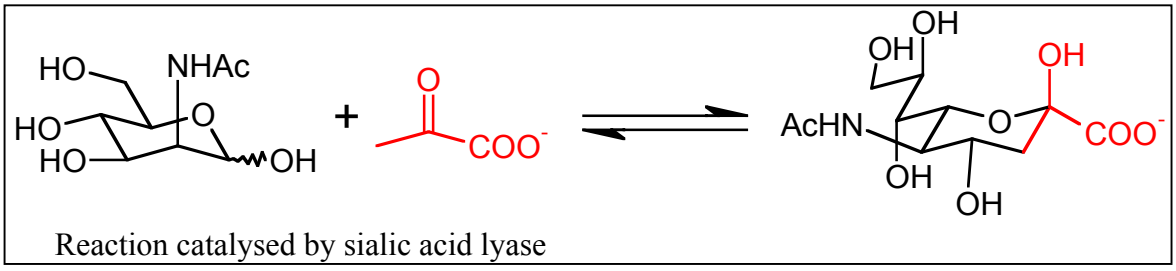
Introduction

Sialic acids are involved in many important cellular recognition and signalling events in a variety of biological processes, for example in cell infection by influenza virus. Sialic acid analogues such as GlaxoSmithKline's Relenza™ which inhibits the influenza virus sialidase offer opportunities to combat disease and study biological processes. Such analogues are however difficult to synthesise chemically. Thus, there is interest in the use of sialic acid synthesising enzymes for the efficient and clean synthesis of sialic acid analogues. Sialic acid aldolase catalyses the condensation of N-acetylmannosamine with pyruvate to generate sialic acid, and forms the target of directed evolution experiments. In addition, sialic acid synthase catalyses the irreversible condensation of phosphoenolpyruvate and N-acetylmannosamine to produce sialic acid, this enzyme is also a potential target for use in the synthesis of analogues.



Reaction catalysed by sialic acid lyase

i) Sialic acid synthase

A number of genes encoding sialic acid synthase from microbial sources were identified from database searches by homology to the *E.coli* enzyme. A number of these genes have been cloned, the enzymes over-expressed and purified and shown to encode sialic acid synthase. However, the gene thought to encode the synthase from *Bacillus subtilis* was shown not to encode a sialic acid synthase and its function remains unknown. Purified synthases have been characterised with respect to metal ion activation, thermostability, pH optima and substrate specificity and are also being used in crystallisation trials. Chemical modification and substrate protection studies indicate that arginine residues play important roles in substrate recognition and site-directed mutagenesis studies are underway to identify those residues responsible. The substrate specificity of these synthases has been determined and has been used to identify targets for improving activity toward non-natural substrates by directed evolution.

ii) Directed evolution of sialic acid aldolase

The technology of directed evolution has the ability to search large areas of sequence space to enable improved or novel activities to be generated. Directed evolution is being used to create mutant sialic acid aldolases with broader substrate specificities than the wild-type enzymes. In addition, enzymes with unnatural stereoselectivity will be created by directed evolution. This project makes use of DNA shuffling to create libraries of mutant enzymes, and novel assays to detect active variants.

Ultimately, these artificially evolved aldolases will be used in the synthesis of inhibitors of influenza virus and other sialic acid mimetics.

Funding: BBSRC and DUE Project Universitas Sebelas Maret Surakarta, Indonesia