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Minireview

Protein dynamics

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Abstract

Modern NMR has revitalized the study of protein dynamics Multidimensional spectra and the heteronuclear spectroscopy allow a substantial gain in resolution Dynamics can be analyzed at individual sites and data on segmental and sequence-dependent flexibility are accumulating This review summarizes the wide variety of NMR approaches for observing internal motions, including the folding processes, and the attempts to correlate dynamics to the biological activity of proteins The implications of mobility on structure determination by NMR is also discussed

Key words Protein, Dynamics, Folding, NMR, Relaxation

1. Introduction

Internal motions and segmental flexibility in proteins are becoming an increasingly important subject for research, as more and more examples are being discovered. Nuclear magnetic resonance (NMR) plays a central role in the observation of these dynamic phenomena. Indeed, the rate of motional processes can be determined quite accurately by NMR over a wide range of frequencies, from subnanosecond to second and hour time scales (Fig. 1) Very high frequencies (nanosecond and subnanosecond range) can be investigated by relaxation measurements in the laboratory frame. Relaxation in the rotating frame is sensitive to fluctuations in the microsecond to millisecond range Lineshape analysis, saturation transfer, backbone proton exchange can be used to investigate motions characterized by millisecond to second time constants

This review is based on presentations given at the FEBS course on Magnetic Resonance and Protein Dynamics held in Erice (Sicily), March 15–21, 1993, where the leading experts in the field have gathered to discuss the potential functional significance of the observed dynamic phenomena and their implications for solution structure determination by NMR.

As reported by O. Jardetzky in his introduction, NMR evidence for the existence of internal flexibility in macromolecules can be obtained from regional differences in the magnitude of the relaxation parameters T1, T2 and NOE, exchange rates of backbone protons, as well as from chemical shifts and line broadening that can be traced to conformational equilibria [1] The existence of internal flexibility in proteins has been known to NMR spectroscopists since the mid 1960s Of major interest was the early identification of flexible segments in the nucleic acid binding domains of Tobacco Mosaic Virus capsid protein and the lac-repressor, as well as in myosin in 1978 [2–4] Abundant new examples of protein dynamics presented at the meeting generally fell into three categories: (1) variations in motional frequencies along the polypeptide backbone, (2) enhanced flexibility of longer segments, particularly in DNA-binding domains of DNA binding proteins, and (3) dynamic phenomena in partially folded structures obtained under denaturing conditions

The discussion of these examples was in the framework of not only NMR evidence, but also evidence provided by other, complementary methods, like differential scanning calorimetry, circular dichroism and photochemical dynamic nuclear polarization Methods of molecular dynamic simulations have proved essential for the evaluation of the nature and the amplitude of the motions

2. Sequence dependent high frequency motions

The interesting, but poorly understood phenomenon of sequence- and structure-dependent variation of the relaxation parameters along the polypeptide backbone was discussed in detail by G Wagner Peng and Wagner [5,6] had developed a method for experimentally deter-

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mining the spectral density functions for CH and NH vectors along the backbone from a combination of ^{13}C or ^{15}N relaxation of various spin states. This permits the mapping of the frequency distribution of rotational motions at the five frequencies of transitions between the levels of the XH spin system, i.e. 0, $f(H)$, $f(X)$, $f(H) \pm f(X)$ Examples of proteins in which variation in rotational motion along the backbone can be detected include EglinC [6] and Gal4 (Lefèvre, Dayie, Peng and Wagner, manuscript in preparation)

It should be emphasized that the reported analysis is entirely empirical and does not invoke any theoretical assumptions It amounts to an experimental measurement of spectral density functions, which need to be explained by a theoretical model of motion The observed variations are puzzling Model-free approaches [7] are too crude to provide a meaningful insight into the detailed maps that can be regenerated empirically. A recent study by Clore and coworkers [8] well illustrates the limited usefulness of the model-free approach. As more and more detailed relaxation measurements become available, more and more arbitrary parameters have to be introduced ad hoc to explain them, losing all relation to physical reality.

