Salt-Dependent Conformational Changes in The Cell Membrane of Halobacterium salinarium

J.C. Hsia*., P.T.S. Wong+ and David H. MacLennan+

Department of Pharmacology* and

Banting and Best Department of Medical Research+

University of Toronto, Toronto 181, Canada

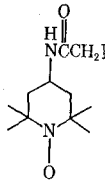
Received February 15, 1971

Summary: Electron spin resonance labels were used to probe for changes in protein and lipid environments before and after the dissolution of the cell membrane of Halobacterium salinarium in dilute salt solution. Two probes were used: N-(1-oxyl-2,2,6,6-tetramethyl-4-piperidinyl) bromoacetamide (I) was bound covalently to protein and N-oxyl-4,4-dimethyl oxazolidine derivative of 12-keto-stearic acid (II) was intercalated into hydrophobic regions of the membrane. Upon dissolution of the membrane, the environment of the protein spin label changed so that the strongly immobilized spectrum became mobile, resembling the spectrum of spin label attached to a random coil. Lesser changes were observed in the spectrum of spin label intercalated into the hydrophobic region of the membrane. The results indicate that a change in protein conformation and a change in the lipid-protein interaction accompanies the dissolution of the membrane in media of low ionic strength.

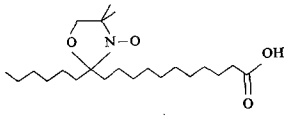
Introduction: The cell membrane of Halobacterium salinarium is insoluble in salt solution greater than about 2 Molar but dissolves as the salt concentration is reduced to less than about 1 Molar. The dissolved membranes are not reduced to subunits but appear to exist as membrane fragments of small size[1]. This fact raises a number of questions about the nature of the bonds holding the membrane together. Lipid-protein interactions in the plasma membrane of halophilic bacteria seem to be nonionic[2]. Anionic phospholipids predominate in the membrane[3] and the protein component contains an unusually high content of dicarboxylic amino acids[4]. It is generally assumed that electrostatic repulsion resulting from this high charge density at the membrane surface is neutralized by high concentrations of counterions and for this reason concentrated salt is required for

structural integrity of the membrane. The solubilization of the membrane in water containing no dissociating reagents offers a rare opportunity for comparing the structure of the intact membrane with its solubilized form.

Application of new physical techniques to natural and model membrane systems has yielded useful information concerning membrane structure[6-11]. The electron spin resonance (ESR) spin-labelling technique is particularly useful in probing for conformation change during the transition from the membranous to the soluble state since resonance spectra of labels (I & II) introduced into the protein or lipid moieties of the membrane reflect changes in the motional freedom and characteristics of the lipid-protein



(I)



(II)

environment but are not directly influenced by the salt concentration of the external environment*. In the present communication we report evidence for conformation changes in the membrane protein and lipid moieties during membrane disintegration.

Materials and Methods: The cell envelope of H. salinarium (19 mg protein/ml) prepared in 4.0 M NaCl according to the procedure of Kushner et al.[4] was incubated in the presence of 10^{-4} M N-(1-oxyl-2,2,6,6-tetramethyl-4-piperidinyl) bromoacetamide (I)[5] at 37° for 1 hr. Unreacted spin label was removed by centrifugation and washing.

Labelling of the membrane lipid moiety was accomplished by an exchange of lipid during incubation of the cell envelope with N-oxyl-4,4-dimethyl oxazolidine derivative of 12-keto-stearic acid[8] (II)-bovine serum albumin

*There is a reduction of signal intensity of the nitroxide spin-label at high salt concentration but there is no difference between the asymmetry of the resonance spectra.

(BSA) complex at 37° for 1 hr. BSA in the supernatant was removed by centrifugation. Electron spin resonance spectra were recorded on a Varian E-6 X-band spectrometer at 22 ± 1°. Average correlation time of the nitroxide spin label was calculated according to Stone et al.[12]

Results and Discussion: In the presence of 4 M NaCl the cell envelope was insoluble and the nitroxide spin label I introduced into the cell envelope through covalent bonding with protein components gave a rather strongly immobilized spectrum (Fig. 1A). The mobility of the label corresponded to an average correlation time (τ_c) of greater than 10 nanosec. In the presence of dilute salt (< 0.1 M NaCl) the cell envelope was soluble and the spin label gave a sharp triplet spectrum (Fig. 1B). A calculated τ_c of 0.33 nanosec. indicated that the binding site of I was

