ELSEVIER

Biochimica et Biophysica Acta 1236 (1995) 331–337

BBA Biochimica et Biophysica Acta

Electrokinetic charge of the anesthetic-induced bR_{480} and bR_{380} spectral forms of bacteriorhodopsin

Stefka G. Taneva [b], José M.M. Caaveiro [a], Ivana B. Petkanchin [c], Félix M. Goñi [a,*]

[a] *Universidad del País Vasco, Departamento de Bioquímica y Biología Molecular, Aptdo. 644, 48080 Bilbao, Spain*
[b] *Central Laboratory of Biophysics, Bulgarian Academy of Sciences, Sofia 1113, Bulgaria*
[c] *Institute of Physical Chemistry, Bulgarian Academy of Sciences, Sofia 1113, Bulgaria*

Received 20 October 1994; accepted 9 March 1995

Abstract

The translational and rotational electrokinetics of the anesthetic-induced spectral transitions $bR_{568} \rightarrow bR_{480} \rightarrow bR_{380}$ of bacteriorhodopsin have been investigated. Formation of the bR_{480} form is associated with an increase of the purple membrane negative electrokinetic charge, while the transformation of bR_{480} into bR_{380} is accompanied by a decrease of the membrane negative charge as compared to that of the 480 nm-absorbing form. Removal of anesthetics leads to the back transitions $bR_{480} \rightarrow bR_{568}$ and (in part) $bR_{380} \rightarrow bR_{568}$; however, the electrokinetic charge of the native membranes is not restored. A strong decrease in the electric polarizability and the appearance of a slow polarizability component are also observed in anesthetic-treated membranes. Comparison with the electrokinetic behaviour of partially delipidated membranes and with that of liposomes composed of purple membrane total lipids suggests that: (i) anesthetic molecules partition mainly at the protein/lipid interface inducing irreversible rearrangement of the boundary lipid layer, and (ii) different mode(s) or site(s) of interaction are responsible for the spectral and surface charge effects. The data are compatible with the hypothesis of anesthetics acting through partial dehydration of the membrane surface.

Keywords: Purple membrane; Anesthetic; Electrophoretic mobility; Electric light scattering

1. Introduction

Characterizing the interaction of anesthetics with lipids, proteins and membranes, and establishing the resulting changes in their physical properties and function is an essential part of the investigation of the mechanisms of anesthesia. While numerous studies suggest that anesthetics interact mainly with lipids and that their effect on membrane proteins is lipid-mediated [1,2] experimental evidence also exists in favor of a direct anesthetic–protein interaction [1,3]. Binding of the halogenated anesthetic halothane to soluble proteins has been recently demonstrated by photoaffinity labelling [4]. A number of electrical properties of model lipid membranes like capacitance and conductance, surface charge density and counter-ion association [1,5–7] has been shown to be affected by general anesthetics.

In this work, the translational and rotational electrokinetics of anesthetic-treated purple membranes have been studied in an attempt to understand the anesthetic–membrane interaction. The disk-shaped PM fragments consist of a highly ordered crystalline lattice of bacteriorhodopsin (bR) molecules [8,9] and possess a high charge asymmetry [10]. The retinylidene chromophore is covalently attached to the ϵ-amino group of the Lys-216 residue of bR via a protonated Schiff base [11]. Purple membranes have been already used in the study of anesthetic–membrane interactions [12–15]. Volatile anesthetics interact with PM causing a large reversible blue shift in the pigment absorption band [15] associated with disturbed exciton coupling among bacteriorhodopsin (bR) molecules [12] and strongly altered photochemistry and proton pumping activity [14]. Nakagawa et al. [16] have recently suggested a specific binding of volatile anesthetics to the protein/lipid interface on the basis of X-ray diffraction experiments.

In order to improve our understanding of the mechanism of action of anesthetics as modulators of bacterio-

Abbreviations: PM, purple membranes; DL-PM, delipidated purple membranes; bR_{568}, native bacteriorhodopsin; bR_{480}, red absorbing form of bacteriorhodopsin; bR_{380}, denatured bacteriorhodopsin; u_e, electrophoretic mobility; B_{ave}, average diameter.

* Corresponding author. Fax: +34 4 4648500.

SSDI 0005-2736(95)00074-7

rhodopsin spectral properties and charge transfer function, we have investigated the variation of electric parameters (electrophoretic mobility and electric dipole moments) of native and partially delipidated purple membranes during the anesthetic-induced $bR_{568} \rightarrow bR_{480} \rightarrow bR_{380}$ spectral transitions. In order to ascertain the role of the protein in the observed purple membrane effects, similar studies have been carried out on liposomes composed of PM lipids. Anesthetics induce a significant increase in the purple membrane negative electrokinetic charge during the conversion of bR to the red absorbing species bR_{480}, followed by a decrease when formation of the bR_{380} form is induced.