In general, large amplitude motions are observed in loops, while structured regions are less flexible The internal motions in the loops may involve all residues, leading to a poor definition of structure as determined by NMR. In other instances, the apparent mobility arises from high flexibility points restricted to few residues at each end of the loop, the rest of the loop being well structured, like in EglinC [6]. Examples of flexible regions in other proteins – notably the human transforming growth factor alpha [9], the Fe(II) cytochrome c_{551} [10] and kistrin [11,12] were given by G. Wagner [13]

However, the need for a clear physical picture of the observed backbone motions and their variations within a given structure has still not been met completely. An additional example was presented for staphylococcal nuclease by J.L Markley [14–17] There clearly exist structures in which little variation is observed along the polypeptide chain, but then there are others in which it is substantial and follows no clearly recognizable pattern.

The analysis of these high frequency motions may well be complicated by the anisotropy of the overall motion of the protein. Recent work of Czaplicki et al at Stanford has shown that a large part of the variation of relaxation parameters along the backbone can be explained by taking the anisotropy of protein completely into account [18,19].

Finally, to quote Gerhard Wagner, for this type of motion, 'It is not obvious whether protein mobility is generally important for protein function or a functionally irrelevant consequence of the protein architecture '

3. Slow conformational exchange

A somewhat clearer picture is emerging from the study of disordered, or flexible segments, which exist part of the time in an ordered (e.g helical) and part of the time in a disordered conformation. Such flexible segments are

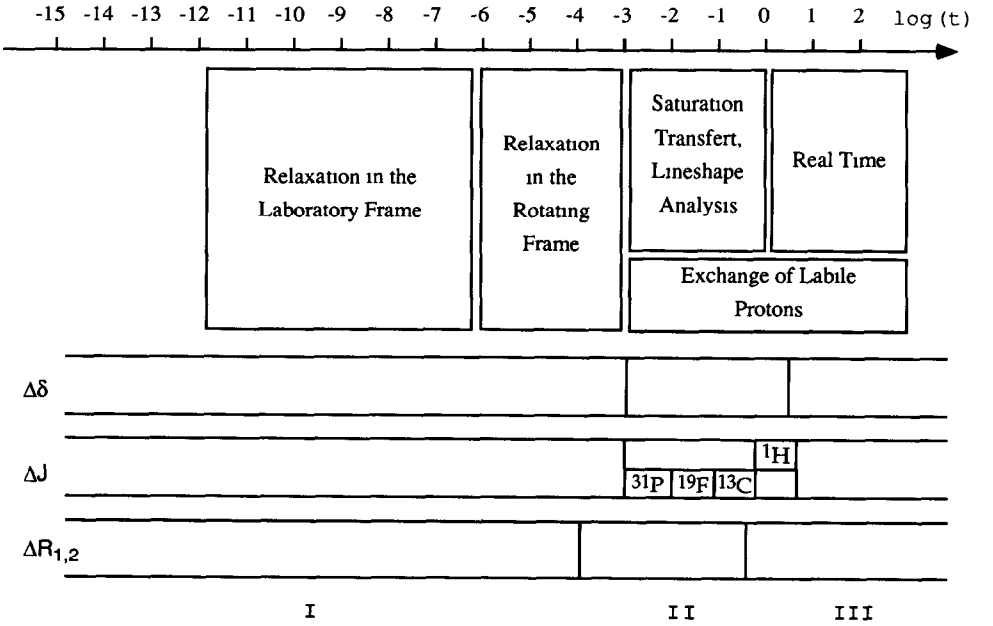

Fig 1 Time scales (in log (sec)) defined by NMR parameters, and NMR methods of observation of exchange at various rates Moving a molecule between two or more conformations may produce a variation of the chemical shift, of the coupling constant or of the relaxation rate constants The amplitude of the variations ($\Delta\delta$, ΔJ or $\Delta R_{1,2}$ respectively) each define a time scale I, II and III define, with respect to these time scales, the regions of fast (where only an average value of the NMR parameter is observed), intermediate and slow exchange (where the NMR parameter of each population can be separated), respectively [31]

ever more frequently found in binding regions, notably DNA binding regions, which suggests that the flexibility may be necessary to facilitate an 'induced fit' of the two ligands to each other upon complex formation