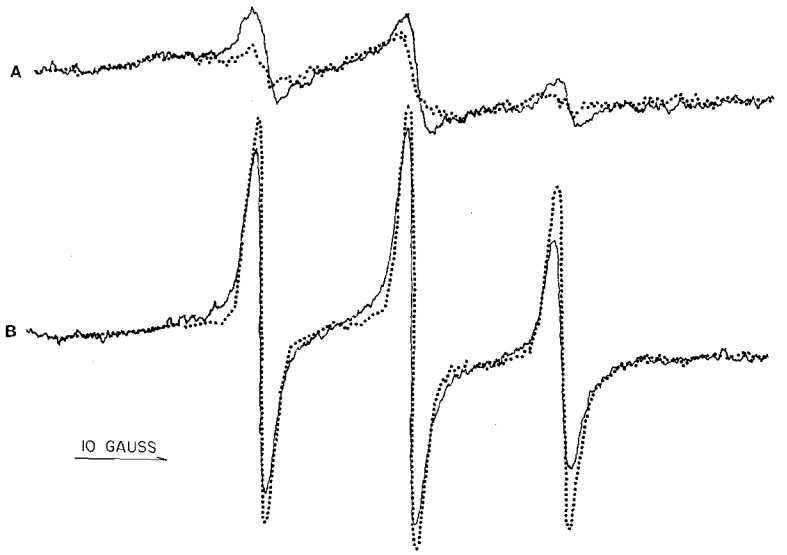


Figure 1. Electron spin resonance spectra of N-(1-oxyl-2,2,6,6-tetramethyl-4-piperidinyl) bromoacetamide covalently bonded to H. salinarium cell envelope.
(A) Dotted line (.....): Resonance spectrum in 4 M NaCl. Solid line (———): Resonance spectrum in 4 M NaCl after exposure to distilled water (compare to (B) solid line spectrum).
(B) Solid line (———): Resonance spectrum in distilled water. Dotted line (.....): Resonance spectrum in distilled water after treatment with trypsin (1 mg/ml) for 15-30 min. All resonance spectra were recorded at identical instrument setting.

exposed. We suggest that this represents a loss of structural integrity of the tertiary structure of the membrane protein moiety. Hydrolysis of the labelled membrane protein with trypsin (1 mg/ml) at both high and low salt concentration further increased the correlation time of the label to a limiting value of 0.096 nanosec. The addition of 4 M NaCl induced a reversal of the loose protein conformation to a compact structure as indicated by a return to the immobilized spectrum, however, complete renaturation could not be achieved (Fig. 1A). The addition of NaCl did

