Vol. 114, No. 2, 1983 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
July 29, 1983 Pages 713-720

CHARACTERIZATION OF THE MOBILITY OF VARIOUS CHEMICAL GROUPS IN THE PURPLE MEMBRANE OF HALOBACTERIUM HALOBIUM BY ^{13}C, ^{31}P AND ^{2}H SOLID STATE N M R

K.-H. Spohn and R. Kimmich

Sektion Kernresonanzspektroskopie Universität Ulm
7900 Ulm, Fed. Rep. Germany

Received June 9, 1983

Lyophilized purple membrane sheets have been investigated by C-13- and P-31-cross polarization/magic angle spinning N M R spectroscopy. The high-resolution C-13 spectrum and its non-quaternary suppression version indicate fast protein side-chain motions but a rigid backbone structure on a time scale of roughly < 0.001 to 0.01 s. Three components of exchangeable hydrogen have been detected by deuterium N M R . The mean exchange time of the peptide hydrogens must be longer than 1 μs. The medium component is attributed to mobile side-chains. In addition a narrow line has been observed which is assigned to the residual hydration water.

The purple membrane of halobacterium halobium (1) is an aggregate of the protein bacteriorhodopsin (75 %) and diverse lipids (25 %) which predominantly possess a phosphorus containing polar headgroup (2). The relatively simple composition provides an easy access to certain positions by diverse N M R techniques.

In literature a series of N M R applications to purple membrane, dissolved brh or fragments of it were published. Thus liquid state ^{13}C N M R has been applied to synthetic brh fragments (3) and to ^{13}C enriched retinals incorporated in brh monomers (4). Growing of halobacteria in media containing deuterium enriched amino acids permitted investigations of the mobility of certain side-chain groups in solid purple membranes with deuterium resonance (5). The phospholipid component of purple membrane has been studied by ^{31}P resonance (6a, 6b). From solid-state

ABBREVIATIONS

C P / M A S	cross polarization/magic angle spinning
N Q S	non-quaternary suppression
brh	bacteriorhodopsin

0006-291X/83 $1.50

^{15}N-spectra of ε- [^{15}N] lysyl-brh it is known that the Schiff base linkage is protonated (7).

C P / M A S spectroscopy permits to record high-resolution ^{13}C and ^{31}P spectra in spite of the solid nature of purple membrane sheets. Deuterium magnetic resonance enables one to study the exchangeable hydrogens. In this paper we report the partly first applications of these techniques to purple membrane. The main interest is dedicated to the characterization of the molecular mobility at diverse positions. We restrict ourselves to lyophilized purple membrane.

EXPERIMENTAL

Purple membrane sheets have been prepared by growing halobacteria in complex (8) or synthetic (9) media. The cells have been harvested and the purple membrane separated according to standard procedures. The purity was tested by absorption spectroscopy.

The C P / M A S - spectra have been recorded with a Bruker CXP 300 spectrometer. The spinning rate was 4.5 kHz, the proton decoupling power 300 - 400 W.

The deuteron spectrum has been recorded at 55 MHz using a home built apparatus (5).

RESULTS AND DISCUSSION

Fig. 1 shows a ^{13}C-spectrum obtained from lyophilized purple membrane sheets by the aid of the C P / M A S - technique. The chemical shifts can be subdivided into three regions (10, 11). The saturated carbons of the protein and the lipid constituents are represented in the 10 to 75 ppm range. Unsaturated carbons of the protein appear between 115 and 160 ppm. The carbonyl lines finally are situated between 170 and 195 ppm. The relatively great width of the carbonyl line compared with the methyl group signals for instance, indicates that it must be due to a certain distribution of chemical shifts. This can be caused by a conformational distribution of the secondary structure (12), i.e. by helical and non-helical regions. Variation of the spinning frequency ensured that no rotational sideband is visible in the spectral range.

Signals of the non-quaternary carbons can be suppressed by the N Q S - technique (13), provided their dipolar broadening is large compared with that of the quaternary carbons.