2. Materials and methods

PM fragments were isolated from *Halobacterium halobium* S9 strain according to a standard procedure [17]. Partially delipidated PM (DL-PM) were prepared by three successive treatments of purple membranes with CHAPS (3-(3-cholamidopropyl)dimethylammonio-1-propane sulfonate), followed by five times washing by centrifugation as described by Szundi and Stoeckenius [18]. Total lipids were extracted from PM as in [19]. Negatively charged multilamellar liposomes were prepared from the total PM lipids in 10 mM Tris-HCl buffer (pH 6.5 and pH 8), the final phospholipid content being 0.25 mM. Halothane (ICI-Farma, Spain) and isoflurane (Abbott Laboratories, Madrid) were added in microliter aliquots to the samples.

The hydrodynamic diameter (B_{ave}) and electrophoretic mobility (u_e) of the membrane fragments were measured using a Zeta Sizer 4 apparatus (Malvern Instruments) combining a cross beam laser anemometer and a parallel beam along the axis of the measuring cell for optimum electrophoresis and sizing, respectively. The electrophoretic mobility was measured by the shift of the frequency of the scattered laser light (Doppler shift) by heterodyning with the incident beam. The light scattering measurements for particle size distribution were made in the photon correlation mode. Electrophoretic and quasi-elastic light scattering measurements were made at 90° scattering angle. The total charge (Q_e) of the membrane fragments was estimated from the experimental electrophoretic mobility values using the equation [20]:

$$Q_e = 6\pi\eta a u_e (1 + ka)/f(ka) \quad (1)$$

where η is the medium viscosity, a is the radius of the electrokinetic unit (i.e., of the membrane fragment), u_e is the electrophoretic mobility, k is the Debye screening length. The Henry function $f(ka)$, which accounts for the fact that the external field is superimposed on the local field around the fragments, depends on the particle shape and approaches 1 and 1.5 for small and large ka, respectively [21].

The relative changes in the intensity of the scattered light by the suspension of purple membrane fragments at application of electric pulses:

$$\alpha = (I_E - I_0)/I_0 \quad (2)$$

was measured at 90°, the direction of the orienting electric pulses being perpendicular to the plane of observation. The light source was an incandescent lamp followed by an interference filter. I_E is the scattered light intensity when an electric field of strength E is applied to the suspension and I_0 is the light intensity without an external field. The light scattering changes at application of electric pulses result from the alignment of the disc-shaped membrane fragments, the orienting moments being due to the interaction of the membrane electric moments (permanent and/or induced) with the externally applied field. The experimental setup and the theory of the electro-optic phenomena are described elsewhere [22,23]. The frequency dependence of the electric light scattering obtained at application of a.c. electric pulses allows the mechanisms of different polarization processes contributing to the particle orientation to be distinguished [22]. Fragment diameter can be determined by the transient process of fragment disorientation after switching off the external field [22,23].

Electrophoretic mobility measurements were performed on suspensions of PM at 2 μM bR concentration either in a neutral electrolyte (1 mM NaCl) or in buffer (either 10 mM Tris-HCl, pH 8 or 10 mM sodium phosphate, pH 8). 1 μM concentration of bR was used for the electric light scattering measurements, a concentration low enough as to avoid the interaction between membrane fragments.

Absorption spectra were measured in a Uvikon 491 Kontron UV-Vis spectrophotometer.

3. Results

3.1. Anesthetic-induced changes in the electrophoretic mobility of purple membranes

The electrophoretic mobility (u_e) of PM fragments is measured as a function of the concentration of two volatile anesthetics (halothane and isoflurane) either in a slightly alkaline (pH 8) buffer or in unbuffered 1 mM NaCl.