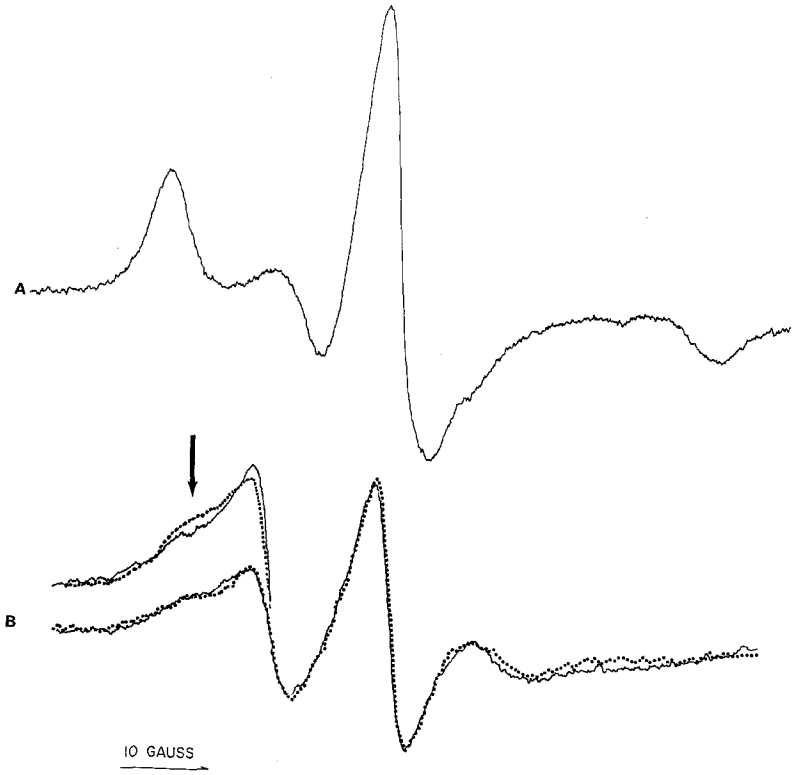


Figure 2. Electron spin resonance spectra of N-oxyl-4,4-dimethyl-oxazolidine derivatives of 12-keto-stearic acid. (A) Complex with BSA (1 x 10^{-4}M) in 4 M NaCl (B) The upper expanded peaks represent the difference of the resonance spectra of II in H. salinarium membrane in 4 M NaCl (————) and distilled water (.....) as indicated by a down arrow (↓). The lower solid (————) resonance spectrum represents H. salinarium membrane in 4 M NaCl. Dotted line (.....) resonance spectrum represents renaturation by addition of 4 M NaCl after exposure to distilled water. The signal intensities were adjusted to facilitate direct comparison.

not increase the turbidity of the sample and presumably did not cause a return to the membranous state.

Resonance spectra of the N-oxyl-4,4-dimethyl-oxazolidine derivative of 12-keto stearic acid (II) - BSA complex (Fig. 2A) were strongly immobilized; a characteristic of label held rigidly by protein molecules. Under these conditions mobility of the label is dictated by the rotational diffusion of the protein molecule. When compound II was intercalated in the cell envelope, a considerable increase of mobility was evident from the sharp resonance spectra with $\tau_c \simeq 3.1$ nanosec. (Fig. 2B). This is characteristic of spin label within a fluid hydrophobic interior of the membrane lipid or lipoprotein. The resonance spectrum in this case was relatively insensitive to exposure to distilled water and trypsin treatment ($\tau_c \simeq 3.7$ nanosec.). However, the appearance of a new peak in the low fields as indicated by an arrow in Fig. 2B suggests that the spin label was not in a homogeneous lipid environment. The more immobilized environment corresponding to the new resonance peak was probably due to a direct interaction between the spin label and the membrane protein. The low field resonance peak persisted when the suspension was again made 4 M in NaCl. This result indicates that there was some perturbation of the lipid-protein interaction during the transition from the insoluble state.

Conclusions: Changes in the spectrum of spin label attached covalently to the protein moiety of the cell membrane of H. salinarium suggest that at least 95% of the binding sites lose their structural integrity upon dissolution of the membrane in low salt. We interpret these changes as resulting from a protein conformational change in response to lowering of the ionic strength of the media. When the protein undergoes a conformational change, the nature of the lipid-protein interaction is slightly altered as indicated by the appearance of a new resonance peak in the spectrum of the spin label intercalated into hydrophobic region of the

membrane. This new peak results from an immobilizing environment and probably results from a direct interaction between the hydrophobic spin label and membrane protein. Neither the protein conformational change nor the change in protein-lipid interaction were completely reversible upon the addition of 4 M NaCl.

Acknowledgements: This investigation was supported by Grants MA 4129, MA 4289, MA 3399 and MA 4166 from the Medical Research Council of Canada.

References

1. Stoeckenius, W., and R. Rowen, J. Cell. Biol. 34, 365 (1967).
2. Brown, A.D., J. Mol. Biol. 12, 491 (1965).
3. Marshall, C.L., and A.D. Brown, Biochem. J. 110, 441 (1968).
4. Kushner, D.J., S.T. Bayley, J. Boring, M. Kates and N.E. Gibbons, Can. J. Microbiol. 10, 483 (1964).
5. Compound I was prepared by one of the authors (J.C. Hsia) at Professor L.H. Piette's Laboratory in 1968.
6. Kamato, V.B., and D. Chapman, Biochim. Biophys. Acta 163, 411 (1968).
7. Hubbell, W.L. and H.M. McConnell, Proc. Natl. Acad. Sci. (U.S.) 63, 16, (1969).
8. Waggoner, A.S., T.J. Kingzett, S. Rottschaefer., O.H. Griffith and A.D. Keith, Chem. Phys. Lipids 3, 245 (1969).
9. Glaser, M., H. Simpkins, S.J. Singer, M. Sheetz and S.I. Chan, Proc. Natl. Acad. Sci. U.S. 65, 721 (1970).
10. Tourtellotte, M.E., D. Branton and A.D. Keith. Ibid 66, 909 (1970).
11. Rottem, S., W. Hubbell, L. Hayflick and H. McConnell, Biochim. Biophys. Acta. 219, 104 (1970).
12. Stone, T.J., T. Buckman, P.L. Nordio, and H.M. McConnell, Proc. Natl. Acad. Sci. (U.S.) 54, 1010 (1965).