COOPERATIVE INTERACTION AMONG SURFACE β- AND γ-CARBOXYL GROUPS MEDIATING THE PERMEATION OF IONS INTO FROG MUSCLE CELLS

GILBERT N. LING
Department of Molecular Biology, Pennsylvania Hospital, Eighth and Spruce Streets, Philadelphia, PA 19107

In 1951 and 1952, departing from the then widely-held belief of full ionic dissociation in electrolyte solutions, I suggested that the degree of association with its counterion becomes greatly increased if one species of the ions is fixed in space (Ling, 1951, 1952). With this enhanced association, K^+, which differs from Na^+ only in short-range attributes (e.g., polarizability, Born repulsion constants), can become preferentially adsorbed on the fixed β- and γ-carboxyl groups of cell proteins. Such selective adsorption on cytoplasmic β- and γ-carboxyl groups served as the foundation for a new theory for selective accumulation of K' over Na^+ in many living cells (Ling, 1952). Similar selective adsorption on cell surface membrane β- and y-carboxyl groups offered the basis for new theories for the selective permeability to K' (Ling, 1953, 1955a, 1960) and for the cellular resting potentials (Ling, 1955b, 1960). Theoretical explanations, both enthalpic and entropic, for the postulated enhanced association of fixed anions with their counterions were offered and other independent evidence supporting this view cited (Ling, 1962).

In years following, extensive testing of these basic concepts and their logical extensions have taken place. The results of the testing have been recently reviewed (Ling, 1984a). However, because of their special relevance to the present work, I shall mention here the following:

(i) fixed anions on the frog muscle cell surface which mediate K' permeation, have a pK_a of 4.6 to 4.7 in agreement with the known pK_a's of β- and γ-carboxyl groups of proteins (Ling and Ochsenfeld, 1965);

(ii) counterion association becomes nearly total when monomeric acids are polymerized and fixed into linear polymer chains (Kern, 1948; Ling and Zhang, 1983), and when a native protein, which adsorbs little alkali-metal ion, adsorbs alkali-metal ion — one ion to one site on all its β- and γ-carboxyl groups when competing fixed cations (e.g., ε-amino and guanidyl groups) have been deionized by OH- (Ling and Zhang, 1984);

(iii) the same cell surface fixed β- and γ-carboxyl groups mediating K' permeation into living cells are also instrumental in generating the cell resting potentials (Edelmann, 1973).

The association-induction (AI) hypothesis, under which title much of the above theoretical and experimental work was presented, also deals with the control of ionic accumulation, permeability and electrical potentials (Ling, 1962, 1984a). In the AI hypothesis, the basic mechanism for this control by drugs, transmitters, hormones, ATP, etc., collectively called "cardinal adsorbents", was electronic polarization and depolarization, or induction of cell proteins in an all-or-none manner. In the AI hypothesis, this all-or-none behavior arises from "cooperative" interaction in the "statistical mechanics" sense of the word, among various protein sites.

Cooperative interaction, long known in the oxygen uptake of hemoglobin solution or

erythrocytes, was observed in the equilibrium distribution of K and Na^+ in frog muscles (Ling, 1966) and, in years immediately following, also seen in a wide variety of other cells (Ling, 1984a). Two new developments have further strengthened the evidence that the cooperative interaction exists on cytoplasmic proteins specifically adsorbing K^+ (and Na^+): (i) the establishment of the adsorbed state of the bulk of K in frog muscle cells (for review, see Edelmann, 1984; see also Ling, 1984a); (ii) that both ion specificity and cooperativity were demonstrated in vitro in the adsorption of alkali-metal ions on the cytoplasmic protein, hemoglobin (Ling and Zhang, 1984).

More recently, my coworkers and I have also shown that the changes of the resting potential of living cells, which behaved differently than that predicted by the Hodgkin-Katz equation (Hodgkin and Katz, 1949) can be quantitatively explained on the basis of the surface adsorption theory (a subsidiary hypothesis of the AI hypothesis), when cooperative interaction among the surface β- and γ-carboxyl groups are taken into consideration (Ling and Fisher, 1983; Ling et al., 1984; Ling, 1984b). This finding raises the issue: "If there is cooperative interaction among the cell surface β- and γ-carboxyl groups generating the resting potential and if the same carboxyl groups also mediate the selective permeation of alkali-metal ions, then there must be cooperative interaction among the same β- and γ-carboxyl groups when ionic permeation rather than resting potential is monitored."