We now have a detailed description of flexibility in the DNA-binding helix–turn–helix of the trp repressor, discussed by C Arrowsmith and O Jardetzky [20,21] The repressor is an intertwined dimer, consisting of two identical chains, each containing six α-helices. Both the solution structure [20,21] and the crystal structure [22] are known. In the solution structure of the free repressor the DNA-binding helices are ill defined They are better defined in the complex with the corepressor tryptophan and best defined in the ternary repressor–Trp–DNA complex In contrast to the core of the dimer, where backbone proton exchange rates of the order of days are observed, the proton exchange rates in the DNA-binding helices are of the order of milliseconds [23], and it is possible to estimate that these segments are helical no more than 90–95% of the time The N-terminal segment of this protein is also disordered, but it is not known whether it makes any contacts with DNA. The importance of site-directed mutagenesis for deciphering the mechanisms of segmental flexibility was underscored by a contribution from M. Gryk [24] for the helix–turn–helix of the Trp-repressor A single amino acid substitution (e.g Cys or Val for Ala) in the turn of the helix–turn–helix domain dramatically stabilizes the entire domain

Slow motion in the microsecond range can be detected by the method of spectral density function analysis described above, as reported for the DNA binding domain of Gal4 (J F Lefèvre, Dayie, Peng and Wagner, manuscript in preparation) The slow conformational exchange localized around the cysteins which bind the two zinc atoms of the protein observation is confirmed by the analysis of the B1 field strength dependence of the T1 relaxation in the rotating frame The measurement of this so called $T1\rho$ relaxation time constant was also recently used in the analysis of slow motion in BPTI [25]

A further example of disorder in a DNA binding protein, that of the Antennapedia homeodomain, has more recently been described by Qian et al. [26] The existence of extensive flexible regions in histones, known since the pioneering NMR studies of E M Bradbury [27,28], have been discussed by C Cerf [29]

4. The significance of conformational flexibility

A possible functional significance for the disorder observed for residues 20–30 of the B-chain of an insulin mutant was proposed by M Weiss In a series of insulin analogues such disorder can be seen both by NMR and crystallography and its role in facilitating a conformational adjustment upon receptor binding represents an attractive hypothesis [30] A somewhat different type of binding site flexibility was described by G.C.K. Roberts for dihydrofolate reductase, where different NAD and NADP analogues are found to bind in different orientations, requiring a corresponding rearrangement of groups within the protein binding site [31–33]. Still another variant of flexibility in the sense of being able to adopt different structures in different environments was shown by H. Oschkinat in the comparison of the solution structure to the crystal structure of the protein chicken egg white cystatin, where a segment appears as a helix in the crystal, and as a β-turn in the NMR structure [34] It is becoming increasingly clear that simple rigid-body docking of drug molecules or other ligands in a fixed conformation to a rigid binding site, both independently determined by X-rays or NMR, will not provide an accurate picture of the structure of biologically important molecular complexes Docking algorithms, some of which were discussed at the meeting, will increasingly need to take either pre-existing or induced flexibility into account

Other examples of conformational readjustment upon ligand binding is given in the previous study of the Ca^{2+} binding loop in the superfamily proteins reported by S Forsén and coworkers [35] The conformational change induced by Ca^{2+} binding to troponin C was described in detail by B Sykes [36,37]. Interestingly, it was shown that the peptides forming the binding sites could interact and fold in the presence of Ca^{2+} Similarly, a Ca^{2+}-induced conformational change in the EGF-like domain of the coagulation factor X was described by M Sunnerhagen [35] In the latter case the ion binding site is near the linker region and the reduction of flexibility caused by calcium binding is thought to induce domain reorganization

5. The protein folding problem

The current understanding, and the complexity, of the protein folding problem were brought out in several presentations and the ensuing discussion W Englander presented the latest developments on backbone proton exchange and its use in quenching experiments to detect intermediates in protein folding on cytochrome *c* [38], calling attention to the fact that not taking into account the observed sequence-dependent variation in the intrinsic exchange rates can introduce serious errors into calculation of secondary structure lifetimes [39].

