Bohuslav Klíma, is that the burial is a reproduction of a real event — a failed birth. The man on the female's left holds her arm to give comfort, but cannot bear to look, while the man on her right tries to help the delivery, but in vain. Klíma suggests that the ochre around the female's pelvis may mark the original location of a fourth skull or the complete skeleton of a newborn child whose bones have disintegrated.

The idea is appealing, but the supposedly complete disappearance of the newborn baby's remains is made slightly doubtful by the good preservation and virtually complete state of the three skeletons. In addition, there are other enigmas: for example, the male with his hand on the female's pelvis was skewered to his sacrum by a large piece of wood, while the other male's skull was smashed (and further deformed by the weight of earth on it). Research is under way to test the hypothesis that these two apparently healthy and powerful men met violent deaths, perhaps sacrificed to accompany the dead female. Moreover, this handicapped female, like the dwarf[5], the crippled old Neanderthaler from Shanidar, Iraq[7] and others, adds further proof to the already well-established notion that communities of the Middle and Upper Palaeolithic took good care of — and perhaps even accorded special importance to — physically disadvantaged individuals. □

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Protein structure

An extra dimension to NMR

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TWO-DIMENSIONAL nuclear magnetic resonance (2D-NMR) techniques, developed by Ernst and Wüthrich[1,2], have greatly advanced the determination of protein structures in solution. The geometric information that is needed to solve the three-dimensional structure of a protein by NMR spectroscopy is obtained from interproton distances assigned from correlation and nuclear Overhauser effect (NOE) experiments. The quality of the structure obtained depends critically on how well the extensive spectral overlap that is observed can be resolved so as to enable the resonances to be assigned unambiguously to specific protons. On page 374 of this issue, Oschkinat *et al.*[3] describe a technique of further improving the resolution of the cross-peaks by extending 2D-NMR into a third dimension.

NMR is proving to be an important complement to X-ray crystallography in the determination of protein structure. Although there is as yet no concrete evidence to indicate that the structures of proteins in solution, determined by NMR, are substantially different from those of proteins in crystal form, determined by X-ray diffraction, provided both techniques have been applied correctly, there is, in principle, the possibility that the restraints imposed on a protein structure by the crystal environment could affect the tertiary structure of segments of the polypeptide chain. In addition, although water of crystallization is observed as a bound surface layer in protein crystals, structural information obtained crystallographically cannot give any extensive information about the effect of bulk solvent on the conformations and mobilities of many surface residues of a protein. Furthermore, although some information on the spatial distribution of the dynamics of a protein can be obtained by X-ray crystallography[4], such information is practically intrinsic to NMR spectroscopy and is therefore more readily accessible. And finally, the range of sizes of polypeptides and proteins for which NMR data are most obtainable lies in precisely that region for which X-ray structures are often hard, or impossible, to obtain because small proteins are difficult to crystallize, present few reactive surface residues for derivatization with heavy metals, and are too large for phasing by direct methods. Application of NMR spectroscopy to larger proteins has developed slowly; the effective upper limit is still 10 kilodaltons in the absence of X-ray crystallographic information to help make assignments.

A protein structure is obtained from NMR data by applying interproton distance information to a distance geometry algorithm[5] or to a molecular dynamics simulation starting from the unfolded polypeptide chain[6]. Because the NOE data required for the solution of a three-dimensional structure by NMR can only give information on short-distance interactions (less than about 5 Å), it becomes critically important to be able to resolve as many of the peaks arising from such interactions as possible. In general, the resolution of the cross-peaks arising from interproton interactions in a β-sheet is relatively straightforward. But cross-peaks that arise from interactions between elements of secondary structure, particularly those arising from interactions of less well-defined secondary structure such as loops or from α-helices, are very often not well resolved, partly because they are weak and partly because they overlap cross-peaks from other interactions. Although the technique that Oschkinat *et al.* describe cannot improve the former, it can significantly improve the latter.

The authors have chosen α1-purothionin, a protein of 46 residues, to illustrate the utility of their technique. In particular, a NOE spectroscopy experiment (detection of inter-residue and intra-residue NOE connectivities between NH and $C^{\alpha}H$ protons) was combined with a total-correlated spectroscopy experiment (detection of J-coupling of intra-residue connectivities between $C^{\alpha}H(i)$ and $NH(i)$ protons) in a restricted region of the NMR spectrum. Recording a complete 3D-NMR spectrum is not possible because it would take too long. But the technique can be applied selectively in regions with extensive overlap of cross-peaks. Thus, the combined experiments described in the paper allowed the assignment of inter-residue cross-peaks that could not be assigned uniquely by 2D-NMR, owing to overlap with other cross-peaks.

Another means to extend resolution has recently been reported, with thioredoxin (108 amino-acid residues) as the example[7]. The thioredoxin was randomly labelled with deuterium to the extent of approximately 75 per cent. The resulting protein gives a significantly better resolved 2D-NMR spectrum than the protein with natural abundance hydrogens. It is conceivable that a combination of the isotope labelling method and extension to 3D-NMR will make it possible to tackle proteins substantially above the 10 kilodalton range, including many that carry out interesting reactions or that undergo conformational changes which cannot be studied by crystallographic means. The only drawback of the three-dimensional method would seem to be that readers of journals and those attending conferences, who have been confounded by the incomprehensible 2D-NMR plots so dear to the hearts of NMR spectroscopists, will now be even further confounded by stereo diagrams of three-dimensional arrays of spots. □

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