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An approach to understanding conformational mobility in peptides and proteins

Considerable success has been achieved using interproton distances obtained from nuclear Overhauser enhancements (NOEs) to generate three-dimensional structures for medium sized biological molecules in solution [1]. These structures are akin to those obtained by X-ray crystallography in that they represent averages, though the NMR average is taken over time rather than over the ensemble of diffracting molecules. Close examination of the scalar coupling constants and NOEs predicted from such coordinates tends to reveal significant deviations from experimental values [2], and various methods are being tried to allow for the effect of averaging over different conformations. These include the introduction of a time dependence for the NOE constraints in molecular dynamics (MD) simulations [3] and the simultaneous energy minimisation of pairs of closely related conformations. However, the computing power available is not sufficient to allow such simulations to follow gross conformational changes, and these methods are proposed principally for the purpose of improving the refinement of a unique structure. Such gross conformational fluctuations, both of proteins and their ligands, may be essential to the process of recognition and binding: NMR is ideally suited for their elucidation since detailed conformational information can be obtained under physiological conditions in solution.

A protocol is described here for exploring the range of conformations accessible to a molecule in solution and for estimating their relative energies by measuring the populations of each class of conformations. The chosen model comprises a set of rigid structures which span the low energy conformational space of the molecule and interconvert rapidly on the NMR time scale. A flow diagram

