

728—THE ION-PAIR CONCEPT IN MACROMOLECULAR DYNAMICS *

E. NEUMANN, K. TSUJI and D. SCHALLREUTER

Physical and Biophysical Chemistry, University of Bielefeld, P.O. Box 8640, D-4800 Bielefeld 1 (F.R.G.)

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SUMMARY

Ion-pairing appears to be a functionally essential interaction element in macromolecular structures such as proteins, nucleic acids and membranes. Recent progress in understanding bioelectric field effects in macromolecular and cellular organisations is summarized in terms of the ion-pair concept. A rigorous general treatment of electric field effects in dipolar chemical systems is outlined in terms of a dielectrochemical potential.

INTRODUCTION

The dynamic behavior of biological macromolecules and membranes in electric fields appears to involve a special electrostatic interaction element: ion-pairing. Associations of cations and anions are well known in strong electrolytes or in the counterion association of linear polyelectrolytes and polyelectrolyte complexes such as the various nucleic acids.

In the case of membrane proteins, cation–anion pair configurations may be of particular functional relevance, especially for the ion flow gating proteins in excitable membranes. The fixed ionic groups and dipoles of membrane components are the direct preferred interaction partners of the electric field of biological membranes. In this context it is recalled that all biomembranes under *in vivo* conditions appear not only to have an electric potential difference (membrane potential) but also to experience changes of this electrical property. The electric potential difference is equivalent to an electric field across the membrane components. The action potential of nerve excitation represents a transient change of the electric membrane field in magnitude and direction from approximately $+70 \text{ kV cm}^{-1}$ to -40 kV cm^{-1} , *i.e.* in the opposite direction [1].

A further example of functionally important changes in the electric membrane field is encountered in the hyperpolarizing increase of the absolute value of the

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membrane potential during light-induced proton pumping by bacteriorhodopsin in halobacteria; this increase in the membrane field is concomitant with a decrease in the proton transport [2]. There is a variety of epiphenomena where structure and function of membrane components appear to be strongly coupled to the electric field of biological membranes. Indeed the presence of a membrane field, *i.e.* membrane polarization, is vital; prolonged depolarization of the plasma membrane causes cell death.

Experimental progress in chemical relaxation kinetics in the presence of high electric fields [3] has brought new information on ion-pairing processes in simple inorganic salts and in linear polyelectrolytes [4] as well as on membrane transport proteins [5–7]; these advances will be briefly summarized.

EXPERIMENTAL

We have developed an electric field-jump relaxation spectrometer that permits the simultaneous measurements of field-induced changes in electric conductance and in optical properties of solutions in the ns–ms time range with high resolution [3,4]. Rectangular field pulses up to 100 kV cm^{-1} and of variable pulse length ($0.5 \mu\text{s}$ –1 ms) can be applied by cable discharge in a single pulse mode to solutions and suspensions.

RESULTS AND DISCUSSION

Ion pairs in MgSO_4 solutions

The electric conductance relaxation spectrum, as response to a rectangular field pulse, of an aqueous MgSO_4 solution exhibits a rapid ($0.1 \mu\text{s}$) part and a slow phase in the $1 \mu\text{s}$ time range. The slow increase in conductance represents a discrete normal mode, depending on the salt concentration and on the electric field strength. The electric data are consistent with familiar concepts of ionic atmosphere relaxation and of the Onsager field dissociation effect. The analysis of the completely analyzable slow relaxation mode yields an electric dipole moment (~ 50 Debye) which is consistent with the presence of the outer-sphere complex $\text{Mg}^{2+} \cdot \text{H}_2\text{O} \cdot \text{SO}_4^{2-}$: an ion-pair involving at least one H_2O dipole shell between the ionic charge carriers of the ion-pair dipole [3,4]. Since the treatment of polarizable dipoles in polar solvents is intricate and notoriously difficult, the experimental determination of dipole moments of ion-pairs in aqueous solution represents an important fundamental information. At present the Fröhlich–Kirkwood approximation for dipolar species in polar solvents like water is experimentally tested.

