Journal of Biomolecular NMR, 1 (1991) 313–322
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J-Bio NMR 036

Two-dimensional NMR study of the conformation of (34-65)bacterioopsin polypeptide in SDS micelles

Konstantine V. Pervushin, Alexander S. Arseniev*, Alexander T. Kozhich and Vadim T. Ivanov

M.M. Shemyakin Institute of Bioorganic Chemistry, U.S.S.R. Academy of Sciences, Ul. Miklukho-Maklaya 16/10, Moscow, V-437, U.S.S.R.

Dedicated to the memory of Professor V.F. Bystrov

Received 25 April 1991
Accepted 16 July 1991

Keywords: Bacterioopsin; α-Helix; Micelles; NMR

SUMMARY

The conformation of the synthetic 32-residue polypeptide, an analog of the membrane spanning segment B (residues 34-65) of *Halobacterium halobium* bacterioopsin, incorporated into perdeuterated sodium dodecyl sulfate micelles in the presence of trifluoroethanol was investigated by ^{1}H NMR spectroscopy. The spectrum resonances were assigned by means of phase-sensitive DQF-COSY, TOCSY and NOESY techniques. Interproton nuclear Overhauser effects and deuterium exchange rates of individual NH groups were derived from two-dimensional NMR spectra. Analysis of the obtained data showed that segment B has a right-handed α-helical stretch from Lys^{41} to Leu^{62} with a kink at Pro^{50}. The α-helix in the C-terminal part is terminated at Gly^{63}, which adopts a conformation typical of amino acid residues in a left-handed helix. The N-terminal part (residues 34-40) has no ordered conformation. NMR data are provided for comparison of the segment B conformation in the isotropic system of an organic solvent, in SDS micelles and in the purple membrane bacterioopsin. Factors affecting the conformation of membrane spanning segment B in various milieus are discussed.

INTRODUCTION

Bacteriorhodopsin (BR) is a protein of the purple membrane of *Halobacterium halobium*. BR consists of one polypeptide chain of 248 amino acid residues and the retinal chromophore bound via Schiff base to Lys^{216} (Bayley et al., 1981). Light absorption by the chromophore triggers a photocycle with the resulting proton translocation across the cell membrane.

* To whom correspondence should be addressed.

Recently a three-dimensional density map of bacterioopsin has been obtained by electron cryomicroscopy (Henderson et al., 1990) with 3.5 Å resolution in a direction parallel to the membrane plane. In the perpendicular direction the resolution was lower (about 10 Å). According to the derived model (Henderson et al., 1990) BR consists of seven α-helical membrane spanning segments A–G. Their rough topology and relative arrangement have also been established.

High-resolution NMR spectroscopy is an elaborate method for conformational analysis of polypeptides in solution (Wüthrich, 1986). Unfortunately, the proton resonances of BR in the bilayer membrane or small liposomes are too broad to be resolved individually. Therefore, a choice of an artificial medium conserving the protein structure and providing high-resolution NMR spectra is crucial for such studies. It has been previously shown by CD and ^{19}F NMR spectroscopy (Arseniev et al., 1987) that BR solubilized in methanol/chloroform (1:1) retains the secondary structure of fully active chromoprotein in the purple membrane and possesses a specific tertiary structure. Moreover, individual fragments of BR isolated after polypeptide chain cleavage retain their native-like conformations in organic solvent (Arseniev et al., 1987; Barsukov et al., 1990; Abdulaeva et al., 1991).

The spatial structure of the peptide mimicking the 34-65 segment of bacterioopsin in methanol/chloroform (1:1) solution has been reported (Arseniev et al., 1988). The peptide conformation is defined as a right-handed α-helix, rigid in the central region Phe^{42}–Nle^{60} and flexible in N- and C-terminal parts.

This paper deals with the conformation of the same peptide, called sB, solubilized in sodium dodecyl-d_{25} sulfate micelles modeling the bilayer membrane. The ^1H NMR spectrum has been completely assigned and the sB secondary structure has been delineated and compared with that in an isotropic organic solvent (Arseniev et al., 1988) and in the native purple membrane (Henderson et al., 1990).