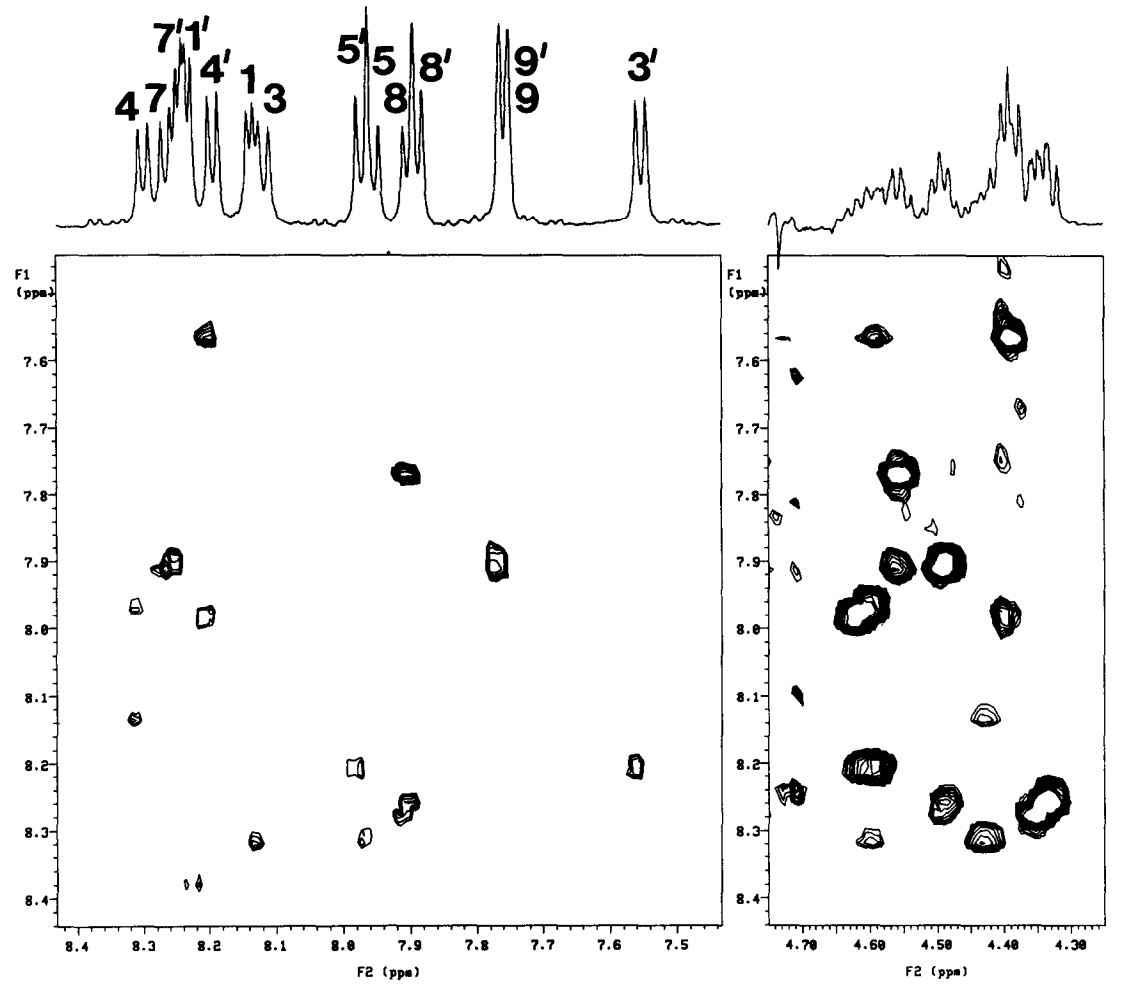


Fig. 1. Portions of the 500 MHz ROESY spectrum of AcYPYDVPDYA obtained with a 300 msec mixing time in 90% H_2O/10% D_2O at pH 6.1 and 22°. Only negative peaks are shown. NH–NH correlations appear in the section on the left, and NH–alpha correlations on the right. The NH assignments are indicated by residue numbers with a prime to indicate the form with Pro2 *trans*.

outlining the process for generating these structures is shown below. The details of the experiments and computational packages used will depend on the molecular weight of the system being investigated: the ordinary Overhauser experiment is suitable for small molecules, and constrained molecular dynamics runs are sufficient to explore their limited range of conformations. The NOE is also suitable for small globular proteins, though a distance geometry calculation [4] is then necessary for a preliminary definition of the structure.

The example taken here is an antigenic acetyl nonapeptide [5], AcYPYDVPDYA, which represents residues 98–106 of influenza virus haemagglutinin. Ordinary NOEs are small because of the intermediate tumbling rate in solution, so the rotating frame Overhauser spectroscopy (ROESY) experiment [6] is used instead. The two-spin approximation is applied to the ROESY experiment to extract approximate distance constraints in the same way as with ordinary NOEs. Portions of the two-dimensional spectrum used are shown in Fig. 1. An unusually high level of overlap occurs because Pro2 exists in both *cis* (44%) and *trans* (56%) forms in slow exchange and the chemical shifts of the backbone protons of residues 5–9 are similar in both forms. The conformational behaviour of the two forms is also assumed to be similar, so that 56% of the volume of the unresolved cross peaks can be ascribed to the *trans* form which will be the one considered here.

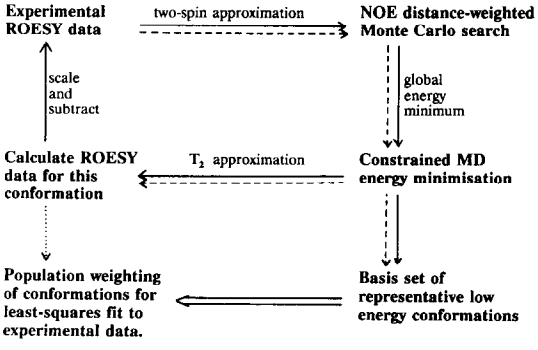
The flexibility of this peptide is too great for distance geometry to be useful and yet the size is sufficient to preclude simulation of all possible conformations by molecular dynamics. The approach adopted is, therefore, a Monte Carlo search of conformational space with NOE pseudo-potentials included to find approximate low energy conformations. These conformations are then refined by constrained molecular dynamics. A similar approach would be suitable for mobile regions of enzymes or hormones once the essential folding had been established by more traditional methods.

The lowest energy conformation obtained in this way is approximately a beta sheet. This is the "NMR structure" which would normally be presented, but here it is used to provide the first element of a conformational basis set. At this point, the quality of an NMR structure might be evaluated according to the distance constraints satisfied: it is better to compare the experiment with the complete set of NOEs calculated from the structure since this also takes account of NOEs which should have been observed but were not. The two-spin approximation is unnecessary for the back calculation since cross-relaxation among the full set of protons can be computed. The approximation of a weak spin-locking field [6] is used here to calculate NOEs in the rotating frame with a modified version of the program CORMA [7]. The first stage of calculation involves adjusting the effective correlation time of the rigid structure to match the measured rotating frame relaxation rate (average T_{1rho} for backbone protons 0.23 sec, effective correlation time 0.8 nsec). Further refinements are possible, including fast and slow correlation times to approximate internal motions and allowance for anisotropic tumbling of the molecule. The computed cross peak intensities are compared with the experiment in Fig. 2. The worst positive mismatch in this data set occurs for the cross peak between the NH of Val5 and the alpha proton of Asp4. The experimental value is just 75% of that calculated, and the computed values are scaled down accordingly before subtracting them from the experimental data. This may be regarded as assigning a 75% probability, or population, to the beta sheet.