Counterion flow coupling in polyelectrolytes

The simultaneous measurements of electric conductance and U.V. electric dichroism of the linear polyelectrolyte poly(riboadenylate, K^+) have shown that:

(1) The K^+ -counterions of the inner ionic atmosphere of poly(rA, K^+) dissociate from the polyion with the same rate as the rod-like ends and the middle part of the polyelectrolyte rotate into the field direction;

(2) counterion dissociation induces increased destacking of the adenine bases of which the rate is also limited by the orientation process;

(3) rotation rate and amplitudes of orientation, of counterion dissociation, and of base destacking strongly decrease with increasing concentration of free counterions (KCl);

(4) *end effects* are important and counterion-polyion polarization causes a large induced dipole moment which is the origin of the orientation processes.

The electrooptical data of poly(rA, K^+) can be described consistently in terms of a flow concept developed by Katchalsky and Neumann [8,9], involving saturation in the counterion polarization (electro-diffusion limit) and concentration effects in terms of a simple mass action formalism [3,4].

According to the coupled flow concept the external electric field, E , causes a counterion flow $J_{pol} = v[c^+]_{pol}$ along the polyion; where $v = u_{pol} \cdot E$ is the velocity, u_{pol} is the mobility and $[c^+]_{pol}$ the local concentration of counterions in the inner (condensed) counterion layer. The counterion polarization and the large induced-dipole moment finally result from flow imbalances at the two ends of the polyelectrolyte rod.

The polarization flow J_{pol} causes a local depletion of counterions at one end of the rod (negative dipole charge) and a local accumulation at the other end (positive dipole charge). The local depletion range gives rise to an influx $J_- = v[c^+]$ of free counterions ($[c^+] \leq 1 \text{ mM}$) from the solution. Because $[c^+] < [c^+]_{pol}$, the negative dipole charge is caused by the flow difference $\Delta J_- = v([c^+] - [c^+]_{pol})$. On the other hand the positive dipole charge arises from $\Delta J_+ = v([c^+]_{pol} - [c^+])$ because the outflow $J_+ = v[c^+]$ is not balanced by the local influx J_{pol} into the accumulation range.

When $[c^+]$ is increased (by KCl addition) all processes which are coupled to counterion polarization, *i.e.* orientation, counterion dissociation and base-destacking are reduced in electric fields. In the limiting case, when $[c^+] = [c^+]_{pol}$, neither polarization, nor any electrooptical or chemical-conformational reaction flows appear.

The anisotropy of the counterion movement along polyionic surfaces suggests that counterion exchange as well as influx and efflux of counterionic substrates or hormones occur preferably at the border line of the ionic atmospheres which cover polyionic regions on macromolecular enzyme and receptor proteins and membranes. Once part of the ion cloud, such substrates and activator substances may reach the active sites *via* surface diffusion [10,11].

In this model, the border regions of the counterion atmosphere serve as a preferable cross section for trapping counterionic substrates, probably in diffuse ion-pair configurations which, coupled to surface diffusion, will appreciably accelerate the effective rates of counterionic ligand binding.

Membrane proteins in electric fields

Bacteriorhodopsin of purple membrane fragments

An instructive example for the action of electric fields on membrane proteins and ion transport processes is the bacteriorhodopsin of the purple membranes of halobacteria. The light-driven proton pump activity of this protein appears to be feedback regulated by a hyperpolarizing increase in the electric membrane field [6].

Recent electrooptical data of aqueous suspensions of purple membranes demonstrate that high electric field pulses ($1-30 \times 10^5 \text{ V m}^{-1}$, $1-100 \mu\text{s}$) cause structural transitions in two kinetically different phases. The rapid mode ($\approx 1 \mu\text{s}$) represents a concerted change in the orientations of both the retinal and some tyrosine and/or tryptophan side chains concomitant with alterations of the local protein environment of these chromophores. The slower mode ($\approx 100 \mu\text{s}$) involves changes in the micro-environment of aromatic amino acids at fixed chromophore orientations and in the pK values of at least two types of proton binding sites leading to a sequential uptake and release of protons [6].

