Alanine Probes of Supra-Molecular Structure and Dynamics

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The development of new protein labeling strategies, along with optimized experiments that exploit the label, have significantly impacted on the types of biochemical problems that can now be addressed by solution NMR spectroscopy. One popular strategy in studies of high molecular weight proteins involves the use of a pair of α-ketoacids, α-ketobutyrate and α-ketoisovalerate, which serve as the biosynthetic precursors for the production of Ile and Leu/Val, respectively.[1] Addition of these precursors to highly deuterated protein expression media produces U-^{2}H, Ile, Leu, Val- methyl-labeled proteins. These precursors are available with different methyl isotopomers ($^{13}CH_3$, $^{13}CH_2D$, $^{13}CHD_2$) so that a large variety of labeled proteins can be produced[2] and a correspondingly large number of experiments can be performed using ^{1}H, ^{13}C or ^{2}H nuclei.

Ile, Leu, Val methyl groups are powerful probes of side-chain structure and dynamics and their utility has been described in a significant number of papers.[2] Ala methyls, on the other hand, report on properties of the backbone and hence provide important complementary information. A number of recent publications outline approaches for the production of highly deuterated, Ala-[$^{13}CH_3$] labeled proteins.[3,4] Ala labeling is challenging since this residue is produced directly as a result of transamination of pyruvate, which is also a precursor in the production of the branched-chain amino acids. Transamination is reversible so even if free methyl-labeled Ala is provided to the media, scrambling will occur with label incorporated at a variety of potentially undesired locations. Recently Boisbouvier and coworkers have developed a procedure to generate methyl labeling at Ala side chains with minimal (<1%) scrambling.[3] This was achieved by adding 2-[^{2}H],3-[^{13}C]-Ala (800 mg/L) as well as precursors for other pathways in which the scrambled amino acids are produced.

The ability to produce highly deuterated, Ala-[$^{13}CH_3$] labeled proteins further increases the number of methyl probes available for studies of very high molecular weight systems.[5] A number of applications involving Ala methyl probes can be envisioned, including measurement of backbone dynamics through relaxation studies, probing structure via residual dipolar couplings, methyl-methyl NOEs (Nuclear Overhauser Effect) or PREs (Paramagnetic Relaxation Enhancement) and studies of molecular interactions.

References

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