Biochimica et Biophysica Acta, 465 (1977) 421—425

BBA Report

BBA 71288

ION TRACER FLOWS AND FLUX RATIOS IN HETEROGENEOUS MEMBRANES

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(Received December 6th, 1976)

Summary

Ion tracer flows and flux ratios at various electrical potentials were studied in heterogeneous membranes comprising parallel pathways of different intrinsic resistance. The total resistance to net flow exceeded the tracer exchange resistance, and the flux ratio was abnormal, as in exchange diffusion commonly attributed to a mobile carrier.

In the study of membrane transport processes it is common to observe discrepancies between resistance coefficients determined from tracer exchange and net flow, as well as "abnormality" of the flux ratio [1]. These effects are commonly attributed either to "single file diffusion" through a narrow channel [2], or "exchange diffusion" by way of a mobile carrier [3]. From the point of view of non-equilibrium thermodynamics these are manifestations of isotope interaction (coupling of flows of abundant and tracer isotopic species). However, this is merely a phenomenological characterization, and in principle various mechanisms are possible [1,4]. We have recently demonstrated that for non-electrolyte flow in the absence of net volume flow, heterogeneity of parallel pathways can account for either positive or negative isotope interaction [5]. We show here that in the case of electrolyte flow, heterogeneity can result in negative isotope interaction, just as could be seen with a membrane carrier.

In the absence of net flow of the test substance and other species the tracer exchange resistance is given by [4]

$$R^* = (-\underline{R}T\Delta\rho/J^*)_{J=0} \tag{1}$$

where J^* and J are the tracer flow and net flow, respectively, $\Delta\rho$ is the difference in specific activity across the membrane, and $\underline{R}$ and T are the gas constant and absolute temperature. The phenomenological resistance coefficient for net flow is given by

$$R = X/J \tag{2}$$

where X is the negative electrochemical potential difference of the test substance. In the absence of isotope interaction $R^* = R$, and in homogeneous membranes the ratio of unidirectional fluxes is given by

$$f = \overrightarrow{J}/\overleftarrow{J} = \exp[JR/\underline{R}T] = \exp[X/\underline{R}T] \tag{3}$$

whereas in the presence of isotope interaction $R^* \neq R$, and the flux ratio is "abnormal", being given by

$$f = \exp[JR^*/\underline{R}T] = \exp[(R^*/R)(X/\underline{R}T)] \tag{4}$$

For membranes with significantly different resistances of parallel pathways, the use of conventional techniques to produce a transmembrane electrical potential difference $\Delta\psi$ will not always create an equipotential surface at the membrane. In the presence of a net flow, as is necessary to determine R, the potential differences across different pathways will in general vary. Since R^* is determined in the absence of potential difference, the effects of membrane heterogeneity on R^* and R may well differ, resulting in abnormality of the flux ratio.

In order to investigate the importance of such effects highly permselective heterogeneous membranes of low electroosmotic permeability were prepared from collodion membranes activated with quaternary ammonium polyelectrolyte(polyvinyl-benzyl-trimethyl ammonium chloride (PVBT, Dow Chemical)) [6—8] by two means: (a) pairs of PVBT membrane elements were assembled in series, employing elements with equivalent values of R^* and R (within 10%); the two elements differed markedly in resistance. (The perforation of the element of higher resistance then resulted in parallel channels of significantly different resistance.) (b) Collodion membranes were activated with PVBT for different periods peripherally and centrally, again resulting in parallel channels differing markedly in resistance. Both types of membranes showed a high degree of permselectivity for anions: membrane (a1) showed $R^*_{\mathrm{Cl}}/R^*_{\mathrm{Na}}$ to be <0.001; membrane (b1) developed nearly ideal Nernst potentials of 54 and 55 mV in a 0.10 M:0.01 M KCl concentration cell. Hence it was possible to identify net anion flow with the electrical current.

With 0.10 M KCl solutions at each surface R^* and R for Cl^- were evaluated according to Eqns. 1 and 2. In 3 membranes of type (a) values of R^*/R were 0.09, 0.11, and 0.60. In 2 membranes of type (b) values were 0.61 and 0.18. Lowering the KCl concentration to 0.01 M lowered R^*/R in membrane (b1) from 0.61 to 0.23, as was consistent with earlier findings [8]. Thus R^* was consistently appreciably less than R. (It should be recalled that in the two components of each type (a) membrane, $R^* \simeq R$.)

The inequality of R^* and R allowed us to test the compatibility of unidirectional fluxes and flux ratios with Eqns. 3 and 4. Unidirectional fluxes were determined from tracer fluxes at different values of $\Delta\psi$. As is seen in

TABLE I

Theoretical and observed flux ratios and ratios of influx in heterogeneous PVBT-collodion membranes.

