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Biocatalytic Synthesis of Selectively Isotopically Labelled Molecules for Specialised Protein NMR Spectroscopy

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PURPOSE OF THE ABSTRACT
Enantioselective synthesis of isotopically labelled biomolecules is not trivial, with most chemical methods requiring precious-metal catalysts and expensive starting materials. Issues such as limited selectivity and low isotopic purities can also create downstream purification steps and waste. Biocatalytic synthesis offers improved selectivity, but typically relies upon costly precursors, such as labelled formate or glucose. In this project, we explore cost-effective routes towards synthesising isotopically labelled biomolecules (such as amino acids and sugars) using chemo- and biocatalytic approaches.

The application we target is Nuclear Magnetic Resonance study of large proteins. NMR is widely used in studying the structure and dynamics of proteins in solution. However, it is inherently limited by the upper size limit, where fast relaxation contributes to complex and uninterpretable spectra. Selective isotopic labelling (2H, 15N, 13C) of proteins using designer amino acid and sugars (and their precursors thereof) can improve this limit, and also enables use of specialised relaxation-optimised NMR techniques such as methyl-TROSY [1].

Particularly, biocatalysis can offer benefits such as mild reaction conditions, high inherent selectivities, and biodegradable catalysts, while chemo-catalysts can be exploited at selective steps for their high productivities and where biocatalytic scope remains limited. Previous work in the Vincent group has established NAD+ cofactor deuteration using [NiFe] hydrogenase enzymes, which can be further applied in redox cascades (fig. 1) for the synthesis of various isotopically labelled chemicals [2], [3]. Further research in this area would enable more practical, routine use of NMR-labelled designer proteins, and contribute towards areas encompassing sustainable manufacturing, biochemical discoveries, and development of novel therapeutics and pharmaceuticals.

FIGURES

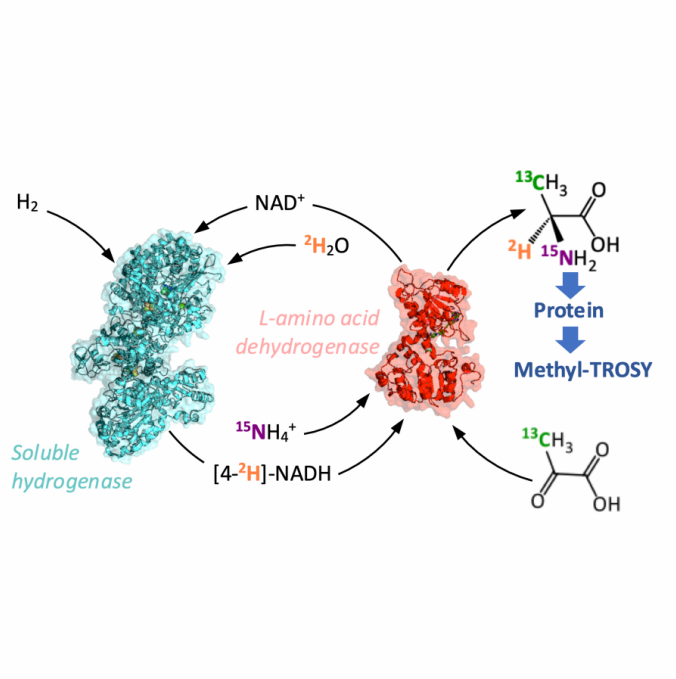

FIGURE 1

Figure 1

Reaction scheme for the synthesis of isotopically labelled L-alanine through use of deuterated NAD2H cofactor, generated in situ

FIGURE 2

KEYWORDS

Isotopic labelling | Cofactor recycling | Amino acid synthesis | Sugar synthesis

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