Volume 318, number 1, 77–79 FEBS 12136 February 1993

The secondary structure of bacteriorhodopsin in organic solution

A Fourier transform infrared study

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Received 15 December 1992

Fourier transform infrared spectroscopy is used to estimate the secondary structure of bacteriorhodopsin dissolved in chloroform-methanol (1:1 v/v), 0.1 M $LiClO_4$. Curve-fitting of the deconvolved spectra in the amide I region shows that the total content of α-helices, reverse turns and β-sheets are similar to the native state. However, the α_{II}-helices, which are the major helical class in native bacteriorhodopsin, are greatly decreased in the solubilized sample. Similarly, the reverse turns and the β-sheets are strongly altered.

Bacteriorhodopsin; Secondary structure; Infrared spectroscopy; α-Helix; Reverse turn; Organic solvent

1. INTRODUCTION

Bacteriorhodopsin, the unique protein present in the purple membrane patches of *Halobacterium halobium*, has been the subject of extensive structural studies in recent years [1–3]. To date, high-resolution three-dimensional structures have only been obtained at a near-atomic resolution [3]. On the other hand, the very low mobility of the BR amino acids has made difficult the application of NMR techniques [4]. Two similar strategies have been reported to overcome this problem: (i) incorporation of bacteriorhodopsin into detergent micelles [5,6], and (ii) solubilization of bacteriorhodopsin in organic solvents [7–10]. In both cases, the major problem is that the bacteriorhodopsin conformation can be significantly altered by the medium, especially in the case of BR dissolved in organic solvents. It thus becomes desirable to obtain structural data from different techniques.

Fourier-Transform infrared spectroscopy is one of the most powerful techniques to obtain information about the secondary structure of proteins, owing to the specific hydrogen bonding of the C=O and N-H groups for each kind of structure. However, the conformation-sensitive bands overlap, and thus several techniques have been developed in order to obtain quantitative estimations of the secondary structural classes [11–15]. We have recently applied an improved version of curve-fitting of the deconvoluted spectrum, to the estimation of the native bacteriorhodopsin secondary structure. Our results have shown the presence of about 63% α-helix, 16% reverse turns, 14% β-sheets and 5% unordered structures [16].

In the present work, we use this FTIR method to estimate the secondary structure of bacteriorhodopsin dissolved in the chloroform/methanol/$LiClO_4$ solvent as used by Arseniev et al. [7]. Both NMR and CD studies have indicated a remarkable similarity of the α-helical content of bacteriorhodopsin in this solvent and in the native state [7,17].

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Abbreviations: BR, bacteriorhodopsin; IR, infrared; FTIR, Fourier transform infrared; organic medium, chloroform/methanol (1:1 v/v), 0.1 M $LiClO_4$.

2. MATERIALS AND METHODS

Purple membrane was isolated from *Halobacterium halobium* strain S9 as described [18] and was solubilized in chloroform/methanol (1:1, v/v), 0.1 M $LiClO_4$ (organic medium) after lyophilization. For FTIR experiments, aliquots at a protein concentration of about 5 mg/ml were placed in 50 μm pathlength CaF_2 IR cells fitted with teflon spacers. IR spectra were acquired on a Mattson Polaris FTIR spectrometer equipped with a MCT detector, working at an instrumental resolution of 1 cm^{-1}, as described in [16].

The spectra were Fourier-derivated and self-deconvoluted using the programs developed by Moffatt et al. [19]. The signal-to-noise ratio in the spectra was greater than 2,000. Fourier derivatives were obtained using a breakpoint of 0.1. Fourier self-deconvolution was carried out using a Lorentzian line-shape, a full width at half height of 14 cm^{-1} and a resolution enhancement factor of 2.7 (this value is less than log S/N, see Mantsch et al. [20]). Using these parameters we avoided the appearance of negative side lobes that are normally indicative of over-deconvolution.

