3D $^{13}$C--$^{15}$N-heteronuclear two-spin coherence spectroscopy for polypeptide backbone assignments in $^{13}$C--$^{15}$N-double-labeled proteins

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SUMMARY

The pulse sequence of a new constant-time 3D triple-resonance experiment, ct-HAICAN[HN], is presented. This experiment delineates exclusively scalar connectivities and uses $^{13}$C--$^{15}$N heteronuclear two-spin coherence to overlay the chemical shift evolution periods of the $^1$C and $^1$N nuclei, thereby providing the four resonance frequencies of the $\alpha$-proton, the $\alpha$-carbon, the amide nitrogen, and the amide proton of a given amino acid residue in three dimensions. This experiment promises to be a valid alternative to 4D experiments, providing the same information on intrarresidue polypeptide backbone connectivities in $^{13}$C--$^{15}$N-double-labeled proteins.

Sequence-specific $^1$H NMR assignments, which provide the basis for 3D protein structure determinations in solution (Wüthrich et al., 1982), have conventionally been obtained by analysis of $^1$H--$^1$H sequential NOEs (Wüthrich, 1986). Although work with homonuclear $^1$H NMR is usually limited to proteins with molecular weights below 10 000 to 12 000, this strategy can be applied to larger proteins with the use of isotope labeling and higher-dimensional, heteronuclear-resolved ($^1$H,$^1$H)-NOESY experiments (e.g., Torchia et al., 1989; Wüthrich et al., 1991). As an alternative, sequential assignments have been obtained using exclusively heteronuclear scalar couplings along the polypeptide backbone in $^{13}$C--$^{15}$N-double-labeled proteins (Igo et al., 1990).

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Abbreviations: 3D, 4D, three-dimensional, four-dimensional; TPPLE, time-proportional phase incrementation; ct, constant-time; rf, radiofrequency; NOE, nuclear Overhauser enhancement; NOESY, two-dimensional nuclear Overhauser enhancement spectroscopy; glutaredoxin(C145), mutant E. coli glyoxalase with the cysteine at position 14 replaced by serine.

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