MATERIALS AND METHODS

sB (primary structure shown in Fig. 1) was synthesized by conventional techniques in solution (Kozhich et al., 1984). Methionine residues 56 and 60, present in bacterioopsin, were substituted in the peptide by norleucines, since such isosteric replacements usually do not affect the polypeptide conformation and function (Wunsch et al., 1982), while simplifying the synthesis.

The peptide was incorporated into micelles of perdeuterated sodium dodecyl-d_{25} sulfate (SDS-d_{25}) (98% deuterium, MSD, U.S.A.) as follows: the 50 mM peptide in trifluoroethanol-d_3 (TFE-d_3) (99.8% deuterium, Stohler Isotope Chemicals, U.S.A.) was added dropwise under stirring to 0.5 M SDS-d_{25} in ^2H_2O (99.96% deuterium, Stohler/KOR Stable Isotopes, U.S.A.) or in H_2O. The sample was diluted with water to final concentrations: 3 mM peptide and 240 mM SDS-d_{25} in the water/TFE-d_3 (15% by volume) mixture. This peptide/SDS mole ratio ensures the incorporation of less than one peptide molecule per SDS micelle (Chang and Kaler, 1985).

^1H NMR spectra (600 MHz) were obtained at 30°C and 40°C on a Varian UNITY-600 NMR spectrometer. Chemical shifts were measured relative to the water resonance taken as 4.75 ppm at 30°C and 4.80 ppm at 40°C. The following spectra were recorded: DQF-COSY (Rance et al., 1983), TOCSY (Bax and Davis, 1985a,b) with mixing times of 30 and 45 ms, NOESY (Jeener et al., 1979; Marion and Wüthrich, 1983) with mixing times of 100 and 200 ms, ROESY (Bax and Davis, 1985a) with mixing times of 50 and 100 ms, and NOESY-1-1-echo (Sklenar and Bax, 1987)

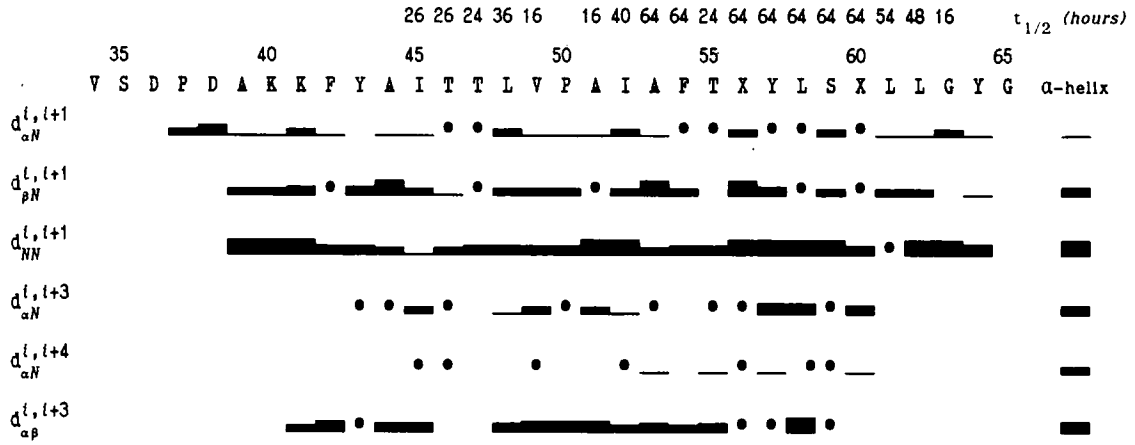

Fig. 1. Amino acid sequence of segment B and survey of NOEs involving NH, $C^{\alpha}H$ and $C^{\beta}H$ protons ($C^{\delta}H_2$ protons of proline residues are considered as honored NH protons). One-letter code of amino acid residues is used, X denotes the norleucine residue. The observed NOEs classified as strong, medium and weak according to cross-peak intensities measured in the NOESY spectrum (τ_m = 200 ms) are shown by thick, medium and thin lines, respectively. If the presence of a cross peak is somewhat doubtful, it is indicated by a black circle. To the right of the NOEs the pattern expected for a residue in a regular right-handed α-helix (Wüthrich et al., 1982; Wagner et al., 1986; Neuhaus et al., 1985) is shown for comparison. Half-exchange times (in hours) of amide protons with solvent deuterium are shown above the sequence. Where not shown, this time is less than 5.5 h, i.e. the corresponding NH/$C^{\alpha}H$ cross peak is absent in the first TOCSY spectrum recorded after dissolving micelles in ^2H_2O/TFE-d_3.