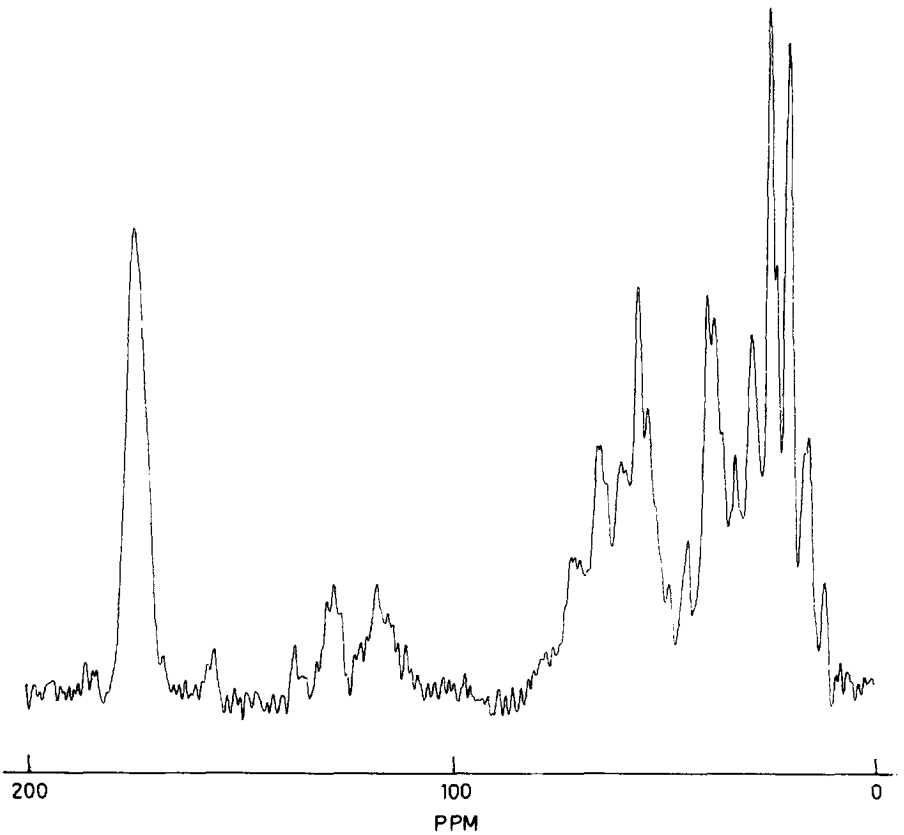

fig. 1: ^{13}C-CP/MAS spectrum of lyophilized purple membrane sheets. (0 ppm corresponds to tetra methyl silane.)

This condition is more or less fulfilled depending on the rate of molecular motions, i.e. on the degree of motional narrowing. The resulting spectrum is shown in fig. 2. As expected, the N Q S - sequence has almost no effect on the intensity of the carbonyl line (175 ppm). The effect is also small for the methyl groups (ca. 20 ppm) which rotate so fast at room temperature that their linewidths become comparable to those of the quaternary carbons.

In the methylene region (25 to 45 ppm) intensity is strongly reduced indicating a reduced mobility even of side-chain and lipid methylene groups. The strongest suppression effect has been observed between 45 and 75 ppm, a range involving the backbone α-carbons of the protein. This confirms the strong rigidity of the helices of which bacteriorhodopsin consists. For these carbons the condition $\Delta\omega\ \tau_c >> 1$ must be valid, where $\Delta\omega$ is the dipolar linewidth and τ_c is the correlation time of molecular motion. We conclude that the rigidity of