Structural intermediates in folding were characterized by a combination of physical techniques in the case of insulin analogues, as reported by M Weiss [40] C Dobson described a complete folding pathway for the assembly of hen egg white lysozyme, beginning with (1) formation of α-helices, (2) formation of two 2-helix bundles, (3) the addition of a β-structure to form a 'molten globule', and finally (4) the rearrangement to a compact glob-

ular structure. This study has yielded the most complete description of the folding process, but the picture is unfortunately complicated by the evidence that parallel pathways also exist [41].

6. Modelling the motions

The description of a motional process includes the rate constant and the amplitude of the structural fluctuations. NMR provides the first parameter quite directly. The latter is not easily extractable from the NMR data and several models of the spectral density or the correlation functions [42–44] (Zhang, Zheng and Jardetzky, manuscript in preparation) have been proposed in the past besides the already cited model-free approach [7]. Also, the dynamical information given by NMR is very local For large molecules like peptides and proteins, it is desirable to reach a global view of the motions in order to recognize concerted movements of large fragments

M. Levitt discussed the role of molecular dynamics (MD) simulations in understanding internal motions in proteins and brought home the point that, to achieve a realistic picture of protein dynamics, simulations including the solvent are essential It is now possible to account for the very fast motions (sub-nanosecond) reflected in NMR parameters by MD [45–48], but we are still in search of a method that would give an accurate theoretical account of the slower segmental motions and conformational equilibria on the micro-millisecond time scale Dynamic techniques can be used also for evaluating thermodynamic properties of a protein evolving from one conformation to another [49] Matching the thermodynamic properties deduced from NMR measurements of the fluctuation rate constants to those calculated may provide a way of modelling slow motions in proteins.

7. The implication of internal motion and flexibility on structure determination by NMR

The implication of dynamics and conformational equilibria for the determination of solution structures of proteins by NMR is well illustrated by the structural investigation of rat galanin reported by R. Rigler [50,51]. The distribution function of the 3D solution structures of this small protein was studied using both the Förster resonant energy transfer and NMR NOE measurements This study reveals the existence of a discrete set of subpopulations, ranging from folded to extended structures. As is well known, the 'average' structure determined under such circumstances is devoid of physical meaning [44] The issues of the accuracy and precision of NMR structures were raised by A Elofson [52], who carried out a comparison of structures calculated from simulated NOESY spectra of six different crystal structures of the bovine pancreatic trypsin inhibitor (BPTI) The comparison led to two important conclusions: [1] the five BPTI crystal structures with average RMSDs < 1.14 Å could not be distinguished by NMR and [2] without additional information from molecular modelling potential functions, it is possible to obtain structures that agree with the NMR data, but are very different from the true structures This result is consistent with a recent study of Liu et al [53], comparing 'NMR' structures calculated from the same set of simulated NOEs derived from the crystal structures The findings in that study were that all three methods (distance geometry, restrained molecular dynamics and optimal filtering) reproduced the overall fold of crambin and nuclear staphylococcal nuclease equally well, but the means of structure families calculated by different methods were about an RMSD of 1 Å apart from each other and from the 'gold standard' structure, even though the precision of each calculation was of the order of 0 5–0 7 Å All these findings strongly suggest that the family of structures reflected in the NMR data may be considerably larger than any one particular calculation may show

8. Conclusion

The increasing application of high resolution NMR to the study of protein dynamics has led to the discovery of a variety of interesting dynamic properties of proteins not suspected in the initial phase of protein structure determination, where it has to be assumed that the protein is essentially rigid. The functional significance of these phenomena remains for the most part to be elucidated An important methodological lesson has clearly emerged from the work already reported.

The juxtaposition of the many ways of detecting protein dynamics by NMR, the many emerging examples of dynamic phenomena, and the effects of dynamics on the accuracy of NMR structures can serve as a reminder of the complementarity of X-ray diffraction and NMR as high resolution methods for the study of macromolecules X-ray diffraction provides accurate and precise geometric information and indirect evidence for mobility NMR provides direct and detailed evidence on dynamics and approximate geometric information

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