The fraction of the red bR_{480} species and the electrophoretic mobility are shown in Fig. 1 as a function of anesthetic concentration. For halothane-treated PM, at concentrations producing formation of bR_{380}, the fraction of this pigment form is also presented (Fig. 1A). A complete $bR_{568} \rightarrow bR_{480}$ transition is induced by halothane in pH 8 buffer at concentration ca. 0.3% (v/v) (results shown for Tris-HCl buffer). Concentrations higher than 0.3% (v/v) induce the formation of bR_{380}. A similar spectral transition to the 480 nm absorbing species is induced by isoflurane but at considerably higher concentrations (Fig. 1A) and no conversion of bR_{480} to bR_{380} pigment form is induced in

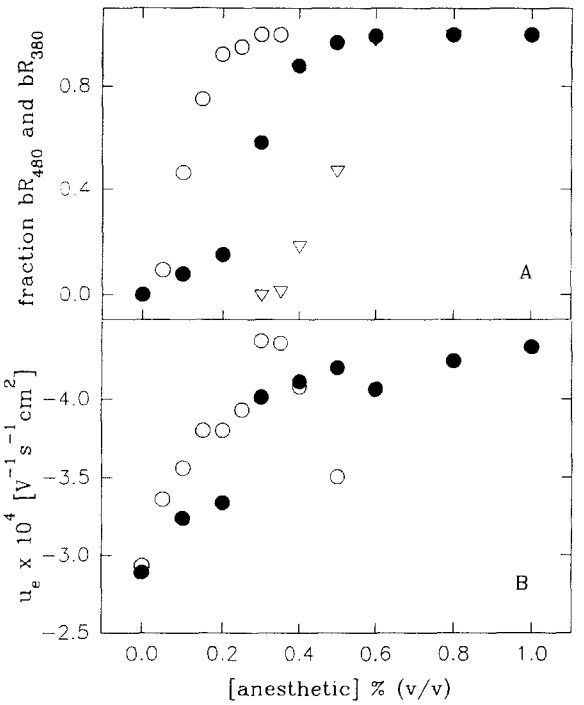

Fig. 1. Fraction of bR_{480} (○, halothane; ●, isoflurane) and bR_{380} (▽, halothane) pigment forms (A) and electrophoretic mobilities of PM (halothane (○) and isoflurane (●)) (B) as a function of anesthetic concentration measured in 10 mM Tris-HCl, pH 8.

the isoflurane concentration range studied. This may be related to the smaller anesthetic potency of isoflurane.

Removal of anesthetics results in a back $bR_{480} \rightarrow bR_{568}$ transition. The original purple color is restored from bR_{480} species either produced by halothane or isoflurane (Fig. 2A). On the contrary, once the bR_{380} species is produced by halothane only partial restoration of the original spectral characteristics of the pigment is observed (Fig. 2A). These results are in agreement with previous studies showing an anesthetic-induced reversible shift of the bR visible absorption maxima from 568 to 480 nm [15,24].

In turn, addition of halothane to a slightly alkaline PM suspension leads to a sharp increase in the electrophoretic mobility, i.e., an increase in the negative charges on the membrane fragments, in a concentration range $0 < C_{hal} < 0.3\%$ (v/v), followed by a decrease of u_e at higher concentrations (Fig. 1B). These two regions of halothane concentration correspond to the $bR_{568} \rightarrow bR_{480} \rightarrow bR_{380}$ spectroscopic transitions. The increase in mobility correlates with the progressive formation of bR_{480} form, while the successive formation of the 380 nm form at higher halothane concentrations is associated with a decrease in the electrophoretic mobility as compared to the bR_{480} form.

An increase in u_e of PM is also induced by isoflurane, the u_e vs. isoflurane concentration dependence being shifted to higher concentrations (almost 2-fold) as compared to halothane (Fig. 1B). In contrast, the addition of saturating concentrations of isoflurane do not cause a decrease of u_e as observed for halothane, instead u_e attains a constant value above 0.5% (v/v) isoflurane. This is an additional evidence that the second part of the u_e vs. halothane concentration dependence, i.e., the decrease in u_e, is related to the formation of the 380 nm absorbing form.

In unbuffered solution (1 mM NaCl), as in pH 8 buffer, increasing concentrations of halothane and isoflurane cause a drop in the absorbance at 568 nm and an increase at 480 nm. However, a smaller amount of the pigment undergoes transition to 480 nm and a small shoulder at 568 nm remains present in the absorption spectra (results not shown). Under these conditions the electrophoretic mobility of PM increases as observed in buffer and reaches a constant value at high anesthetic concentrations. The maximal increase of the electrophoretic mobility is the same irrespective of the anesthetic in both suspending media.

While the removal of anesthetics results in a complete recovery of the bR absorbing properties from bR_{480} species (Fig. 2A) the shift of the electrophoretic mobility to more negative values is completely irreversible (Fig. 2B). The partial original color restoration upon removal of halothane once 380 nm absorbing form was produced (Fig. 2A) corresponds to a slight shift of the electrophoretic mobility to lower values (Fig. 2B).