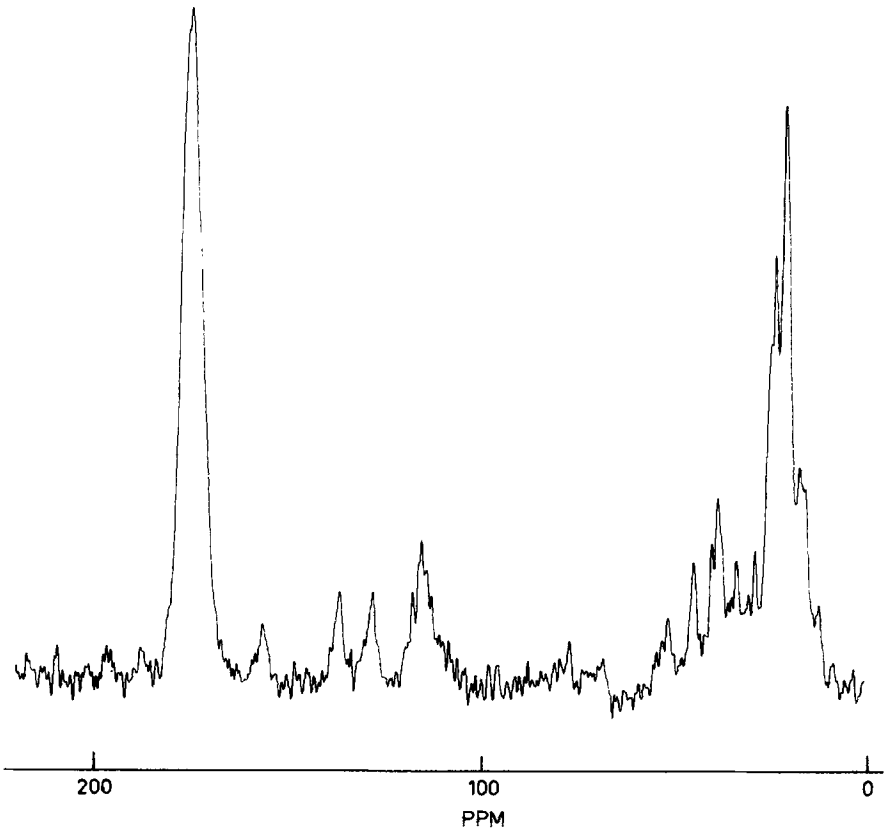

fig. 2: ^{13}C-spectrum of purple membrane recorded with the NQS version of the CP/MAS-technique.

the helical parts of the protein backbone is confirmed on a time scale of roughly < 10^{-3} to 10^{-2} s. The more mobile situation found at the non-helical chain-parts at the membrane surface is discussed in (14).

The rigidity of the purple membrane is also confirmed by ^{31}P C P / M A S spectra. Fig. 3 shows an example. As already shown in (6a) the chemical shift anisotropy reveals itself in its full extent. While Ekiel et al. (6a) recorded the powder pattern of the ^{31}P resonance, we have observed the spinning side band spectrum, the envelope of which corresponds to the powder lineshape. The spectrum is extended over about 200 ppm corresponding to about 24 kHz. We conclude that the headgroups of the phospholipids incorporated in the purple membrane sheets are rigid on a time scale < 10^{-6} - 10^{-5} s.

From the spectra discussed so far it follows that the lipid/protein structure of purple membrane is rather immobile. Mobile groups are formed by certain side chains as