Electric gating of ion transport

The dependence of the electrooptical relaxation amplitudes of purple membrane suspensions on the field strength suggests a saturable induced dipole moment mechanism [5,7]. The electric field is apparently able to increase, in a strongly cooperative manner, the mean distance between positively and negatively charged amino acid side groups (ion-pairs) so that the mean dipole moment increases (Fig. 1). The kinetics of the electrooptical changes and the steep field dependence indicate a rather large reaction dipole moment, $\sim 1.1 \times 10^{-25} \text{ C m}$ ($= 3.3 \times 10^4$ Debye) per cooperative unit at a field strength of $1.3 \times 10^5 \text{ V m}^{-1}$ (which is the transition midpoint) [7].

These results not only suggest a possible control function of the electric field of the bacterial membrane during the photocycle of bacteriorhodopsin, but also a

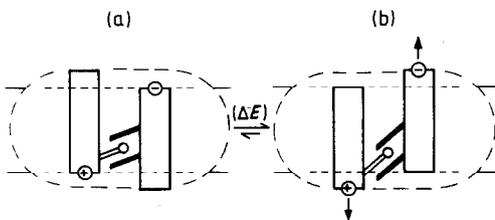


Fig. 1. Principle of the saturable induced-dipole mechanism causing positional changes of side chains in helical membrane proteins. In bacteriorhodopsin helical parts with different net charge may move transversal to the membrane plane in opposite directions when the electric membrane-field is increased, (a) \rightarrow (b). The geometrically limited increase in the distance of the charge centers is equivalent to a saturable induced-dipole moment [7]. The transversal displacement of at least one of the two helical parts can thereby cause a concerted rotational shift of the retinal (=O) and of aromatic amino acid side chains which may sandwich (T.H. Haines) the retinal chromophore.

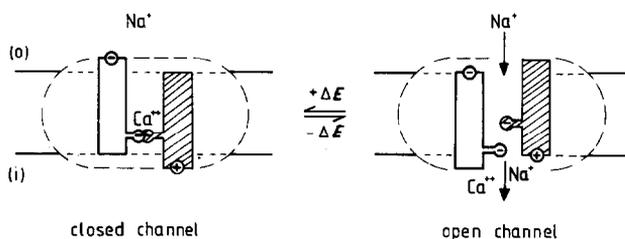


Fig. 2. Model for a gating element of the axonal Na^+ channel. At high electric field (resting membrane potential) between the outside (O) and the inside (i) of the excitable membrane, the gating protein is in the closed conformation. Ca^{2+} ions are bound to anionic amino acid side chains of helical parts kept in a high dipole moment configuration (large distance between the cationic and anionic groups) by the membrane field. Depolarization reduces the membrane's electric field which, in turn, reduces the electric polarization by transversal movements of the helical parts, decreasing the distance between the fixed charges. The Ca^{2+} ion of the former bridge is exchanged for Na^+ ions which flow through the open, field-relaxed conformation into the interior of the cell.

possibly general, induced dipole mechanism for electric field dependent structural changes in membrane transport proteins such as the gating proteins in the excitable membranes of nerve and muscle cells. By analogy with the scheme of Fig. 1, we may suggest an induced dipole gating mechanism for the axonal Na^+ channel in a highly schematic form (Fig. 2), also incorporating $\text{Ca}^{2+}/\text{Na}^+$ ion exchange [12].

Long-lived electric imprint into structure

A further kinetic aspect of the bacteriorhodopsin data may also be of general fundamental importance. Compared to the rapid induction of the electric field-mediated structural changes in the purple membranes (μs range), annealing of the changes after the electric impulse is very slow. The field-induced conformational transitions in bacteriorhodopsin are thus long-lived compared to the pulse duration; these transitions therefore exhibit memory properties.