By the use of Eq. (1) and the experimentally obtained u_e values the electrokinetic charge Q_e of the bR_{480} and bR_{568} forms of the pigment is estimated. The estimated surface charge density of purple membrane of $-5.8 \cdot 10^{-4}$

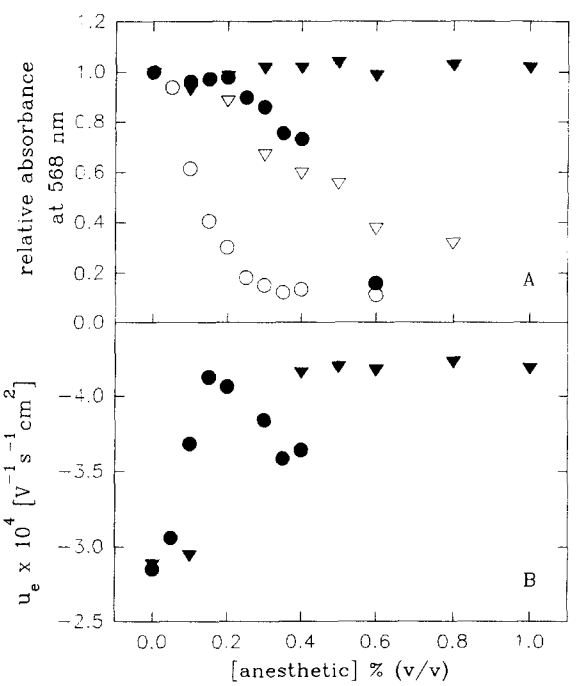

Fig. 2. (A) Absorbance at 568 nm in the presence (empty symbols) and after removal (full symbols) of halothane (○, ●) and isoflurane (▽, ▼). (B) Electrophoretic mobility of PM after removal of halothane (●) and isoflurane (▼).

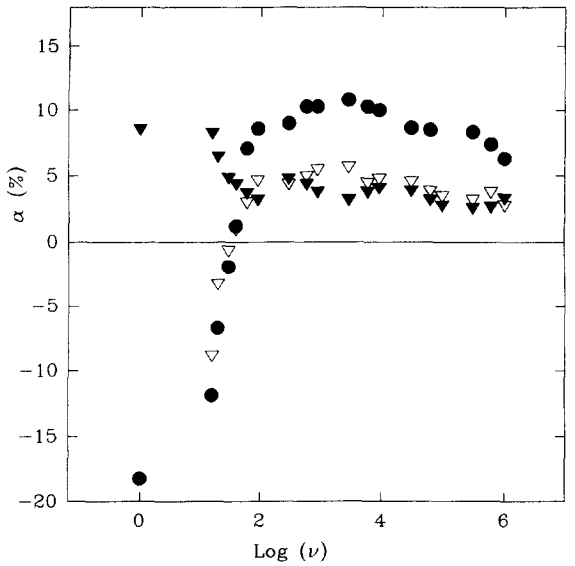

Fig. 3. Frequency dependence of electric light scattering effect of native (●) and halothane-added PM (0.05% (▽) and 0.1% (▼) (v/v)). Electric field strength $E = 3.9 \cdot 10^2$ V/m. Sample conditions as specified in Fig. 1.

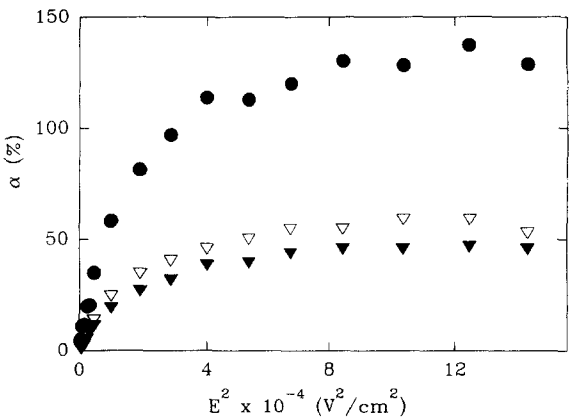

Fig. 4. Field dependence of the electro-optical effect measured at frequency 1 kHz. (●) native PM, (▽) 0.05% (v/v) and (▼) 0.1% (v/v) halothane.

electric charges/Å^2 (i.e., −0.66 electric charges/bR assuming 1140 Å^2 for the surface area of a bR molecule), is in keeping with previously reported values [25] and is lower than those obtained by techniques estimating local surface charge density [26,27]. The anesthetic-induced bR_{480} form has a higher effective negative charge (−1.26 electric charges/bR) than the native bR_{568}. Note that the average size of the membrane fragments does not change in the presence of anesthetics throughout the transition $bR_{568} \rightarrow bR_{480}$ (B_{ave} is ca. 600 nm).