Curve-fitting was done as follows: Fourier self-deconvolution and derivation gave the number and position of the component bands. The

bandwidth and intensities were also estimated from the deconvoluted and derivated curves. These were the input parameters for an iterative curve-fitting that was performed to fit the bands with Gaussian lineshape, to the deconvoluted spectrum, using a Spectra Calc program (Galactic Inc.). The peak positions, heights and bandwidths were allowed to vary simultaneously until a good fit was achieved. The relative area of each band gave the corresponding secondary structure percentage. The amino-acid side-chain contribution [21] was not taken into account because its contribution to the spectrum is very weak [16]. The results shown represent the mean area and the standard deviation of four independent experiments.

3. RESULTS AND DISCUSSION

Fig. 1 (upper trace) shows the absorption spectrum of BR dissolved in chloroform/methanol (1:1, v/v), 0.1 M $LiClO_4$, in the 1,800–1,500 cm^{-1} region. Unlike samples dissolved in water, solvent subtraction is a straightforward step, because the organic solvent does not have absorption bands in the amide I region. This spectrum shows few details, due to band overlapping. Further information can be obtained through Fourier deconvolution, which increases mathematically the band resolution [11]. Fig. 1 also shows the deconvoluted curve (bottom trace), which reveals additional peaks at the sides of the principal bands. In order to quantify the bands of the amide I region, a curve-fitting procedure over the deconvoluted spectrum was performed. Fig. 2 shows a representative curve-fitted result, and Table I lists the constituent bands with the mean area values and the standard deviations. Previous results from native purple membrane suspensions in water [16] are also included for comparison. Two major bands appear at 1,652 and 1,658 cm^{-1}, which lie in a region characteristic of α-helices, although unordered structures can also contribute to the 1,652 cm^{-1} band [12]. The α_{II} band of native BR, with a major component appearing at about 1,665 cm^{-1}, is greatly decreased in BR solubilized in the organic medium (Table I). Taking the 1,644 cm^{-1} band as due to 3_{10} turns as in native BR [16] and the 1,665 cm^{-1} band as due to residual α_{II}-helix, the total percentage of the four α-helical bands plus unordered structures is 75%. Assuming that the estimated 5% of unordered structures for the native purple membrane samples remains unchanged, the total α-helix appears to be about 70%. This value is slightly higher than that obtained for the native bacteriorhodopsin using the same method (about 63%), and could represent a tendency to form higher proportions of α-helices in this medium. Thus, our results show that although the overall α-helical con-

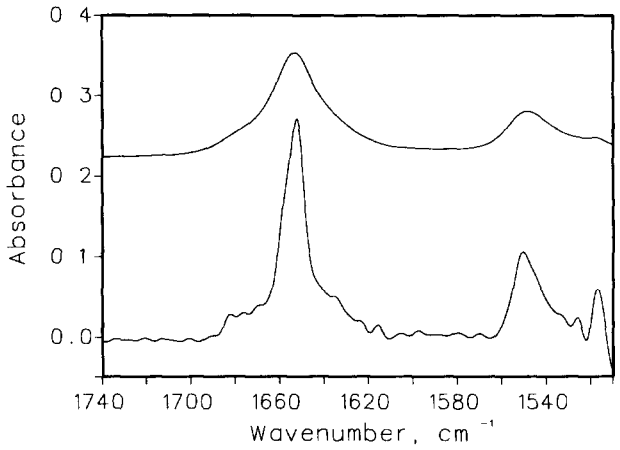

Fig. 1. Absorption (upper trace) and deconvoluted (lower trace) FTIR spectra of purple membrane dissolved in chloroform/methanol (1:1, v/v), 0.1 M $LiClO_4$. Bacteriorhodopsin concentration, 2 mg/ml; instrumental resolution, 1 cm^{-1}. Absorption spectrum was obtained by co-adding 1,000 scans in blocks of 20 of the shuttle accessory, in order to minimize the water vapour bands. Deconvolution was done using a bandwidth of 14 cm^{-1} and a resolution enhancement factor of 2.7

Table I

Position, fractional areas and assignments of the amide I bands of bacteriorhodopsin