with a mixing time of 100 ms, where the last 90° pulse was replaced by a 90°-τ-90°-δ-90°-2τ-90°-δ train with τ = 100 μs and δ = 100 μs delays. Data obtained by the NOESY-1-1-echo technique were used to check for a bleach-out effect of presaturation on amide NH to $C^{\alpha}H$ NOE cross-peak intensities. All the two-dimensional ^1H NMR spectra were recorded in the pure phase absorption mode using the phase-sensitive technique (States et al., 1982). Time delay for relaxation was 1.2 s. The spectra were processed using the standard VNMR Varian supplement and a modified version of FELIX from Hare Research Inc., U.S.A. Cross-peak intensities (volumes) in 2D spectra were measured by summing up in a defined rectangle ($\Delta\omega1 \times \Delta\omega2$) around the cross peak (typically 50 × 40 Hz), where $\Delta\omega1$ and $\Delta\omega2$ are the sizes of the rectangle in ω1 and ω2 direction, respectively.

In order to minimize the rate of the proton exchange between amide protons and bulk water, solutions with pH 3.0 and 4.6 were used. To measure the deuterium exchange rates of sB amide groups, SDS micelles containing sB were lyophilized from H_2O/TFE-d_3 and dissolved in ^2H_2O/TFE-d_3. Then a series of 12 TOCSY spectra was recorded over 66 hours. Temperature was adjusted at 30°C and pH measured after the experiment was 3.0. The half-exchange times of amide protons were found by fitting the cross-peak volume time dependences to the equation:

$$V(t) = V(0)*\exp(-\ln2*t/t_{1/2})$$

where V(t) is the cross-peak volume (integration limits are the same for all TOCSY spectra) in the 2D spectrum. Its recording was started at time t once the sample has been placed into the spectrometer.