3.2. Electric light scattering effect of PM in the presence of halothane

Analysis of the frequency dependence of the electric light scattering effect (Fig. 3) measured at electric field strength $E = 3.9 \cdot 10^2$ V/m, corresponding to the linear part of the field dependence ($\alpha(E^2)$) shows that: (i) PM fragments have a frequency dependence typical for colloid particles with a positive electro-optical effect in the kHz region and a negative effect, relaxing in the low frequency range (Hz region). This reflects an orientation of the native membrane fragments due to orienting moments in transversal direction at low frequencies and along the membrane surface in the kHz-region, respectively. (ii) The frequency dependence of the electro-optical effect is strongly influenced by anesthetic interaction with PM. The magnitude of the low frequency electro-optical effect decreases upon addition of 0.05% (v/v) halothane and even changes its sign upon addition of higher halothane concentrations. The magnitude of the positive effect increases with the frequency decrease in the Hz-region at 0.1% (v/v) (and 0.2% (v/v) results not shown) halothane. The low frequency part of the dispersion curves of halothane-treated PM

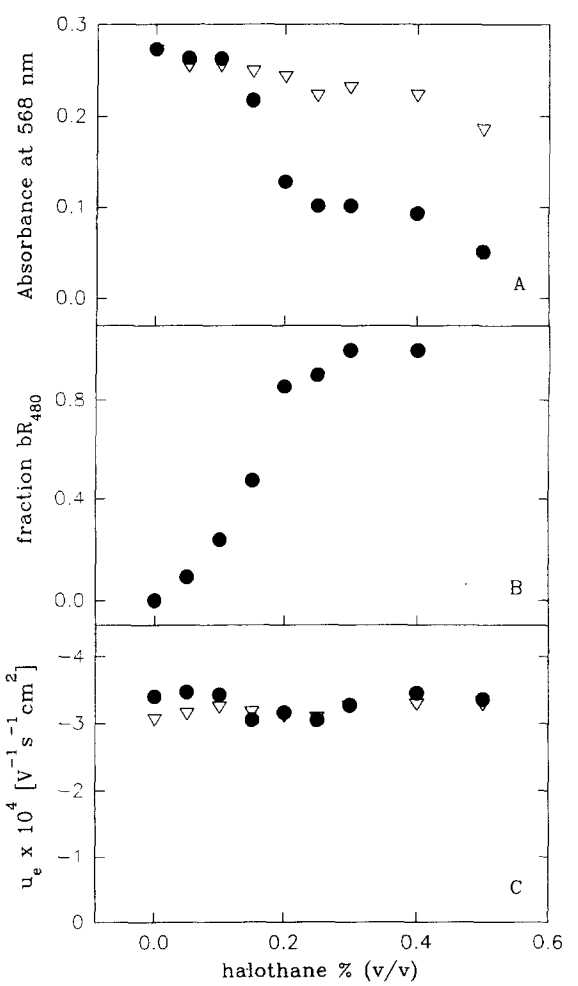

Fig. 5. Effect of increasing concentration of halothane (●) on the absorbance at 568 nm (A), the fraction of bR_{480} (B) and the electrophoretic mobility (C) of delipidated PM in 10 mM Tris-HCl, pH 8. Open symbols after the removal of the anesthetic.

(0.1% (v/v)) suggests that a slow polarizability component dominates particle orientation under these conditions. In the plateau region the magnitude of the effect decreases with the increase of anesthetic concentration. Correspondingly, the initial slope of the field dependence $\alpha(E^2)$, measured at a constant frequency of 1 kHz, becomes smaller in the presence of anesthetic (Fig. 4).

3.3. Influence of membrane delipidation on the anesthetic-induced absorbance and electrophoretic mobility changes

The effect of anesthetics on the spectral features of delipidated PM is qualitatively similar to that on native membranes, namely an increase in the absorbance at 480 nm and a decrease at 560 nm (Fig. 5A). However, the conversion of bR_{568} to bR_{480} takes place at higher anesthetics concentrations (Fig. 5B). This reflects a somewhat higher stability of lipid-depleted purple membranes to the action of anesthetics on the bR light absorbing properties. Similar effects require about twice as much halothane in delipidated than in native PM. However, an appreciable difference between purple and delipidated membranes is the negligible change in the electrophoretic mobility of DL-PM (Fig. 5C). Thus, after removal of a large fraction of the membrane lipids the anesthetic-induced spectral transition is not associated to an increase in electrophoretic mobility as in the case of the native membranes.