Longevity of field-induced structural changes may also offer a basis for the interpretation of ATP formation by thylakoid membranes [13,14] and by sub-mitochondrial particles [15] after the exposure to electric fields. When the lifetime of the electric field-induced ATPase activation (to synthesize ATP) is greater than the pulse duration, *after-field effects* may occur in a way similar to that of the after-field pH changes [6] observed in bacteriorhodopsin of purple membranes. The electric field-induced energization [16] of ATP synthetase would thus be caused by an electric field-induced, long-lived structural transition to the active enzyme conformation.

It may be recalled that electric field-induced, long-lived structural changes have been suggested as a mechanism for the initial, physical step of biological memory recording [8].

Thermodynamics of chemical transformations in electric fields

Structural transitions of the type $B_0 \rightleftharpoons B_1$, induced by an electric field E at

constant pressure p and Kelvin temperature T may be described by [17]:

$$\left(\frac{\partial \ln K}{\partial E}\right)_{p,T} = \frac{\Delta M}{RT} \quad (1)$$

where $K = [B_1]/[B_0]$ is the apparent equilibrium constant (concentration ratio of the conformers), R is the gas constant and $E = |E|$. The apparent molar reaction moment ΔM is the difference between the components of the molar (permanent and induced) dipole moments parallel to the field of the conformers: $\Delta M = M_1 - M_0$. Thus $\Delta M \neq 0$, if $M_1 \neq M_0$.

In terms of the degree of transition $\Theta = [B_1]/([B_1] + [B_0])$ and with $K = \Theta/(1 - \Theta)$ equation (1) leads to:

$$\left(\frac{\partial \Theta}{\partial E}\right)_{p,T} = \frac{\Theta(1 - \Theta)\Delta M}{RT} \quad (2)$$

With $\bar{\epsilon}_0$ and $\bar{\epsilon}_1$ being the (average) molar absorption coefficients of the conformers B_0 and B_1 , respectively, the chemical transition may be measured as an absorbance change [5,7]. Per cm light path, $\Delta A^{(ch)} = (\bar{\epsilon}_1 - \bar{\epsilon}_0)c\Delta\Theta$, where $c = [B_1] + [B_0]$ is the total concentration of B. It is now readily shown that at a given field strength,

$$\Delta M = \frac{RT(1 - \Theta^\circ)}{\Theta(1 - \Theta)} \left(\frac{\partial(\Delta A/\Delta A_s)}{\partial E}\right)_{p,T} \quad (3)$$

where Θ° refers to $E = 0$ and ΔA_s is the saturation value at $\Theta = 1$.

Dielectrochemical affinity

Chemical electric field effects of dipole equilibria can be treated rigorously in terms of the dielectrochemical potential of the dipolar charge configuration B_j with the dipole moment

$$\mathbf{m}_j = |z_j|e\mathbf{l}_j \quad (4)$$

where e is the (positive) elementary charge, z_j is the charge number of one of the charges of the dipole and \mathbf{l}_j is the charge distance vector. In a macromolecule, oppositely charged groups may be grouped together into ion-pairs. The total dipole moment, \mathbf{M} , is then the vector sum over all the individual contributions \mathbf{m}_j , of the various ion pairs.

For plate condenser geometries the measurable polarization \mathbf{M} is the parallel component of the total macroscopic dipole component $\mathbf{M} = \mathbf{P} \cdot \mathbf{V}$, where \mathbf{P} is the polarization per unit volume and \mathbf{V} is the volume. It is obvious that \mathbf{M} is given by the statistical average $\langle \mathbf{m}_j \cos \vartheta_j \rangle$ of \mathbf{m}_j according to

$$\mathbf{M} = (\mathbf{M})_{\parallel} = N_A \sum_j n_j \langle \mathbf{m}_j \cos \vartheta_j \rangle = N_A \sum_j n_j \mathbf{M}_j \quad (5)$$

where N_A is the Avogadro-Loschmidt constant, n_j is the amount (mol) of substance j , \mathbf{M}_j is the average partial molar dipole moment given by

$$\mathbf{M}_j = \left(\frac{\partial \mathbf{M}}{\partial n_j}\right)_{n \neq n_j} = N_A \langle \mathbf{m}_j \cos \vartheta_j \rangle \quad (6)$$

and ϑ_j is the angle between the dipole vector m_j and the electric field [18].

