



Fermenter production, purification and characterization of enzymes involved in the ILV biosynthesis pathway

Nuclear Magnetic Resonance Spectroscopy (NMR) is a powerful technique used systematically in "Drug Discovery" for the identification / validation of therapeutic targets or drug molecules, which is of great interest for the pharmaceutical industry. However, standard NMR techniques are characterized by low sensitivity and are limited only to proteins of small molecular sizes (less than 30 kDa). Recent advances in selective isotopic labelling of methyl groups allowed to push upwards the boundaries of this spectroscopy making possible the NMR study of several supra-molecular assemblies with high molecular weight (up to 1 MDa).

NMR-Bio is a local start-up, hosted by IBS, operating in the structural biology field. Its activity relies, among others, on the distribution of precursors of amino acids enriched with stable isotopes. These precursors, when fed to bacteria, allow the production of proteins selectively labeled on their methyl groups.

Protocols describing the synthesis of precursors to selectively label the methyl groups of isoleucines (I), leucines (L) and valines (V) of proteins expressed in *E. coli*, are already known in the literature and mastered by NMR-Bio's scientists. However, if many proteins are expressed in bacteria, others, including more complex and important proteins (phosphorylated, glycosylated) for the biopharmaceutical research, must be expressed in more "advanced" systems such as eukaryotic expression (insect and mammalian cells). In eukaryotic cells, the aforementioned amino acids (I, L and V) are essential and must be provided to these cells to be able to divide and thus express the protein target. Therefore, the use of precursors already developed, whose cost is affordable, is not possible to obtain the desired isotopic labelling.

In this context, NMR-Bio proposes an internship with the aim to develop protocols for expression and purification of certain bacterial enzymes, already identified, which ensure the

conversion of NMR-Bio precursors to selectively labelled isoleucine, leucine and valine amino acids.

The internship will have the following objectives:

1. The development of expression / purification protocols of Formate dehydrogenase (FDH).
2. The biochemical and functional characterization of the obtained FDH.
3. The covalent immobilization of FDH on an agarose support to stabilize the protein
4. The development of expression / purification protocols of Leucine dehydrogenase (LDH)
5. The biochemical and functional characterization of the obtained LDH.
6. The scale-up of the set-up protocols for fermentor productions.

We are looking for a motivated M1 or M2 student, with basic knowledge of protein expression/purification in bacterial system. This internship will allow the student to develop his knowledge in biochemistry, to acquire notions in NMR and to be in contact with the industrial research.

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