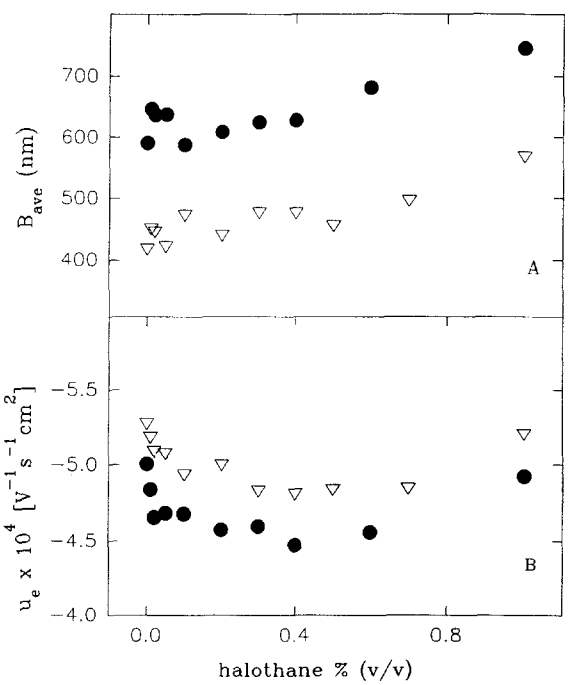

Fig. 6. Variation of the average hydrodynamic diameter (A) and the electrophoretic mobility (B) of multilamellar liposomes from total PM lipid extract with halothane concentration. 10 mM Tris-HCl buffer pH 6.5 (●) and pH 8 (▽); phospholipid concentration 0.25 mM.

3.4. Electrophoretic mobility of halothane-treated liposomes

The electrophoretic mobility and the hydrodynamic diameter of negatively charged multilamellar vesicles of total PM lipids were measured under identical experimental conditions as purple membranes. The electrophoretic mobility of the lipid vesicles drops upon addition of halothane up to 0.3% (v/v), reaches a plateau in the concentration range 0.3–0.8% (v/v) and increases after addition of 1% (v/v) halothane (Fig. 6B). Vesicle diameter is found to increase with increasing halothane concentrations (Fig. 6A). Therefore, halothane induces changes in the electrophoretic mobility of the lipid vesicles in the opposite direction to that observed for native purple membranes. The decrease in u_e of the lipid vesicles is estimated to correspond to a decrease in the negative charge of the liposomes from $-3.79\ \mu C/cm^2$ to $-3.03\ \mu C/cm^2$ upon addition of 0.4% (v/v) halothane. This result is in agreement with the previously suggested decrease in the surface charge density of liposomes induced by anesthetics [1,7].

4. Discussion

Electrostatic interactions of the positively charged Schiff base with its counterion environment (charged and polar amino acid residues and water molecules) stabilize the spectral properties of the pigment in the ground state [28–30]. Previous studies showed that a large shift of bR absorption maximum to 480 nm can be induced by a number of treatments: general and local anesthetics [12,13,15], high temperature [31], organic solvents [32], dimethyl sulfoxide [33], membrane dehydration [34,35] and in bR variants with substituted Trp 182, Pro 186 [36–38]. The covalent linkage between the helices A and B has been shown to be important for the equilibrium between the 560 nm and 480 nm pigment forms in reconstituted protein [39].

In an attempt to correlate the chromophore spectral features with the membrane electric properties we studied the electrokinetic and electrooptical behaviour of PM upon modification of the pigment visual absorbance induced by anesthetics in native and delipidated membranes.

The first abrupt increase in u_e of native PM (Fig. 1B) may be explained through the partitioning of anesthetic molecules at the lipid/protein interface, near the membrane surface, and the corresponding perturbation of lipid–protein interactions, i.e., a change in the protein conformation and/or the arrangement of the lipid polar head groups, leading to the exposure of additional charged residues. Alternatively, a shift of the slipping plane towards the membrane surface connected with changed hydrodynamics in presence of anesthetics could be the reason for the observed increase in the electrophoretic mobility.

The change in the sign and the increase of the electro-

optical effect with decreasing frequencies (the Hz-region of the frequency dependence (Fig. 3)) indicate a slow polarization process in the Stern layer, induced by anesthetics. The decrease in the magnitude of the electro-optical effect in the kHz-region indicates a smaller induced dipole moment of anesthetic-treated PM and reflects a variation of the polarization of the diffuse double layer ions. These results support the concept of anesthetic adsorption at the membrane/water interface [1,40].