The molar reaction moment in equation (2) for the chemical change ($0 = \sum \nu_j B_j$) at constant T and p and at a given field strength E is defined by

$$\Delta M = \left(\frac{\partial M}{\partial \xi} \right)_{E,p,T} = \sum_j \nu_j M_j \quad (7)$$

where ν_j is the stoichiometric coefficient (with sign) of species B_j and $d\xi = dn_j/\nu_j$ is the differential DeDonder advancement (mol) of the reaction.

The derivation of equation (1) requires the introduction of Guggenheim's characteristic Gibbs function [19] defined as the difference

$$\tilde{G} = G - EM \quad (8)$$

of the ordinary Gibbs free energy at E and the electric work $\int dw_{el} = \int M dE = ME$.

With E_{in} and S denoting the inner energy and the total entropy in the field, equation (8) is rewritten as [18]:

$$\tilde{G} = E_{in} - TS + pV - EM \quad (9)$$

The general Gibbs equation for chemical changes in electric fields is:

$$dE_{in} = TdS - pdV + \sum_j \tilde{\mu}_j dn_j + EdM \quad (10)$$

where $\tilde{\mu}_j$ denotes the *chemical potential* of species B_j in the field. Equations (9) and (10) then yield

$$d\tilde{G} = dG - d(EM) = -SdT + Vdp + \sum_j \tilde{\mu}_j dn_j - MdE \quad (11)$$

At constant p , T equation (11) reduces to

$$d\tilde{G} = \sum_j \tilde{\mu}_j dn_j - MdE \quad (12)$$

Consistent with ordinary thermodynamics we obtain from equation (12):

$$\tilde{\mu}_j = \left(\frac{\partial \tilde{G}}{\partial n_j} \right)_{p,T,E,n \neq n_j} \quad (13)$$

where all n except n_j are constant. By cross differentiation equation (12) yields

$$\left(\frac{\partial \tilde{\mu}_j}{\partial E} \right)_{p,T} = - \left(\frac{\partial M}{\partial n_j} \right)_{p,T,E,n \neq n_j} = -M_j \quad (14)$$

where equation (6) was applied to obtain the partial molar dipole moment M_j .

According to Kirkwood and Oppenheim [21], integration of equation (14) between E and $E = 0$ yields

$$\tilde{\mu}_j = \mu_j - \int M_j dE \quad (15)$$

where $\tilde{\mu}_j(0) = \mu_j$ is the ordinary chemical potential at $E = 0$

By analogy with Guggenheim's electrochemical potential we may call $\tilde{\mu}_j$ the *dielectrochemical potential* [11,18] of the dipolar species. Similar to a previous treatment [18] we introduce a standard dielectrochemical potential $\tilde{\mu}_j^\ominus$ and write

$$\tilde{\mu}_j = \tilde{\mu}_j^\ominus + RT \ln \tilde{a}_j \quad (16)$$

where $\tilde{a}_j = c_j \tilde{y}_j$ is the activity and \tilde{y}_j the activity coefficient *in the field*; evidently $\tilde{\mu}_j^\ominus = \mu_j^\ominus - \int M_j^\ominus dE$ [18].