In the following, I shall briefly introduce a newer version of the equation describing ionic permeation with cooperative interaction. I shall then describe some simple experimental data which affirm the prediction of this new theory.

THEORY

Using the one-dimensional Ising method, Yang and Ling (Ling, 1964) derived an adsorption isotherm that has since been known as the Yang-Ling isotherm. Using K^+ adsorption in the presence of Na^+ as an example, we have

$$[K^+]_{ad} = \frac{[f]}{2}\left[1 + \frac{\xi - 1}{\sqrt{(\xi-1)^2 + 4\xi\theta}}\right], \quad (1)$$

where

$$\theta = \exp(\gamma/RT), \quad (2)$$

and $-\gamma/2$ is the nearest neighbor interaction energy, and

$$\xi = \frac{[K^+]_{ex}}{[Na^+]_{ex}} \cdot K^{oo}_{Na\to K}, \quad (3)$$

where $[K^+]_{ex}$ and $[Na^+]_{ex}$ are the external K^+ and Na' concentration. $K^{oo}_{Na\to K}$ is the intrinsic equilibrium constant in the Na–K exchange adsorption.

This isotherm reduces to the simple Langmuir adsorption isotherm, when there is no near neighbor interaction, i.e., $-\gamma/2 = 0$.

The earlier equation for ionic permeation (Ling, 1965), which did not take into account nearest neighbor interaction contains two terms: The first term, called saltatory route, involves ion diffusion through the polarized water in aqueous channels; the second term, called adsorption-desorption route, involves the adsorption of the cation onto surface fixed anions followed by libration to face the inside of the cell and eventual desorption. Again using the rate of K entry (V_K) in the presence of Na^+ as an illustration, we have

$$V_K = \underbrace{A_K [K^+]_{ex}}_{\text{saltatory route}} + \underbrace{\frac{V_K^{max} [K^+]_{ex} \tilde{\tilde{K}}_K}{1 + [K^+]_{ex} \tilde{\tilde{K}}_K + [Na^+]_{ex} \tilde{\tilde{K}}_K}}_{\text{adsorption-desorption route}} \quad (4)$$

where A_K is a constant specific to the ion under study at a specific temperature, pressure, etc. V_K^{max} is the maximum rate of entry when $[K^+]_{ex} \to \infty$. $[K^+]_{ex}$ and $[Na^+]_{ex}$ are the concentration of K' and Na' in the external solution respectively; $\tilde{\tilde{K}}_K$ and $\tilde{\tilde{K}}_{Na}$ are the adsorption constants of K' and Na' respectively on the surface carboxyl groups.

By replacing the Langmuir isotherm with the Yang-Ling isotherm, we have the new equation for ion permeation

$$V_K = A_K[K^+]_{ex} + \frac{V_K^{max}}{2}\left[1 + \frac{\xi' - 1}{\sqrt{(\xi'-1)^2 + 4\xi'\theta'}}\right], \quad (5)$$

where

$$\theta' = \exp(\gamma'/RT), \quad (6)$$

and

$$\xi' = \frac{[K^+]_{ex}}{[Na^+]_{ex}} \cdot K_{Na\to K}^{oo(s)}. \quad (7)$$

$K_{Na\to K}^{oo(s)}$ is the Na^+ to K^+ adsorption exchange constant and $-\gamma'/2$ is the nearest neighbor interaction energy between K' and Na^+ adsorption on the surface anionic sites. Of course, Equation 5 can also be generalized to include a variety of surface sites with different V_{max}, ξ', and θ'.

MATERIALS AND METHODS

Isolated sartorius muscles from Vermont leopard frogs (Rana pipiens pipiens, Schreber) were used.

The modified Ringer's solution used contained neither Mg'' nor Na' but 25 mM K; 1.69 mM PO_4; 0.85 mM Ca''; Cs' labeled with ^{134}Cs from 1.0 to 20 mM; and sucrose making up the total osmotic activity to equal that of 0.118 M NaCl. Additional sucrose was also included in the stock 0.118 M CsCl and 0.118 M KCl used to make the incubation solutions to offset the swelling effect of these salt solutions on frog muscles (0.139 M sucrose for 0.118 M KCl and 0.071 M sucrose for 0.118 CsCl; for details, see Ling and Ochsenfeld, 1965). Mg^{++} is eliminated because its presence promotes precipitation of Cs' probably as a complex salt of Cs', Mg'' and phosphates; in the absence of Mg^{++} and phosphates, Cs^+ remains in solution.

