Multi-conformational peptide dynamics derived from NMR data: A new search algorithm and its application to antamanide

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SUMMARY

A search algorithm, called MEDUSA, is presented which allows the determination of multiple conformations of biomolecules in solution with exchange rate constants typically between 10\textsuperscript{3} and 10\textsuperscript{7} s\textsuperscript{-1} on the basis of experimental high-resolution NMR data. Multiples of structures are generated which are consistent as ensembles with NMR cross-relaxation rates (NOESY, ROESY), scalar J-coupling constants, and T\textsubscript{1p} measurements. The algorithm is applied to the cyclic decapeptide antamanide dissolved in chloroform. The characteristic radio-frequency field dependence of the T\textsubscript{1p} relaxation rates found for the NH protons of Val\textsuperscript{1} and Phe\textsuperscript{6} can be explained by a dynamical exchange between two structures.

INTRODUCTION

Most proteins and peptides are flexible, with a dynamic equilibrium between various local energy minima, sometimes called conformational substates (Austin et al., 1975). It is known that conformational flexibility is of importance for biological function and for the folding process that proceeds through numerous conformations spanning a relatively large phase space volume (Kim and Baldwin, 1990; Wright et al., 1988). Obtaining information on transient conformations is therefore of great interest. Numerous experimental approaches have been proposed, such as using the temperature dependence of B factors from X-ray diffraction (Petsko and Ringe, 1984; Ringe and Petsko, 1985), quasi-elastic neutron scattering (Cusack, 1989), fluorescence depolarization (Beecham and Brand, 1984), ultrasonic absorption measurements (Slutsky et al., 1989) and NMR studies (Lipari and Szabo, 1982; Kopple et al., 1988; Nusser et al., 1988; Clore et al., 1990). All
these techniques have some virtues and many limitations. X-ray diffraction, although informative about local disorder, does not provide rate constants nor insight into correlated motional processes, and the relevance of the results is restricted by the single-crystal environment. Quasi-elastic neutron scattering and ultrasonic absorption allow the measurement of correlation functions of the motional processes but provide no local information.

NMR has some of the most attractive features. It uses local sensors that are sensitive to motion over a wide time scale (Abragam, 1961; Wüthrich, 1986; Ernst et al., 1987). Slow dynamics can be followed in real time after an initial perturbation of the chemical equilibrium, possibly combined with a pulse labelling technique (Roder and Wüthrich, 1986; Roder et al., 1988; Udgaonkar and Baldwin, 1988). Conformational processes with exchange lifetimes \( \tau_e \) in the range \( 10^{-4} \leq \tau_e \leq 10^{-1} \) s can be investigated by line shape studies. Faster intramolecular processes may be monitored through relaxation measurements. The effects on relaxation are dependent on the ratio \( \tau_e/\tau_c \) with the overall molecular tumbling correlation time \( \tau_c \). For \( \tau_e/\tau_c \gg 1 \), population-averaged conformationally averaged relaxation and cross-relaxation rates are observed. For \( 0.1 \leq \tau_e/\tau_c \leq 10 \), intramolecular dynamics becomes directly relaxation-active, while for \( \tau_e/\tau_c \ll 1 \) conformational averaging of the molecular structural parameters occurs that depends on the details of the motional process (Brüschweiler et al., to be published). In addition, it is possible to sense, by rotating frame relaxation measurements (Jones, 1966), processes that modulate the chemical shift in the range \( 10^{-6} \leq \tau_e \leq 10^{-3} \) s (Deverell et al., 1970).

In the standard procedure of NMR structure determination, NOESY or ROESY (Jeener et al., 1979; Kumar et al., 1981; Bothner-By et al., 1984) cross-peak intensities are translated into distances \( r_{kl}^{\text{NOE}} \) between nuclei \( k \) and \( l \) (Wagner and Wüthrich, 1982). In a mono-conformational system, the measured \( r_{kl}^{\text{NOE}} \) can be used in a distance geometry (DG) (Havel and Wüthrich, 1984; Braun and Gö, 1985; Crippen and Havel, 1988) or restrained molecular dynamics (rMD) algorithm (Clore et al., 1985; Kaptein et al., 1985) to determine the three-dimensional biomolecular structure. When it turns out to be impossible to find a structure consistent with all measured NOE/ROEs and the holonomic structural data (bond lengths and bond angles), it is likely that several dynamically interchanging conformations are involved. On the other hand, the finding of a structure which fulfills all constraints does not prove the absence of multiple conformations. An approach to apply rMD also for dynamic structures has recently been proposed by Torda et al. (1990). It uses a time-variable NOE-restrained potential that depends on the past of the trajectory.

This contribution describes a novel algorithm for processing NMR information that allows the extraction of multiple conformations for exchange processes slow compared to the molecular tumbling correlation time \( \tau_e \), i.e. \( \tau_e/\tau_c \gg 1 \), where population-averaged cross-relaxation rates are observed.

SEARCH PROTOCOL

The search protocol is called MEDUSA standing for Multiconformational Evaluation of Distance information Using a Stochastically constrained minimization Algorithm. It interprets experimental cross-relaxation data in the following way:

a) The detection of an NOE/ROE yielding a distance \( r_{kl}^{\text{NOE}} \) implies that there is at least one signifi-