For dipolar interaction partners (according to $0 = \sum \nu_j B_j$) in electric fields, a *dielectrochemical affinity* is defined by

$$\tilde{\mathcal{A}} = - \sum \nu_j \tilde{\mu}_j = - \sum \nu_j \tilde{\mu}_j^\ominus - RT \sum \nu_j \ln \tilde{a}_j \quad (17)$$

The characteristic Gibbs function for isothermal–isobaric chemical changes is then derived from equations (12) and (17),

$$d\tilde{G} = -\tilde{\mathcal{A}}d\xi - MdE \leq 0 \quad (18)$$

and, consistent with general thermodynamics:

$$\tilde{\mathcal{A}} = - \left(\frac{\partial \tilde{G}}{\partial \xi} \right)_{p, T, E} \geq 0 \quad (19)$$

yielding familiar expressions for reversible (equilibrium) processes ($d\tilde{G} = 0$, $\tilde{\mathcal{A}} = 0$) and for irreversible (non-equilibrium) processes ($d\tilde{G} < 0$, $\tilde{\mathcal{A}} > 0$). Note that the ordinary Gibbs free energy $G = \tilde{G} + EM$ increases in the presence of E (!).

Using equation (17) and the definition of the thermodynamic equilibrium constant $K^\ominus = \Pi \tilde{a}_j^{\nu_j} = K \cdot \bar{Y}$ with $K = \Pi c_j^{\nu_j}$ (concentration ratio) and $\bar{Y} = \Pi \tilde{y}_j^{\nu_j}$ together with the non-equilibrium ratio $Q^\ominus = \Pi a_j^{\nu_j}$ we obtain

$$\tilde{\mathcal{A}} = RT(\ln K^\ominus - \ln Q^\ominus) \quad (20)$$

where $RT \ln K^\ominus = -\sum \nu_j \tilde{\mu}_j^\ominus$; the bar denotes equilibrium values of c_j and y_j . By analogy with the treatment of Schwarz [20] it can be shown that, using the equilibrium criteria $d\tilde{G} = 0$ and $\tilde{\mathcal{A}} = 0$, equations (18) and (20) yield, respectively,

$$\left(\frac{\partial \tilde{\mathcal{A}}}{\partial E} \right)_\xi = \left(\frac{\partial M}{\partial \xi} \right)_E = \Delta M \quad (21)$$

and, provided that the dependence of \tilde{Y} on ξ is negligible, equation (1) obtains.

Generally, the van't Hoff relationship for electric field effects on chemical processes is

$$\left(\frac{\partial \ln K^\ominus}{\partial E} \right)_{p, T} = \frac{\Delta M^\ominus}{RT} \quad (22)$$

where

$$\Delta M^\ominus = \Delta M - RT \left(\frac{\partial \ln \tilde{Y}}{\partial E} \right)_{\xi, p, T} \quad (23)$$

derived from $\int (M_j - M_j^\ominus) dE = -RT \ln \tilde{y}_j$ with $\tilde{y}_j = \tilde{a}_j/a_j$; a_j being associated with μ_j (at $E = 0$) according to $\mu_j = \mu_j^\ominus + RT \ln a_j$ [18].

For computer analysis of actual chemical transition curves $\Theta(E)$, see equation (2), it is useful to apply the integrated form of equation (22):

$$K^{\ominus}(E) = K^{\ominus}(0) \exp \left[\frac{\int \Delta M dE}{RT} \right] \quad (24)$$

Equation (24) relates concentration (activity) shifts induced by the presence of an electric field to the change in the molar reaction dipole moments. The value of ΔM^{\ominus} and (from its dependence on E) also the mechanism of the dipole-field interaction can thus be determined.

The thermodynamic formalism outlined here was used to analyze the electric field effects observed in the proton transport protein bacteriorhodopsin of purple membrane fragments. It has been found that this protein is particularly sensitive to the electric field of the membrane. The apparent coupling of the light-induced proton-pumping cycle to the membrane field [2,6] represents an interesting bioelectric phenomenon which may be partially investigated *in vitro*. In summary, the study of bioelectric structures and processes on the molecular level of chemistry and physics may thus be classed as one of the efforts [22] to "translate" (bioelectric) life phenomena into meaningful physical concepts.

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