While anesthetics induce the spectral transition after lipid depletion of PM, only minor changes in the electrophoretic charge (in the opposite direction than those in native PM) are found (Fig. 5). Therefore, the removal of lipids from the intertrimeric space [41] stabilizes the membranes towards the action of anesthetics. This suggests that the lipid–protein and intertrimeric interactions, affected by lipid removal, are important for the perturbing effect of anesthetics. This result is in agreement with the previous finding that the aggregation state and packing of bR molecules as well as the lipid composition affect the formation of the 480 nm pigment [14]. Moreover, the different extent to which lipid removal affects the anesthetic-induced spectral and surface charge changes suggests that different mode(s) or site(s) of interaction are responsible for the color and surface charge changes.

A further indication of the lipid/protein interface as a site of action for the anesthetic molecules comes from the observed change of the electrokinetic charge of lipid vesicles in opposite direction to that of PM. Dehydration and/or decrease in the dielectric constant can lead to the decrease in the negative charge of the lipid vesicles. Taking into consideration the model for the arrangement of the lipid polar head groups in two layers in PM as proposed by Kates [42], we suggest that partitioning of anesthetic molecules at the lipid/protein interface leads to an irreversible rearrangement of the lipid head groups and exposure of the phosphate groups located closer to the hydrophobic interior.

Halogenated anesthetics possess a strong hydrogen-bond breaking activity and can form anesthetic/water dimer complexes either as proton donors or as acceptors [43,44]. Thus it is reasonable to assume that anesthetic interaction with PM may affect the hydrogen bonding networks thought to stabilize the protein conformation [32]. Adsorption of anesthetic molecules at the membrane/water interface might affect the structure of bound water and induce an effect similar to dehydration. Considering that the lipid areas of PM are predominantly hydrated [45] it seems possible that water molecules can be displaced mainly from the lipid surface domain, and thus may account, through changes in surface conductivity, for the difference in the electrophoretic mobility variation induced by anesthetics in native and delipidated membranes. The present results suggest that dehydration of the membrane surface by anesthetic adsorption at the membrane/water interface might take place and above a certain anesthetic concentration this could result in a decrease of the membrane surface charge.

In conclusion during the anesthetic-induced spectral transitions $bR_{568} \rightarrow bR_{480} \rightarrow bR_{380}$ the PM negative surface charge: (i) increases by ca. 0.6 electric charges/bR upon the complete conversion of bR_{568} to the red absorbing species bR_{480}, (ii) decreases under the $bR_{480} \rightarrow bR_{380}$ spectral transition induced by halothane, (iii) it is virtually unchanged after partial removal of the membrane lipids. In liposomes from PM total lipids, the electrokinetic charge is altered in the opposite direction to that of PM fragments. These effects appear to be caused by the binding of anesthetic molecules at the lipid/protein interface, and may be due to a partial dehydration of the PM surface by anesthetics.

Acknowledgements

S.G.T. acknowledges a NATO grant. This work has been supported in part by DGICYT, grant No. PB91-0441.

References

[1] Ueda, I. (1991) in Drug and Anesthetic Effects on Membrane Structure and Function (Aloia, R.C., Curtain, C.C. and Gordon, L.M., eds.), pp. 1–33, Wiley-Liss, New York.
[2] Fesik, S.W. and Makkiyannis, A. (1985) Mol. Pharmacol. 27, 624–629.
[3] Dickinson, R., Franks, N.P. and Lieb, W.R. (1993) Biophys. J. 64, 1264–1271.
[4] Eckenhoff, G. and Shuman, H. (1993) Anesthesiology 79, 96–106.
[5] Yoshida, T., Mori, T. and Ueda, I. (1983) J. Coll. Interface Sci. 96, 39–47.
[6] Yoshida, T., Kamaya, H. and Ueda, I. (1983) J. Coll. Interface Sci. 96, 48–54.
[7] Yoshida, T., Kamaya, H. and Ueda, I. (1985) J. Coll. Interface Sci. 105, 129–135.
[8] Henderson, R. and Unwin, P.N.T. (1975) Nature (London) 257, 28–32.
[9] Blaurock, A.E. and Stoeckenius, W. (1971) Nature (London) New Biol. 233, 152–155.
[10] Jonas, R., Koutalos, Y. and Ebrey, T.G. (1990) Photochem. Photobiol. 52, 1163–1177.
[11] Oesterhelt, D. and Stoeckenius, W. (1971) Nature (London) New Biol. 233, 149–152.
[12] Nishimura, S., Mashimo, T., Hiraki, K., Hamanaka, T., Kito, Y. and Yoshiya, I. (1985) Biochim. Biophys. Acta 818, 421–424.
[13] Henry N., Beaudoin, N., Baribeau, J. and Boucher, F. (1988) Photochem. Photobiol. 47, 85–90.
[14] Uruga, T., Hamanaka, T., Kito, Y., Uchida, I., Nishimura, S. and Mashimo, T. (1991) Biophys. Chem. 41, 157–168.
[15] Messaoudi, S., Lee, K.-H., Beaulieu, D., Baribeau, J. and Boucher, F. (1992) Biochim. Biophys. Acta 1140, 45–52.
[16] Nakagawa, T., Hamanaka, T., Nishimura, S., Uruga, T. and Kito, Y. (1994) J. Mol. Biol. 238, 297–301.
[17] Oesterhelt, D. and Stoeckenius, W. (1974) Methods Enzymol. 31, 667–678.
[18] Szundi, I. and Stoeckenius, W. (1987) Proc. Natl. Acad. Sci. USA 84, 3681–3684.

