Cell-Free Production of Stable Isotope-Labeled Proteins

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Nuclear magnetic resonance (NMR) spectroscopy is used for various purposes in protein science, such as structural biology and drug development. During the last decade, many of the long-standing methodological difficulties of protein NMR spectroscopy, such as molecular size and sensitivity limitations, have been successfully addressed. For example, using the SAIL (stereo-array isotope labeling) method, it is now routinely possible to determine the high-quality structures of proteins as large as 40-50 kDa, as easily as small proteins.[1] These technological breakthroughs emerged by the synergic development of spectroscopic methodologies and preparative methods of protein samples optimized for collecting the necessary NMR parameters in efficient and accurate manners.

A crucial issue was the development of appropriate protein expression systems, to enable NMR spectroscopists to prepare isotope-labeled protein samples in their own laboratories. It is especially important when using sophisticated stable isotope aided NMR approaches, such as the SAIL method, to choose a method that efficiently incorporates the expensive labeled amino acids into the targeted proteins without serious metabolic scrambling. This is where the cell-free protein expression systems have made a major contribution. The cell-free expression systems utilize the extracts of various living cells, which contain all of the cellular components relevant for protein synthesis. One can choose the most appropriate host cells, which can be micro-organism, plant or mammalian cells, depending on the type of protein one needs to express.

The cell-free protein expression system actually has a long history, dating back to the initial successful trial to express a protein *in vitro* using an *E. coli* cell extract in the 1960's, as described in the literature.[2] In those early days, the protein production stopped soon after the expression got started, and therefore it was not possible to prepare the amounts of protein that are required for an NMR or X-ray structural analysis. During the last few decades, however, the expression level has been enormously improved by several key modifications.[3] Now, cell-free protein synthesis methods are commonly used as important alternatives to cellular protein expression systems, for a wide variety of functional and structural studies.[4,5,6] Although most of the current NMR investigations employing cell-free protein production utilize the *E. coli* cell-free extract, it should be noted that the wheat germ extract seems to be quite useful to express "difficult" proteins, such as mammalian proteins or large protein complexes.[7] In addition to the methods using cell-extracts, there is a completely different approach that employs a reconstituted protein expression system: the "PURE system." Since the PURE system is exclusively composed of the purified components necessary for protein synthesis, it will open various new possibilities that cannot be realized by any other methods.[8]

References

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