Chloroform/methanol/$LiClO_4$			H_2O[a]		
Freq.[b]	% Area[c]	Assignment	Freq.[b]	% Area[c]	Assignment
1,689	0.2 ± 0.01	reverse turns	1,689	3.1 ± 0.7	reverse turns
1,683	3.1 ± 0.1	reverse turns	1,680	7.3 ± 0.6	reverse turns
1,677	3.6 ± 0.3	reverse turns	1,673	5.4 ± 0.4	reverse turns
1,670	3.5 ± 0.4	reverse turns	1,665	22.1 ± 0.8	α_{II}
1,665	7.7 ± 1.2	α_{II}	1,658	23.0 ± 0.3	$\alpha_{I} + \alpha_{II}$
1,658	18.1 ± 1.2	$\alpha_{I} + \alpha_{II}$	1,650	11.2 ± 0.2	α + unordered
1,652	39.4 ± 1.3	α + unordered	1,642	9.9 ± 1.4	3_{10} turns
1,644	9.7 ± 0.6	3_{10} turns	1,634	9.1 ± 0.7	β + C=N
1,635	12.3 ± 1.4	β + C=N	1,627	3.4 ± 0.6	β
1,624	2.6 ± 0.9	β	1,619	4.7 ± 0.4	β

[a] These values are included for comparative purposes, and are taken from [16]. [b] Frequency positions are expressed in cm^{-1}, and are rounded off to the nearest integer. [c] Mean relative areas calculated from four independent experiments. The standard deviation is also indicated. The relative area values are also affected by the methodological errors, due to the distortions caused by the deconvolution procedure [16]. In the present case, these methodological errors must be almost the same as in the case of native BR, because the band distributions are similar. As we use only the spectra for comparative purposes, the methodological errors are not considered.

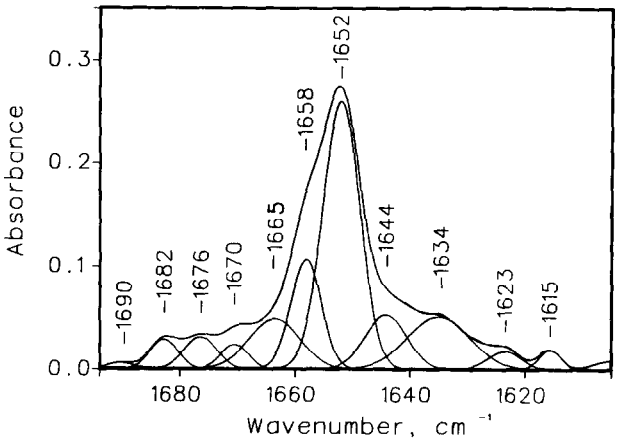

Fig. 2. Deconvoluted spectrum of dissolved purple membrane in the amide I region, with the best-fitted individual component bands. Band-fitting was done as indicated in section 2.

tent remains comparable, the α-helical classes are different from the native BR.

The region below 1,640 cm^{-1} corresponds mainly to β-sheets, with a total content of about 15% (the undetermined C=N contribution must be subtracted from this percentage). As compared to the native protein, there are differences mainly in the band distribution. The region 1,700–1,670 cm^{-1}, corresponding mainly to reverse turns, appears to be strongly changed in comparison with the native purple membrane (Table I). Both the band frequencies and the amounts are at variance with the native values, indicating that important changes have occurred in the loop region.

Our results indicate that transfer of bacteriorhodopsin from the native lipid environment to the organic solvent causes two major changes in the secondary structure. One is an important decrease of the α_{II}-helices and its transformation into a normal α_I-helix. The other one is the presence of great alterations in the reverse turns. Additionally, another change corresponds to some alterations in the β-sheet structures. All these changes can be accounted for by the nature of the medium because the principal characteristics of the membrane bilayer are altered in the organic medium. Conceivably, the most comparable part corresponds to the bilayer interior; although more polar, the chloroform/methanol mixture has similar properties as the lipid tails. Furthermore, it is likely that the lipid molecules that remain in solution are still interacting with BR α-helices. On the contrary, the membrane surface is totally absent in the organic medium. The effects of these changes on the BR secondary structure are difficult to assess, but some hypotheses can be formulated. First, the surface structures must be affected, as our results seem to corroborate. Second, due to the hydrophobic nature of the organic solvent, α helices can equally form, with similar boundaries as the native ones. Also, helix–helix interactions, which represent the second stage in the membrane protein oligomerization model [22], are allowed in the organic solvent. The different helical classes found in the organic medium could be the consequence of differences in the solvent properties, but they could also be due to the absence of the bilayer boundary formed by the lipid headgroup–water interface. In this latter case, the electric field created by the membrane surface potential could have a preponderant role in modulating the exact conformation of the transmembrane α-helices. Whether or not the native conformational characteristics like α_{II}-helices are essential for the BR function remains to be elucidated.