[19] Kates, M., Yengoyan, L.S. and Sastry, P.S. (1965) Biochim. Biophys. Acta 98, 252–268.
[20] Shaw, D. (1969) Electrophoresis, Academic Press, New York.
[21] Henry, D.C. (1931) Proc. R. Soc. Lond. Ser. A 133, 106–129.
[22] Stoylov, S.P., Shilov, V.N., Dukhin, S.S., Sokerov, S. and Petkanchin, I. (1977) Electrooptika Kolloidov, Naukova Dumka, Kiev.
[23] Todorov, G., Sokerov, S. and Stoylov, S.P.S. (1982) Biophys. J. 40, 1–4.
[24] Lee, K.-H., McIntosh, A.R. and Boucher, F. (1991) Biochem. Cell Biol. 69, 178–184.
[25] Packer, L., Arrio, B., Johannin, G. and Volfin, P. (1984) Biochem. Biophys. Res. Commun. 122, 252–258.
[26] Ehrenberg, B., Ebrey, T.G., Friedman, N. and Sheves, M. (1989) FEBS Lett. 250, 179–182.
[27] Renthal, R. and Cha, C.-H. (1984) Biophys. J. 45, 1001–1006.
[28] De Groot, H., Harbison, G., Herzfeld, J. and Griffin, R. (1989) Biochemistry 28, 3346–3353.
[29] Henderson, R., Baldwin, J.M., Ceska, T.A., Zemlin, F., Beckman, E. and Dowing, K.A. (1990) J. Mol. Biol. 213, 899–929.
[30] Papadopoulos, G., Dencher, N.A., Zaccai, G. and Buldt, G. (1990) J. Mol. Biol. 214, 15–19.
[31] Fischer, U. and Oesterhelt, D. (1979) Biophys. J. 28, 211–230.
[32] Mitaku, S., Ikuta, K., Itoh, H., Kataoka, R., Naka, M., Yamada, M. and Suwa, M. (1988) Biophys. Chem. 30, 69–79.
[33] Pande, C., Callender, R., Henderson, R. and Pande, A. (1989) Biochemistry 28, 5971–5978.
[34] Lazerev, Y. and Terpugov, E. (1980) Biochim. Biophys. Acta 590, 324–338.
[35] Hildebrandt, P. and Stockburger, M. (1984) Biochemistry 23, 5539–5548.
[36] Hacket, N.R., Stern, L.J., Chao, B.H., Kronis, K.A. and Khorana, H.G. (1987) J. Biol. Chem. 262, 9277–9284.
[37] Mogi, T., Stern, L.J., Chao, B.H. and Khorana, G.H. (1989) J. Biol. Chem. 264, 14192–14196.
[38] Mogi, T., Marti, T. and Khorana, G.H. (1989) J. Biol. Chem. 264, 14197–14201.
[39] Tsujiuchi, Y., Engelman, D.M. and Tokunaga, F. (1992) Photochem. Photobiol. 56, 895–901.
[40] Yoshida, T., Okabayashi, H., Kamaya, H. and Ueda, I. (1989) Biochim. Biophys. Acta 979, 287–293.
[41] Glaeser, R.M., Jubb, J.S. and Henderson, R. (1985) Biophys. J. 48, 775–780.
[42] Kates, M. (1990) in Glycolipids, Phosphoglycolipids, and Sulfoglycolipids, pp. 93–102, Plenum Press, New York.
[43] Sandorfy, C. (1978) Anesthesiology 48, 357–359.
[44] Hobza, P., Mulder, F. and Sandorfy, S. (1982) J. Am. Chem. Soc. 104, 925–928.
[45] Rogan, P.K. and Zaccai, G. (1981) J. Mol. Biol. 145, 281–284.