(Preparation of the membrane elements involved casting of a porous collodion membrane, activation by adsorption of PVBT, and irreversible shrinking by controlled drying [6—8]. The dried membranes had a thickness of 50—75 μm. Elements were selected on the basis of mechanical strength, high permselectivity, low water permeability, and convenient electrical resistance.) The symbol f is the observed flux ratio, and $\exp[X/\underline{R}T]$ and $\exp[(R^*/R)(X/\underline{R}T)]$ are the theoretical values of the flux ratio predicted by Eqns. 3 and 4, respectively. $\vec{J}_{\Delta\psi}/\vec{J}_0$ is the observed ratio of the influx at electrical potential difference $\Delta\psi$ and zero, and $(X/\underline{R}T)/(1-\exp[-(X/\underline{R}T)])$ and $((R^*/R)(X/\underline{R}T))/(1-\exp[-(R^*/R)(X/\underline{R}T)])$ are the theoretical values of $\vec{J}_{\Delta\psi}/\vec{J}_0$ corresponding to Eqns. 3 and 4, respectively [8]. In the absence of a concentration difference $X = -zF\Delta\psi$, where z is the valence and $\Delta\psi = \psi^{\text{in}} - \psi^{\text{out}}$. Membranes of 6.1 cm^2 area were exposed at each surface to 0.1 M KCl, except for the use of 0.01 M KCl in the case of b1†. The "outer" solution (left side) contained tracer quantities of K^{36}Cl. The electrical potential difference $\Delta\psi$ was measured by means of calomel half cells and 3 M KCl-agar bridges. Ag/AgCl electrodes and a voltage clamp were used to set $\Delta\psi$ at desired values, correcting for solution resistance between the bridge tips and the plane of the membrane. DC electrical resistance R_e was determined from Ohm's law, transiently setting $\Delta\psi$ at ±10 mV. Values of R_e in the various membranes varied from 61 to 264 $\Omega\cdot$cm^2 (in 0.10 M KCl). Because the chloride transport number was >0.99, and electroosmosis was insignificant (water transport numbers were <2 mol$\cdot$equiv.$^{-1}$) [8], the phenomenological resistance coefficient was taken as $R = F^2 R_e$, where F is the Faraday. Unidirectional fluxes were taken as the mean value of $-J^*/\Delta\rho$ during three or four 10—15-min periods in three series of experiments, with $\Delta\psi$ zero, positive or negative. After each series the cold solutions were replaced. At $\Delta\psi = 0$, $J = 0$, permitting the calculation of R^* from Eqn. 1, whereas $(J^*/\Delta\rho)_{\Delta\psi+}$ was taken as the influx and $(J^*/\Delta\rho)_{\Delta\psi-}$ was taken as the efflux. This means of determining the influx and efflux is experimentally simpler than reversal of the hot and cold sides.

Membrane	R^*/R	$\Delta\psi$(mV)	$\exp[X/\underline{R}T]$	f	$\exp[(R^*/R)(X/\underline{R}T)]$	$\dfrac{X/\underline{R}T}{1-\exp[-(X/\underline{R}T)]}$	$\vec{J}_{\Delta\psi}/\vec{J}_0$	$\dfrac{(R^*/R)(X/\underline{R}T)}{1-\exp[-(R^*/R)(X/\underline{R}T)]}$
a1	0.09	−25				0.59	0.96	0.96
		25	2.65	1.09	1.09	1.57	1.05	1.05
		50				2.27	1.13	1.09
b1	0.61	−25				0.59	0.73	0.72
		25	2.65	1.78	1.81	1.57	1.31	1.33
		50				2.27	1.64	1.72
b1†	0.23	−50				0.32	0.77	0.79
		50	7.02	1.72	1.57	2.27	1.32	1.24

Table I, the flux ratio f, abnormal according to Eqn. 3, was consistent with Eqn. 4. Similarly, $\vec{J}_{\Delta\psi}/\vec{J}_0$, the ratio of influx at values of applied electrical potential difference $\Delta\psi$ and zero, was adequately predicted only by the expression incorporating the influence of isotope interaction.

Without knowing the exact geometrical and electrical properties of a membrane it is not possible to derive its transport properties precisely. However, it may be possible to explain the effects of membrane heterogeneity on R^*, R, and the flux ratio in qualitative terms. In the determination of R^* from exchange diffusion in the absence of electrical forces, various pathways will contribute to tracer flow in inverse proportion to their intrinsic resistance, and low resistance pathways will be predominant. In the determination of R with electrical forces the transmembrane potential difference will be lower across pathways of low resistance than across pathways of high resistance. Hence the relative contribution to net flow of the low resistance channels may be less than in the case of exchange diffusion, and thus we might expect that the introduction of channels of low resistance would decrease R less than R^*. Again, in the measurement of the flux ratio we would expect the flows in the low resistance channels to be of major significance, and f to reflect a value of transmembrane electrical potential close to those across the low resistance channels.

Although the effects described in the table are thus explicable qualitatively, the quantitative agreement with formulations developed for homogeneous membranes is perhaps surprising. These results are possibly attributable to the marked differences in resistance of the two types of pathways in our membranes, such that within experimental accuracy J, R^*, $\vec{J}$ and f were all determined essentially by the channels of low resistance. Whether Eqn. 4 remains valid in membranes with less marked heterogeneity remains to be tested both theoretically and experimentally.

Admittedly the membranes examined here are of unusual structure. Nevertheless, our results seem relevant to the study of transport processes in both synthetic and biological systems. In the latter, both intrinsic heterogeneity and artifactual edge damage could contribute to variability of the resistance of different pathways. Parallel active and passive pathways also could induce variation of the transmembrane electrical potential along the membrane surface. Whatever the specific mechanism, it is noteworthy that membrane heterogeneity can result in behavior characteristic of exchange diffusion in the absence of mobile membrane carriers.

This work has been supported by grants from the U.S.P.H.S. (GM-12852, HE-13648, and HL-14322 to the Harvard-MIT Program in Health Sciences and Technology), the National Science Foundation (GB-40704), and the Swiss National Foundation (3.488.75).

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