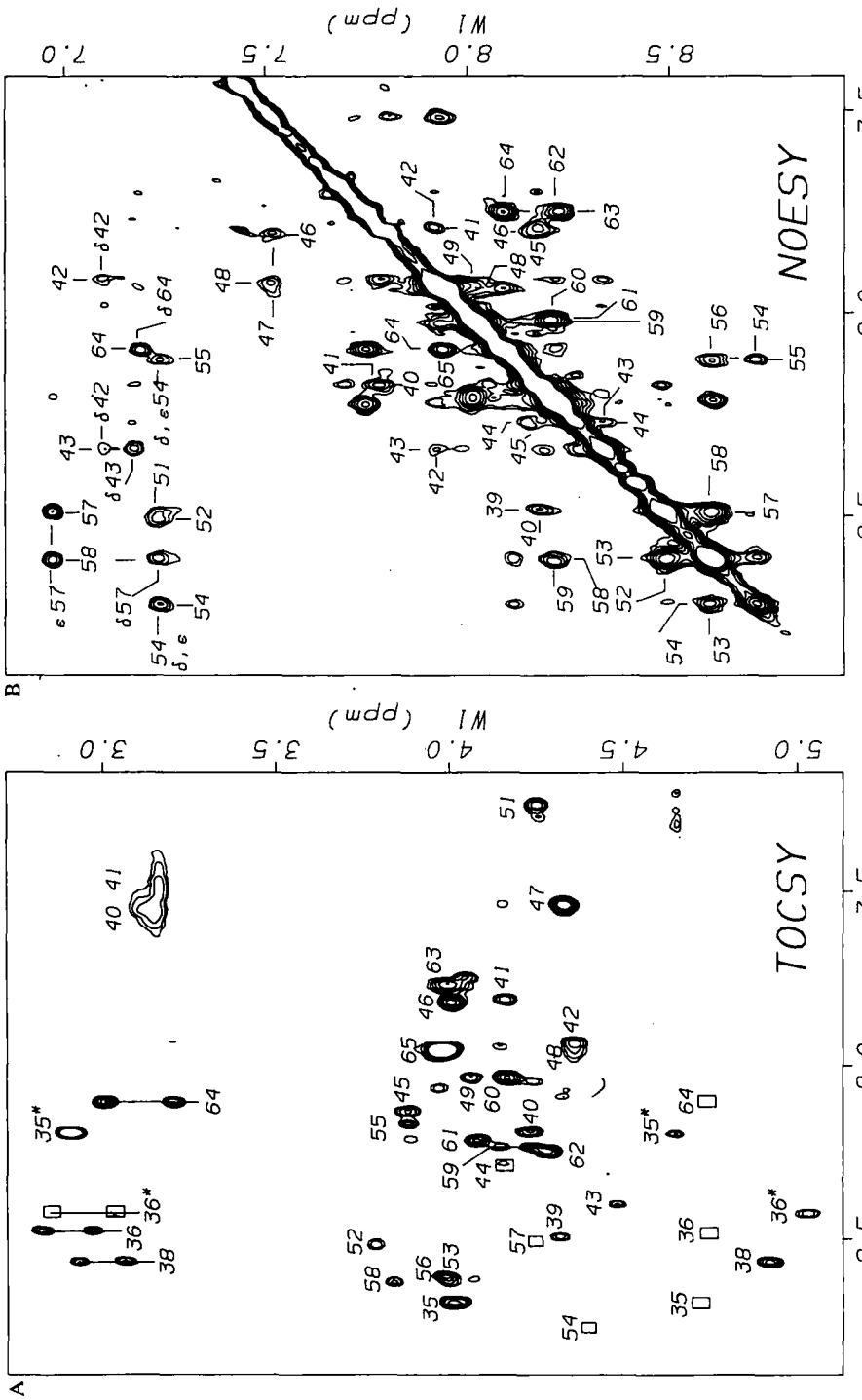

Fig. 2. (A) NH (ω_2 = 7.16 – 8.90 ppm)/$C^{\alpha}H$ (ω_1 = 2.72 – 5.13 ppm) region of the TOCSY spectrum (mixing time 45 ms) and (B) NH (ω_2 = 7.42 – 8.89 ppm)/NH (ω_1 = 6.85 – 8.93 ppm) region of the NOESY spectrum (mixing time 200 ms) of segment B in SDS-d_{25} micelles in H_2O/TFE-d_3 (15% by volume), at pH = 3.0 and 40°C. Assignments of the $N_iH/C^{\alpha}{}_iH$ and $N_iH/C^{\beta}{}_iH$ cross peaks in (A), and $N_iH/N_{i+1}H$ cross peaks in (B) are indicated. Positions of cross peaks with intensities lower than the selected 'cutoff' in the TOCSY spectrum or falling into the band bleached by water presaturation are indicated by empty rectangles. Cross peaks which belong to the second conformational state of Ser^{35} and Asp^{36} are marked with an asterisk. Assignments are indicated by numbers corresponding to the residue position in the bacterioopsin sequence.

RESULTS AND DISCUSSION

High-resolution ^1H NMR spectra (partly shown in Fig. 2) were recorded after solubilization of sB in SDS micelles and overnight incubation at room temperature. The latter condition provided for conformational homogeneity of sB.

Resonance assignment in ^1H NMR spectra

DQF-COSY and TOCSY (spin-lock time 45 ms) were used to delineate the principal spin systems of amino acid residues as described (Wüthrich, 1986). Unfortunately, most of the cross peaks in the NH/$C^\alpha H$ region of phase-sensitive DQF-COSY spectra were not strong enough due to a larger line width of NH resonances (12–16 Hz) as compared to spin-spin coupling constants of vicinal NH-$C^\alpha H$ protons. Therefore, proton spin systems were delineated using both TOCSY spectra and aliphatic and aromatic regions of DQF-COSY spectra. Spin systems of aromatic protons were linked to spin systems of NH-$C^\alpha H$-$C^\beta H_2$ protons of Phe and Tyr residues through NOEs between the $C^{\delta,\varepsilon}H$ and $C^\beta H_2$ protons.

With a few exceptions, the proton spin systems of amino acid residues were completely identified (Table 1). We were unable to distinguish between Nle^{56} and Nle^{60} $C^\delta H_2$-$C^\varepsilon H_3$ resonances due to strong overlap in the aliphatic region of the spectra. In addition, only an approximate value of the chemical shift of the Phe^{54} $C^\zeta H$ proton resonance is indicated and that of Phe^{42} is not reported because of degeneration of the informative cross peaks with the diagonals of 2D spectra.