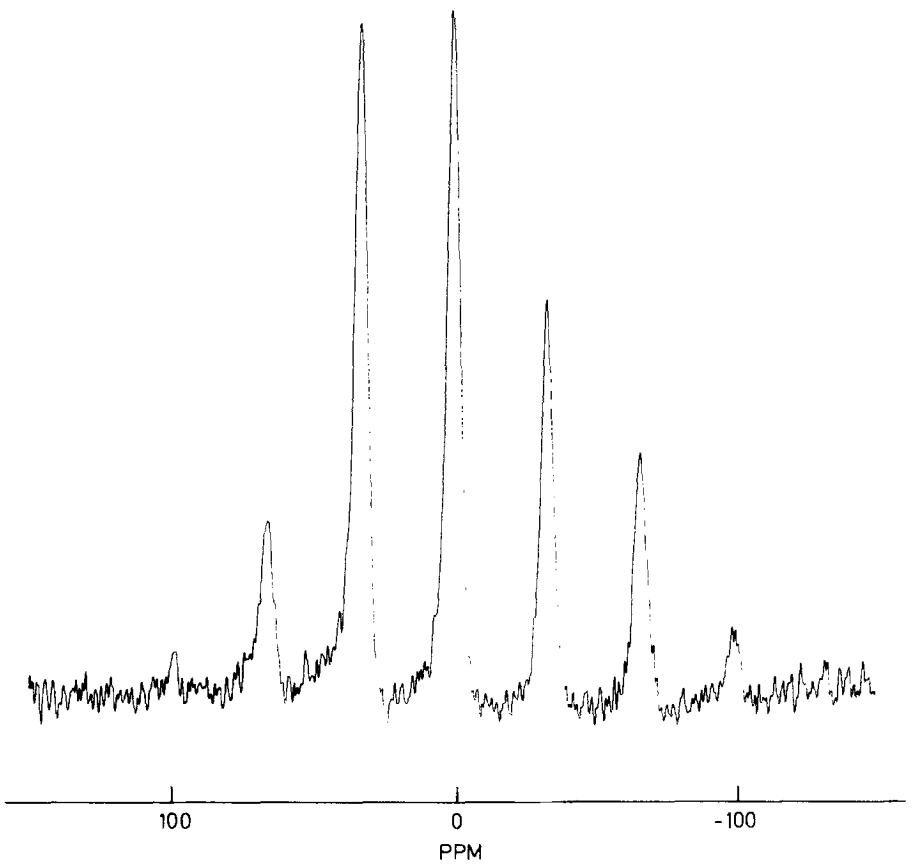

fig. 3: ^{31}P-spinning side band spectrum recorded with the CP/MAS-technique. (0 ppm corresponds to $(NH_4)H_2PO_4$.)

can be determined by deuterium magnetic resonance of labelled bacteriorhodopsin (5). Molecular motions can also be detected by deuterium resonance of the exchangeable hydrogen atoms.

Lyophilizing a purple membrane/D_2O-suspension and recording near room temperature the deuterium spectrum of the residual deuterons which have been incorporated in the membrane sheets by chemical exchange permits the detection of at least three components (fig. 4). The quadrupole splittings are about 120 kHz, 38 kHz and < 6 kHz, respectively.

The 120 kHz component is assigned to deuterons incorporated in peptide bonds. Their mean exchange time must be longer than 10^{-6} s in order to account for the absence of any motional averaging.

The medium component very likely corresponds to mobile ND_2 or ND_3 groups of the side chains. The rotational tumbling

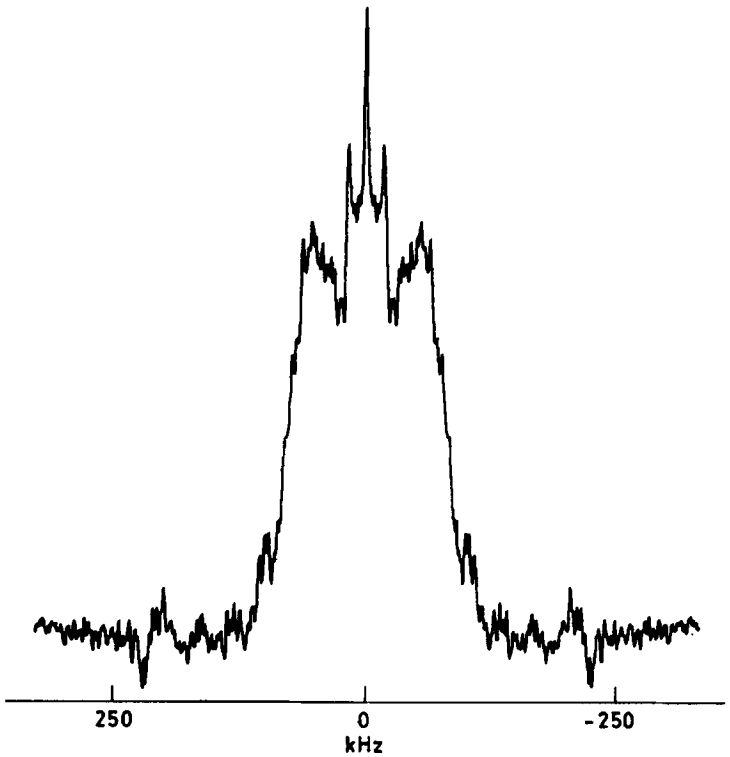

fig. 4: ^{2}H-spectrum of the exchangeable hydrogens of purple membrane.

and/or hydrogen exchange must be fast compared with the deuterium time scale (10^{-6} - 10^{-5} s) in coincidence with the conclusions drawn in (5,15).

