Automated NMR assignment of protein side chain resonances using automated projection spectroscopy (APSY)

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This paper describes an automated method for sequence-specific NMR assignment of the aliphatic resonances of protein side chains in small- and medium-sized globular proteins in aqueous solution. The method requires the recording of a five-dimensional (5D) automated projection spectroscopy (APSY-) NMR experiment and the subsequent analysis of the APSY peak list with the algorithm ALASCA (Algorithm for local and linear assignment of side chains from APSY data). The 5D APSY-HC(CC-TOCSY)CONH experiment yields 5D chemical shift correlations of aliphatic side chain C-H moieties with the backbone atoms H(N), N, and C‘. A simultaneous variation of the TOCSY mixing times and the projection angles in this APSY-type TOCSY experiment gives access to all aliphatic C-H moieties in the 20 proteinogenic amino acids. The correlation peak list resulting from the 5D APSY-HC(CC-TOCSY)CONH experiment together with the backbone assignment of the protein under study is the sole input for the algorithm ALASCA that assigns carbon and proton resonances of protein side chains. The algorithm is described, and it is shown that the aliphatic parts of 17 of the 20 common amino acid side chains are assigned unambiguously, whereas the remaining three amino acids are assigned with a certainty of above 95%. The overall feasibility of the approach is demonstrated with the globular 116-residue protein TM1290, for which reference assignments are known. For this protein, 97% of the expected side chain carbon atoms and 87% of the expected side chain protons were detected with the 5D APSY-HC(CC-TOCSY)CONH experiment in 24 h of spectrometer time, and all these resonances were correctly assigned by ALASCA. Based on the experience with TM1290, we expect that the approach presented in this work is routinely applicable to globular proteins with sizes up to at least 120 amino acids.

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