Isolated sartorius muscles from different frogs were incubated for 10 minutes in 10 ml of the experimental solutions, which were kept at 0° C and bubbled with air. Afterwards the muscles were washed another 10 min. in a Ringer's bicarbonate solution (102.6 mM Na'; 2.5 mM K; 1 mM Ca''; 1.2 mM Mg^{++}; 85.4 mM Cl^-; 3.2 mM PO_4; 17.3 mM HCO_3^-; pH 7.4) bubbled with 95% O_2 and 5% CO_2 and also kept at 0° C. At the end of washing, the muscles were blotted dry, weighed, and projected into 1 ml of 1 N HCl in a Lusteroid counting tube before counting on a Packard Automatic y-Scintillation Counter (Model 250A).

RESULTS

Figure 1 shows the rates of labeled Cs' entry into frog sartorius muscles. Each point is the average of readings from three different muscles: the full length of the vertical lines represents twice the standard error.

The solid line in Figure 1 is theoretically calculated according to Equation 5, with $-\gamma 1/2 = 0.68$ Kcal/mole, which corresponds to a Hill coefficient (n) of 3.4, indicating strong autocooperative interaction among the surface sites adsorbing labeled Cs'.

DISCUSSION

The main purpose of this paper is to present the new equation (Equation 5) describing ionic permeation which takes into account the site to site cooperative inter-

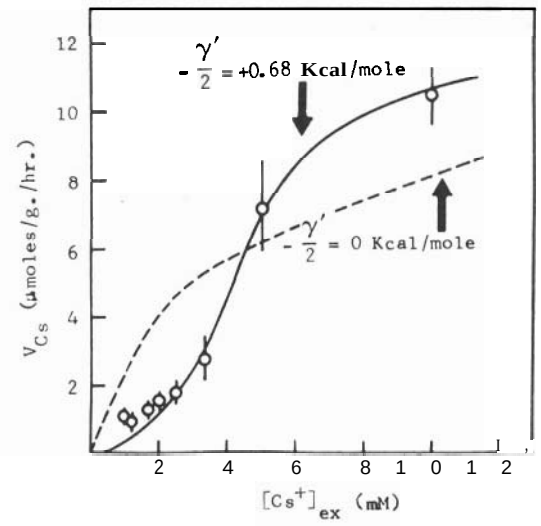

FIGURE I. Rate of labeled Cs' permeation into frog sartorius muscle at different external labeled Cs^+ concentration and a constant K^+ concentration (25 mM). Temperature was 0°C. Solid lines going through or near most of the experimental points are calculated according to Equation 5 with $-\gamma 1/2$ equal to +0.68 Kcal/mole indicating strong autocooperative interaction among the cell surface carboxyl groups adsorbing the ions. Dotted lines calculated from Equation 5 with $-\gamma 1/2$ equal to zero. Theoretical curves calculated without considering Cs'entry via the saltatory route. Labelled Cs'trapped in extracellular space fluid was removed by washing in non-labeled Ringer's solution for 10 minutes.

action. The single set of experimental data (one among a number of similar sets of data on the permeation of other ions under varied conditions to be presented in future publications) is to demonstrate that cooperative interaction among surface anionic sites has now been observed both in the measured resting potential and in the behavior of β-and γ-carboxyl groups mediating Cs^+ ion entry into the cell. This confirmation rounds up the general applicability of the Yang-Ling isotherm (1) to selective ion adsorption on isolated proteins in vitro; (2) to selective accumulation of alkali-metal ions in living cells; (3) to salt-induced swelling of living cells (Ling and Peterson, 1977); (4) to resting potential; and finally (5) to the fixed β- and γ-carboxyl groups mediated cation permeation into living cells. With its broad applicability in such a variety of cell physiological manifestations established, it is most exciting and challenging to look forward to investigating the control of synchronized physiological activities made possible by the autocooperative interaction among spatially separated sites by minute concentration of drugs, hormones, ATP, Ca^{++}, etc., which have been collectively referred to as "cardinal adsorbents".

SUMMARY

A new equation for the ion permeation into living cells is described. This equation, differs from earlier ones, in that cooperative interaction among the fixed surface β- and y-carboxyl groups mediating ion entry via the adsorption-desorption route is taken into account. Results of a single set of experiments describing labeled Cs^+ into frog sartorius muscles at 0°C affirms the existence of the predicted cooperative interaction which endows the cell membrane and other organelles with a mechanism for coherence and control.

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