Proton spin systems of amino acid residues were assigned to specific positions in the amino acid sequence (Fig. 1) through $d_{\alpha N}$, $d_{\beta N}$, d_{NN} NOE connectivities for the amide proton (in case of Pro^{37} and Pro^{50} through $C^\delta H_2$ protons) of residue i + 1 with the $C^\alpha H$, $C^\beta H$ and NH protons of the preceding residue i, respectively. NOE connectivities of the $C^\alpha H$ protons of Ser^{35}, Asp^{36} and Tyr^{64} were found in the NOESY-1-1-echo spectrum because these resonances usually fell within the band bleached by water presaturation (see, for example, Fig. 2A). d-Connectivities of sB are summarized in Fig. 1.

Resonances from residues 38-65 were assigned unequivocally. NH/NH NOEs were observed (Fig. 2B) between all neighboring residues from Ala^{39} to Tyr^{64}. Proton resonances of Val^{34}, Ser^{35} and Asp^{36} were assigned according to the analysis of their spin systems in TOCSY and DQF-COSY spectra but not by means of the NOE contacts between them. Moreover, there are no intraresidue NOE cross peaks for Ser^{35} and Asp^{36} in NOESY spectra. However, the intraresidue NOEs in a rotating frame were found in ROESY spectra. This is possible (Brown and Farmer, 1989) if the N-terminal part of sB is very flexible, i.e. if a number of different conformations of Val^{34}, Ser^{35} and Asp^{36} are in rapid equilibrium. However, two sets of proton resonances for Ser^{35}, Asp^{36} and Pro^{37} were found in TOCSY (see, for example, Fig. 2A) and DQF-COSY spectra. This indicates a slow exchange (on a chemical shift scale) between two conformations or sets of conformations.

Conformation of segment B

The peptide secondary structure can be deduced from the analysis of $d_{\alpha N}$, $d_{\beta N}$, d_{NN} NOE connectivities supplemented with amide proton exchange rate measurements (Wüthrich, 1986). The connectivities observed for sB along with those expected for an α-helix (Wüthrich et al., 1982; Wagner et al., 1986; Neuhaus et al., 1985; Arseniev et al., 1983) are shown in Fig. 1. These connec-

TABLE I
PROTON RESONANCE ASSIGNMENTS OF (34-65)BACTERIOOPSIN (3 mM) IN SDS-d_{25} (240 mM) MICELLES IN H_2O/TFE-d_3 (15% BY VOLUME) AT 40°C, pH 3.0

Residue	δ (± 0.01 ppm)			
	NH	$C^{\alpha}H$	$C^{\beta}H$	Other
Val^{34}		4.02	2.36	$C^{\gamma}H_3$ 1.13, 1.20
Ser^{35}	8.68	4.72	4.00	
Ser^{35*}	8.20	4.65	2.90	
Asp^{36}	8.48	4.74	2.82, 2.97	
Asp^{36*}	8.42	5.03	2.85, 3.04	
Pro^{37}		4.49	2.03, 2.36	$C^{\gamma}H_2$ 2.10, 2.10
				$C^{\delta}H_2$ 3.50, 3.50
Pro^{37*}		4.45	2.20, 2.48	$C^{\gamma}H_2$ 2.23, 2.23
				$C^{\delta}H_2$ 3.52, 3.59
Asp^{38}	8.66	4.90	2.92, 3.05	
Ala^{39}	8.48	4.30	1.58	
Lys^{40}	8.18	4.21	1.85, 1.95	$C^{\gamma}H_2$ 1.55, 1.68
				$C^{\delta}H_2$ 1.84, 1.84
				$C^{\varepsilon}H_2$ 3.13, 3.13
				NH_3^+ 7.56
Lys^{41}	7.79	4.14	1.87, 1.96	$C^{\gamma}H_2$ 1.43, 1.57
				$C^{\delta}H_2$ 1.81, 1.81
				$C^{\varepsilon}H_2$ 3.07, 3.07
				NH_3^+ 7.47
Phe^{42}	7.92	4.33	3.18, 3.18	$C^{\delta}H_2$ 7.10, 7.10
				$C^{\varepsilon}H_2$ 7.28, 7.28
				$C^{\zeta}H$
Tyr^{43}	8.34	4.48	3.19, 3.19	$C^{\delta}H_2$ 7.17, 7.17
				$C^{\varepsilon}H_2$ 6.89, 6.89
Ala^{44}	8.27	4.14	1.62	
Ile^{45}	8.15	3.86	1.99	$C^{\gamma}H_2$ 1.30, 1.83
				$C^{\gamma}H_3$ 0.98
				$C^{\delta}H_3$ 0.83
Thr^{46}	7.80	3.98	4.21	$C^{\gamma}H_3$ 1.06
Thr^{47}	7.51	4.31	4.13	$C^{\gamma}H_3$ 1.22
Leu^{48}	7.93	4.33	1.68, 1.93	$C^{\gamma}H$ 1.84
				$C^{\delta}H_3$ 0.94, 0.98
Val^{49}	8.02	4.06	2.44	$C^{\gamma}H_3$ 0.97, 1.15
Pro^{50}		4.22	1.83, 2.20	$C^{\gamma}H_2$ 1.86, 2.30
				$C^{\delta}H_2$ 3.64, 3.64
Ala^{51}	7.23	4.23	1.62	
Ile^{52}	8.51	3.77	2.10	$C^{\gamma}H_2$ 1.15, 1.92
				$C^{\gamma}H_3$ 0.99
				$C^{\delta}H_3$ 0.90
Ala^{53}	8.60	4.05	1.52	
Phe^{54}	8.72	4.40	3.24, 3.36	$C^{\delta}H_2$ 7.24, 7.24
				$C^{\varepsilon}H_2$ 7.24, 7.24
				$C^{\zeta}H$ 7.27
Thr^{55}	8.17	3.86	4.46	$C^{\gamma}H_3$ 1.33

