Backbone dynamics of (1-71)- and (1-36) bacterioopsin studied by two-dimensional $^1$H-$^{15}$N NMR spectroscopy

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Summary

The backbone dynamics of uniformly $^{15}$N-labelled fragments (residues 1-71 and 1-36) of bacterioopsin, solubilized in two media (methanol-chloroform (1:1), 0.1 M $^2$H$_2$CO$\cdot$NH$_4$, or SDS micelles) have been investigated using 2D proton-detected heteronuclear $^1$H-$^{15}$N NMR spectroscopy at two spectrometer frequencies, 600 and 400 MHz. Contributions of the conformational exchange to the transverse relaxation rates of individual nitrogens were elucidated using a set of different rates of the CPMG spin-lock pulse train and were essentially suppressed by the high-frequency CPMG spin-lock. We found that most of the backbone amide groups of (1-71)bacterioopsin in SDS micelles are involved in the conformational exchange process over a rate range of $10^3$ to $10^5$ s$^{-1}$. This conformational exchange is supposed to be due to an interaction between two $\alpha$-helices of (1-71)bacterioopsin, since the hydrolysis of the peptide bond in the loop region results in the disappearance of exchange line broadening. $^{15}$N relaxation rates and $^1$H-$^{15}$N NOE values were interpreted using the model-free approach of Lipari and Szabo [Lipari, G. and Szabo, A. (1982) J. Am. Chem. Soc., 104, 4546-4559]. In addition to overall rotation of the molecule, the backbone N-H vectors of the peptides are involved in two types of internal motions: fast, on a time scale < 20 ps, and intermediate, on a time scale close to 1 ns. The intermediate dynamics in the $\alpha$-helical stretches was mostly attributed to bending motions. A decrease in the order parameter of intermediate motions was also observed for residues next to Pro in $\alpha$-helices, indicating an anisotropy of the overall rotational diffusion of the molecule. Distinctly mobile regions are identified by a large decrease in the order parameter of intermediate motions and correspond to the N- and C-termini, and to a loop connecting the $\alpha$-helices of (1-71)bacterioopsin. The internal dynamics of the $\alpha$-helices on the millisecond and nanosecond time scales should be taken into account in the development of a model of the functioning bacteriorhodopsin.

Introduction

For many years, measurements of $^{15}$N and $^{13}$C relaxation rates have been used for NMR studies of molecular mobility (Abragam, 1961). However, only since 2D experiments with 'reverse' proton detection have been developed (Bax et al., 1989) and $^{15}$N- and $^{13}$C-enriched proteins have become readily available, it has been possible to perform detailed and extensive heteronuclear relaxation studies on proteins (for a recent review, see Wagner (1993)).

Internal motions faster than the rotational correlation time can be elucidated by analysis of heteronuclear relaxation rates and NOEs (Lipari and Szabo, 1982). Slow conformational exchange in the micro-millisecond time range could increase the transverse relaxation rate. The size of this increase depends on the chemical shift refocusing scheme used during the relaxation delay. Exchange processes on the millisecond time scale could be elucidated by variation of the delay between the 180° pulses in the CPMG pulse train and the exchange rates can thus be
measured (Bloom et al., 1965; Reeves, 1975; Orekhov et al., 1994). The contribution to the transverse relaxation rate caused by conformational exchange on a faster (microsecond) time scale can be evaluated by measuring relaxation rates at different magnetic field strengths (Bar-

che et al., 1994). Thus, NMR spectroscopy can provide fundamental information on intramolecular motions in a wide range of time scales.

By now it is generally believed that membrane proteins possess a flexible spatial structure and exhibit strong conformational dynamics on very broad time scales (Po-
pot et al., 1993), so they are very attractive objects for exploration by dynamic NMR. Comparison of the dyna-
ic parameters of a membrane protein along with its spatial structure in different environments could give insight into protein–protein and protein–membrane interactions in lipid membranes.

Bacteriorhodopsin (or bacterioopsin (BO) for the protein without the retinal chromophore) is a transmembrane protein acting as a light-dependent proton pump in the purple membrane of H. halobium (see Ovchinnikov (1982) for a review). Electron cryomicroscopy data suggested a seven-α-helix structural motif for bacteriorhodopsin (Henderson et al., 1990).