Acknowledgements: We thank M. Duñach for critically reviewing the manuscript and J. Cladera and F. Sepulcre for helpful discussions. This work was supported by the Dirección General de Investigación Científica y Técnica (Grant PB89-0301 to E.P. and fellowship to J.T.).

REFERENCES

[1] Ovchinnikov, Yu.A. (1982) FEBS Lett. 148, 179–191.
[2] Dencher, N.A. (1983) Photochem. Photobiol. 38, 753–767.
[3] Henderson, R., Baldwin, J.M., Ceska, T.A., Zemlin, F., Beckmann, E. and Downing, K.H. (1990) J. Mol. Biol. 213, 899–929.
[4] Knowles, P.F. and Marsh, D. (1991) Biochem. J. 274, 625–641.
[5] Mayo, K.H., Schussheim, A., Vuister, G.W., Boelens, R., Kaptein, R., Engelhard, M. and Hess, B. (1989) FEBS Lett. 235, 163–168.
[6] Seigneuret, M., Neumann, J.-M. and Rigaud, J.-L. (1991) J. Biol. Chem. 66, 10066–10069.
[7] Arseniev, A.S., Kuryatov, A.B., Tsetlin, V.I., Bystrov, V.F., Ivanov, V.T. and Ovchinnikov, Yu.A. (1987) FEBS Lett. 213, 283–288.
[8] Barsukov, I.L., Abdulaeva, G.V., Arseniev, A.S. and Bystrov, V.F. (1990) Eur. J. Biochem. 192, 321–327.
[9] Maslennikov, I.V., Arseniev, A.S., Tchikin, L.D., Kozhich, A.T., Bystrov, V.F. and Ivanov, V.T. (1991) Biol. Membr. (USSR) 8, 156–160.
[10] Pervushin, K.V. and Arseniev, A.S. (1992) FEBS Lett. 308, 190–196.
[11] Byler, D.M. and Susi, H. (1986) Biopolymers 25, 469–487.
[12] Surewicz, W.K. and Mantsch, H.H. (1988) Biochim. Biophys. Acta 952, 115–130
[13] Dong, A., Huang, P. and Caughey, W.S. (1990) Biochemistry 29, 3303–3308.
[14] Dousseau, F. and Pézolet, M. (1990) Biochemistry 29, 8771–8779.
[15] Lee, D.C., Haris, P.I., Chapman, D. and Mitchell, R.C. (1990) Biochemistry 29, 9185–9193.
[16] Cladera, J., Sabés, M. and Padrós, E. (1992) Biochemistry (in press).
[17] Barsukov, I.L., Nolde, D.E., Lomize, A.L. and Arseniev, A.S. (1992) Eur. J. Biochem. 206, 66–672.???
[18] Oesterhelt, D. and Stoeckenius, W. (1974) Methods Enzymol. 31, 667–678.
[19] Moffatt, D.J., Kauppinen, J.K., Cameron, D.G., Mantsch, H.H. and Jones, R.N. (1986) Computer Programs for Infrared Spectrometry, NRC Bulletin No. 18, NRC, Ottawa, Canada.
[20] Mantsch, H.H., Moffatt, D.J. and Casal, H. (1988) J. Mol. Struct. 173, 285–298.
[21] Venyaminov, S.Yu. and Kalnin, N.N. (1990) Biopolymers 30, 1243–1257.
[22] Popot, J.-L. and Engelman, D.M. (1990) Biochemistry 29, 4031–4037