TABLE I (continued)

Residue	δ (± 0.01 ppm)			
	NH	$C^{\alpha}H$	$C^{\beta}H$	Other
Nle^{56}	8.61	3.98	1.88, 2.05	$C^{\gamma}H_2$ 1.34, 1.63 $C^{\delta}H_2$ 1.32, 1.32 $C^{\varepsilon}H_3$ 0.83
Tyr^{57}	8.49	4.22	3.19, 3.22	$C^{\delta}H_2$ 6.97, 6.97 $C^{\varepsilon}H_2$ 6.78, 6.78
Leu^{58}	8.60	3.83	1.51, 1.69	$C^{\gamma}H$ 1.60 $C^{\delta}H_3$ 0.83, 0.91
Ser^{59}	8.22	4.26	4.13, 4.23	
Nle^{60}	8.02	4.14	1.88, 1.96	$C^{\gamma}H_2$ 1.34, 1.68 $C^{\delta}H_2$ 1.32, 1.32 $C^{\varepsilon}H_3$ 0.83
Leu^{61}	8.21	4.06	1.56, 1.68	$C^{\gamma}H$ 1.33 $C^{\delta}H_3$ 0.82, 0.93
Leu^{62}	8.23	4.26	1.58, 1.85	$C^{\gamma}H$ 1.60 $C^{\delta}H_3$ 0.93, 0.93
Gly^{63}	7.75	3.99, 3.99		
Tyr^{64}	8.09	4.74	2.98, 3.18	$C^{\delta}H_2$ 7.19, 7.19 $C^{\varepsilon}H_2$ 6.89, 6.89
Gly^{65}	7.94	3.96, 3.96		

* Second conformational state of Ser^{35}, Asp^{36} and Pro^{37} is denoted by an asterisk.

tivities and the slow amide proton exchange rates in the 45-62 region allow ascribing the α-helical conformation to the 41-62 region of sB.

It is well known that proline destabilizes α-helices because of steric hindrance between the proline ring and the side-chain atoms of the preceding residue (Richardson and Richardson, 1989). Nevertheless, proline is encountered in the middle of α-helical fragments of globular and membrane proteins (Deisenhofer et al., 1989) and produces a kink in the α-helix of about 26°. It could be suggested that the relatively high-energy conformation of the proline region is stabilized in proteins due to the compact packing of α-helices. However, our data unequivocally indicate that the sB α-helix conformation also withstands the presence of Pro^{50} both in organic solvent (Arseniev et al., 1988) and in SDS micelles. Moreover, according to deuterium exchange rate data (Fig. 1) all hydrogen bonds typical of the α-helix are retained, although exchange rates for Val^{49} and Ala^{51} are somewhat faster then those observed for other residues in the α-helical region (Fig. 1).