High-resolution NMR studies of membrane proteins require their solubilization in a membrane-mimicking environment, i.e., an appropriate organic solvent or detergent micelles (Arseniev et al., 1987; Pervushin et al., 1991). From CD (Arseniev et al., 1987) and FT-IR (Torres and Padros, 1993) results, it was concluded that bacteriorhodopsin retains most of its native secondary structure in methanol–chloroform (1:1), 0.1 M LiClO4. It was also shown that some elements of the bacterio-
hoplin native tertiary structure are present in this milieu (Arseniev et al., 1987; Abdulaeva et al., 1991). The de-
tailed structures of bacteriorhodopsin fragments have been obtained by 2D 1H NMR spectroscopy (Arseniev et al., 1988; Masiennikov et al., 1990,1991a,1993; Barsukov et al., 1992; Sobol et al., 1992). The spatial structures of segments A (residues 1–36) and B (residues 34–65), both in organic mixture and in sodium dodecyl(2H2O) sulfate (SDS) micelles, were reconstructed using the available NMR data (Masiennikov et al., 1991b; Lomize et al., 1992; Pervushin et al., 1992; Pervushin and Arseniev, 1992).

Here we present a 15N NMR study of the backbone dynamics of the (1–71) and (1–36) fragments of bacterio-

opsin in methanol–chloroform (1:1), 0.1 M 2HCO2NH4, and in SDS micelles at two spectrometer frequencies, i.e., 400 and 600 MHz. The (1–36)- and (1–71) bacterioopsin fragments reveal the same structural properties as in the whole bacteriorhodopsin molecule and could be regarded as minimal structural units consisting of one ‘free’ α-helix and two interacting transmembrane α-helices, respectively.

Materials and Methods

The uniformly 15N-labeled fragments (1–36)- and (1–71)-bacterioopsin were isolated as described previously (Abdula-
eva et al., 1987; Orekhov et al., 1992) from uniformly 15N-
labeled H. halobium, strain ET1001, which was cultured according to the published procedure (Crespi, 1982) in a medium containing the hydrolysate of 15N-labeled Chlorella vulgaris. NMR samples were prepared as described (Pervushin et al., 1994). The Asp36-Pro 37 peptide bond of (1–71)BO in SDS micelles was hydrolysed by incubation of the NMR sample for several months at room temperature. Electrophoresis indicated almost 100% hydrolysis.

The 15N and 1H resonance assignment of (1–71)BO (Pervushin et al., 1994) was used throughout. The 15N assignment for (1–36)BO was readily obtained from the 15N and 1H resonance assignments of (1–71)BO and the 1H assignment of (1–36)BO (Pervushin et al., 1992). NMR measurements were carried out at 600 and 400 MHz on UNITY-600 and UNITY-plus-400 VARIAN spectrometers at 30 °C and 50 °C for the samples in organic mixture and in micelles, respectively. The pulse sequences used to measure 15N longitudinal (R(S)) and transverse (R(T)) relaxation rates were based on those described previously (Kay et al., 1989; Clore et al., 1990a; Peng and Wagner, 1992), appropriately modified to eliminate cross-correlation between dipolar and chemical shift anisotropy relaxation mechanisms (Palmer et al., 1992).

In the experiments for the measurement of the R(S) and R(T) relaxation rates, a delay of 1.4 s was used before each transient (2.5–5 times the longitudinal relaxation time of the backbone amide protons). Delays 2τ between the centers of the 180° pulses (the pulse length was 140 μs) in the 15N CPMG spin-echo sequence during the transverse relaxation period were 0.2, 0.5, 1.0 and 2.0 ms. R(S) and R(T) relaxation rates were obtained using relaxation delays of 0.01, 0.1, 0.2, 0.4, 0.6, 0.9, 1.2, 1.5 and 1.8 s and 0.001, 0.02, 0.04, 0.06, 0.08, 0.10, 0.12 and 0.14 s, respectively. Sample heating during the pulse sequences was estimated by measuring the separation between the OH and CH3 resonances in the external standard (methanol) and was found not to exceed 3 °C. The 1H–15N NOEs were measured exactly as described previously (Kay et al., 1989; Clore et al., 1990a; Orekhov et al., 1994) with a delay of 4 s before each transient. All spectra were processed by a modified version of the FELIX software (Hare Research Inc., Woodinville, WA). Cross-peak volumes were measured using the program EASY (C. Bartels, ETH Zürich, Zürich). Relaxation rates and the associated errors were calculated by the appropriate utility of the VNMR (VARIAN) software.

calculation of dynamic and chemical exchange parameters was performed by software written in-house. Calculation of the ‘model-free’ parameter set, z (i.e., S2, S2', τ, and Δε, see below), was performed by nonlinear minimization of