The differences, which far exceed the experimental error of ± 0.005, now provide a new set of constraints for generating an alternative conformation which will be quite unlike the first. The new conformation which is obtained approximates an alpha helix, and the ROESY cross peak intensities computed for this structure are compared with the residue of the experimental values, scaled to represent a 100% population, in Fig. 3. The scaled experimental error corresponds to ± 0.02 in this plot, but the differences are still significant, particularly among the NH–NH interactions and close to the proline residues. Further cycles of this iterative procedure would generate structures with beta turns, each with small populations, until the precision of the experimental data is exhausted.

The final step in the analysis is to weight the populations of the conformations in the basis set to minimise the r.m.s. deviation from the experimental values. For the purpose of this illustration, only the first two conformations are considered. The least-squares fit then assigns a 78% population to the beta sheet and 22% to the alpha helix, both of which are overestimated because of the limited basis set. The cross peak intensities computed for this mixture are indicated by horizontal bars in Fig. 2.

It should not be assumed that the conformations gen-



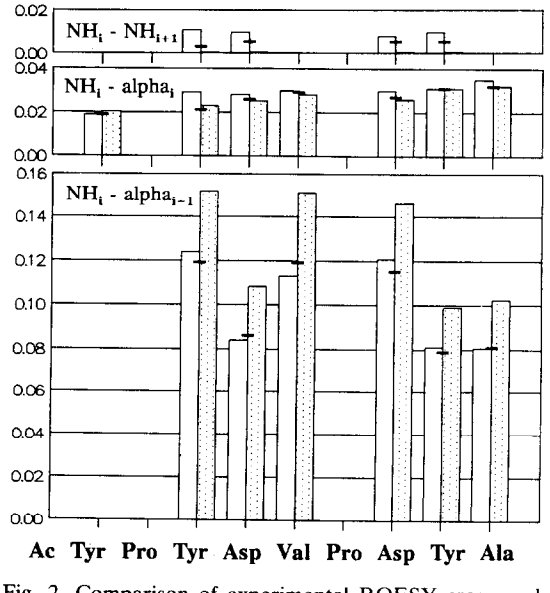


Fig. 2. Comparison of experimental ROESY cross peak intensities with calculation (shaded bars) for the beta sheet conformation. Results of the final least-squares fit are indicated by horizontal bars.

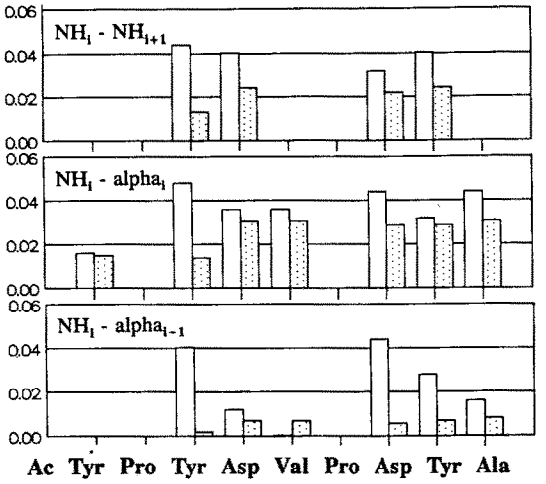


Fig. 3. Comparison of differences taken between experimental ROESY cross peak intensities and those calculated for the first $_4$(beta sheet) conformation with calculation (shaded bars) for the alpha helix conformation.

erated by this method are the only ones which exist, or that they interconvert instantaneously. Rather, they are representative of the virtual continuum of low energy conformations and provide a basis for estimating both the enthalpic and the entropic cost of adopting a single conformation in a tightly bound state. The process lends itself to complete automation, from the measurement of large numbers of cross peaks to the weighting of multiple conformations. The data set down here is necessarily limited since this automation is not yet complete, but it does illustrate the possibility for obtaining detailed information about the conformational behaviour of short peptides which have been disimissed previously as being "random coil." This further admits the possibility of comparing the biological activity of peptide analogues with the populations of representative conformations, and of evaluating the conformational preferences of particular sequences in free solution for the purpose of predicting protein structures. The approach may be still more important in much larger systems for detecting the presence of conformations which are weakly populated but still have relative energies much less than the energy available from interactions with an antibody or receptor.

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