The α-helical part located before Pro^{50} (residues 45-49) appears to be more flexible than the region after Pro^{50} (residues 52-62) as judged from a relative decrease in NOE NH/NH cross-peak intensities and the faster amide proton exchange rates (Fig. 1).

The C-terminal part of the α-helix extends up to Gly^{63}. The NH proton of Gly^{63} is involved in hydrogen bonding as indicated by a slow deuterium exchange rate for this amide group (Fig. 1). In NOESY spectra $C^{\beta}H$ Ser^{59}/$C^{\beta,\delta,\varepsilon}H$ Tyr^{64} and $C^{\beta}H$ Leu^{62}/$C^{\delta,\varepsilon}H$ Tyr^{64} NOEs were found. A wire model of the sB was used to demonstrate that these protons are brought close if Gly^{63} is in the left-handed helical conformation ($\varphi \simeq 80°$, $\psi \simeq 20°$). This conformation of glycine frequently occurs at the C-terminal part of α-helices in proteins (Schellman, 1980).

Duplication of Ser^{35}, Asp^{36} and Pro^{37} resonances leads to the proposal that a fairly high energy barrier separates two low-energy conformation sets. This situation is expected for the sB N-terminal part if *cis-/trans*-isomerization of the Asp^{36}-Pro^{37} peptide bond occurs. Unfortunately, our NOESY and ROESY spectra contain no NOE cross peak, which would unequivocally show of the *cis* or *trans* Asp^{36}-Pro^{37} peptide bond configuration.

Summarizing, the preliminary ^1H NMR analysis shows that sB is a right-handed α-helix in the central part (residues 41-62) with a kink at Pro^{50}. Residues 34-36 do not have a fixed conformation. The peptide bond between Asp^{36}-Pro^{37} slowly interconverts between *cis* and *trans* configuration. At the C-terminus the α-helix is terminated by Gly^{63} which has φ and ψ angles characteristic of a left-handed helix.

Comparison of sB conformations in methanol/chloroform, SDS micelles and BR molecule

Ideas about differences between NMR-derived conformations of sB in the methanol/chloroform mixture and in SDS micelles follow from comparing the chemical shifts of proton resonances. Of particular interest is comparison of chemical shifts of NH and $C^{\alpha}H$ proton resonances, which serve as secondary structure indicators (Bruix et al., 1990; Pastore and Saudek, 1990; Williamson, 1990). Differences between the chemical shifts of these resonances for sB solubilized in methanol/chloroform and in SDS micelles are shown in Fig. 3. While the chemical shifts of $C^{\alpha}H$