The narrow line finally is attributed to D_2O of the residual hydration shells which cannot be removed by prolonged lyophilization (several days) although no significant D_2O bands could be found in the infrared spectrum. However, rehydration by exposure to a D_2O-atmosphere for some hours causes a strong increase of the narrow component.

Partially relaxed ^{2}H-spectra recorded with a repetition rate of 17 s^{-1} show reduced narrow and broad line components. Their longitudinal relaxation rates T_1^{-1} therefore must be less than the repetition rate. The finding that long spin lattice relaxation times appear in connection with both a high and a vanishing degree of motional averaging (in contrast to the medium component) suggests that the relevant fluctuations correspond to situations above and below the T_1-minimum at $\omega\tau_c \approx 1$, respectively. The different fluctuation amplitudes revealing themselves in the diverse quadru-

pole splittings otherwise would be incompatible with the order of the relaxation times.

The slow exchange rates of the deuterons in intra-helical hydrogen bonds indicates a similar behaviour as found with hemoglobin (16). It has been interpreted by the cooperativity in transient and local H-bond cleavage. On the other hand, the conformational flexibility of the protein is reported to be affected by high electric fields which are effective in vivo (17). Thus local unfolding prior to hydrogen exchange might be more likely under natural conditions.

ACKNOWLEDGEMENTS

One of us (R. K.) would like to thank Professors Oldfield and Gutowsky for a fruitful stay at the University of Illinois at Urbana-Champaign. Thanks are also due to Professor Oesterhelt for advice and Professors Kleinschmidt and Klotz for the permission to use their ultracentrifuge laboratory. Dr. Förster, Bruker Analytische Meßtechnik GmbH, is acknowledged for recording the C P / M A S spectra. This work has been supported in part by N.A.T.O. Grant RG 192.80.

REFERENCES

1. Oesterhelt, D. and Stoeckenius, W. (1973) Proc. Nat. Acad. Sci. 70, 2853-2857
2. Kates, M. and Kushwaha, S.C. (1978) Energetics and Structure of Halophilic Microorganisms, Caplan, S.R. and Ginzburg, M. (Eds.) pp. 461-480, Elsevier, Amsterdam
3. Sugihara, T., Blout, E.R. and Wallace, B.A. (1982) Biochem. 21, 3444-3452
4. Yamaguchi, A., Unemoto, T. and Ikegami, A. (1981) Photochem. Photobiol. 33, 511-516
5. Kinsey, R.A., Kintanar, A. and Oldfield, E. (1981) J. Biol. Chem. 256, 9028-9036
6a. Ekiel, I., Marsh, D., Smallbone, B.W., Kates, M. and Smith, I.C.P. (1981) Biochem. Biophys. Res. Commun. 100, 105-110
6b. Rice, D.M., Herzfeld, J. and Griffin, R.G. (1981) Biophys. J. Abstr. 33, 103a
7. Harbison, G.S., Herzfeld, J. and Griffin, R.G. (1983) Biochem. 22, 1-5
8. Oesterhelt, D. and Stoeckenius, W. (1973) Meth. Enzymol. 31, 667-678
9. Lanyi, J.K. and MacDonald, R.E. (1979) Meth. Enzymol. 56, 398-407
10. Allerhand, A., Childers, R.F. and Oldfield, E. (1973) Biochem. 12, 1325-1341
11. Wüthrich, K. (1976) NMR in Biological Research: Peptides and Proteins pp. 358-366, North-Holland/ American-Elsevier, Oxford

12. Jardetzky, O. and Roberts, G.C.K. (1981) NMR in Molecular Biology, p. 235, Academic Press, New York
13. Opella, S.J. and Frey, M.H., (1979) J. Amer. Chem. Soc. 101, 5854-5856
14. Baianu, I.C., Gutowsky, H.S. and Oldfield, E., in press
15. Keniry, M.A., Kintanar, A., Smith, R.L., Gutowsky, H.S. and Oldfield, E. in press
16. Englander, S.W., Calhoun, D.B., Englander, J.J., Kallenbach, N.R., Liem, R.K.H., Malin, E.L., Mandal, C. and Rogero, J.R. (1980) Biophys. J. 10, 577-587
17. Tsuji, K. and Neumann, E. (1983) Biophys. Chem. 17, 153-163