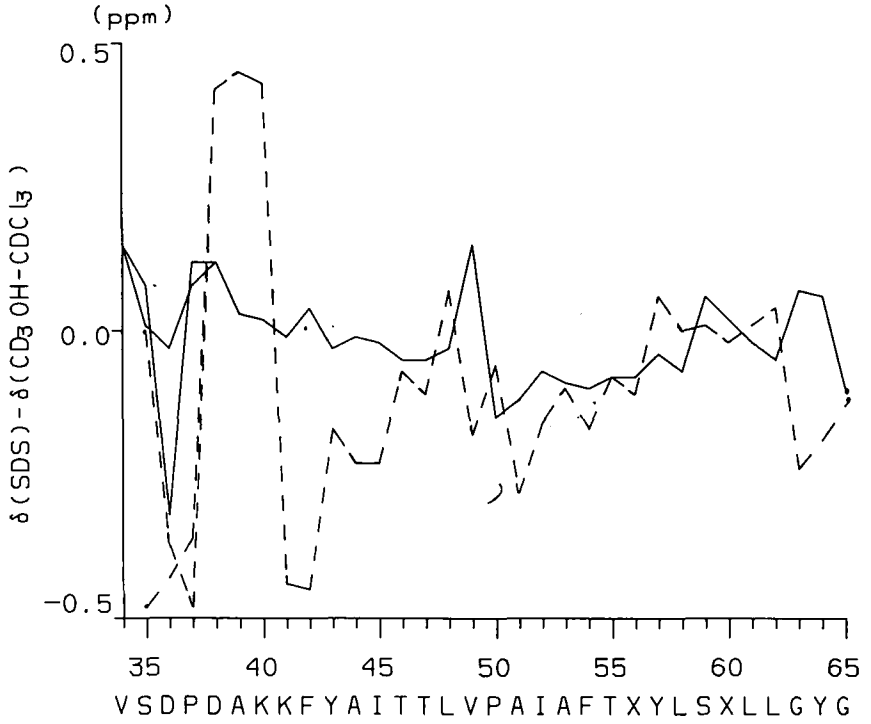

Fig. 3. Differences in the chemical shifts of amide (dashed line) and $C^{\alpha}H$ (solid line) proton resonances ($C^{\delta}H_2$ protons of proline residues are considered as honored NH protons) of segment B in SDS micelles and in methanol/chloroform solution. The values for the solution are taken from Arseniev et al. (1988) but increased by 0.52 ppm if the methanol Me-group resonance (3.50 ppm) is used as an internal reference. For Ser^{35}, Asp^{36} and Pro^{37} both sets of resonances are presented.

proton resonances do not differ significantly in both milieus, the NH shifts are more sensitive to the environment. The most pronounced differences are found in the N-terminal part (residues 35-45), where they reach $\pm$ 0.5 ppm. It seems that this part is more exposed to the polar phase of the SDS/H_2O system than the C-terminal part (residues 46-62) where the chemical shift differences are within 0.1 ppm. Presumably the α-helical 46-62 region is well incorporated into the hydrophobic micelle core. Chemical shifts of $C^{\alpha}H$ protons differ significantly only for one of the Asp^{36} conformers and for the Val^{49} (Fig. 3). We ascribe these differences to conformational changes within Pro^{37} and Pro^{50} residues in these environments. Some chemical shift differences observed for NH protons in the C-terminal 63-65 region (see Fig. 1) are apparently due to differences in the C-terminal part conformations.

From electron cryomicroscopy of purple membranes (Henderson et al., 1990) it follows that the α-helix of segment B is located in the Asp^{38}-Leu^{62} region. In contrast to the C-terminal part of the stretch, where the α-helix is clearly delineated, in case of Asp^{38}, Ala^{39} and the side chains of Lys^{40} and Lys^{41} a clear electron density pattern was not obtained and high-temperature factors were assigned to these residues. At Pro^{50} the α-helix also shows a kink. As seen from the above discussion our conclusions are very similar but not identical.

The membrane creates an anisotropic environment, hydrophobic in the middle and polar outside the membrane. The isotropic medium of the methanol/chloroform solution simulates only the hydrophobic part of the membranes. Therefore, the anisotropic medium of SDS micelles appears to mimic better the environment of the membrane spanning segment B in BR, than the methanol/chloroform solution. As sB contains hydrophilic N-terminal residues Ser^{35}, Asp^{36}, Asp^{38}, Lys^{40} and Lys^{41}, the conformation of this region is affected by the polar phase of the micelle-water or membrane-water system. A rigid α-helical region of sB in methanol/chloroform is localized at residues 42-60. Others have a tendency to continue the α-helix, although weaker towards both ends of sB. On the other hand, the peptide in SDS micelles has no ordered structure at the N-terminal part, but the stable α-helical region is extended towards the C-terminus to include residues 41-62.

It follows from the above discussions that rigid α-helical conformations of segment B in artificial media are close to that of the intact BR molecule in the purple membrane. Consequently, the location of the α-helix region in the amino acid sequence and its length are determined by the sequence itself and, to a lesser extent, by membrane anisotropy. It seems that the anisotropic micelle (membrane)–water interface just amplifies the structural tendencies coded for in the primary structure. In turn, the tertiary structure is determined by interactions between the surfaces of self-organized α-helices. This hypothesis, if correct, opens a wide perspective for reconstruction of the spatial structure of membrane proteins based on conformations of isolated fragments and the restricted set of constraints for fragment packing. This might lead to development of a strategy of *a priori* calculation of the membrane protein spatial structure. In order to verify this hypothesis we have already investigated by 2D ^1H NMR spectroscopy conformations of a set of synthetic (Arseniev et al., 1988; Maslennikov et al., 1990, 1991) and proteolytic bacterioopsin fragments (Arseniev et al., 1987; Barsukov et al., 1990; Abdulaeva et al., 1991). The present work is a part of that study.

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