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Na/K competitive transport selectivity of (221)C_{10}-cryptand: effects of pH and carrier concentration

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Abstract

The kinetics of the competitive transport of Na^+ and K^+ ions across the membrane of large unilamellar vesicles (LUV) were determined when transport was induced by (221)C_{10}-cryptand, an ionizable mobile carrier. The experiments were performed at various pH values (7.7 and 8.7) and carrier concentrations (0.1, 0.5 and 1.0 μM) in order to quantify the effects of these parameters on the Na/K competitive transport selectivity of this mobile carrier. At any given pH and carrier concentration, the apparent affinity of (221)C_{10} for Na^+ was higher and less dependent on the concentration of the other competing ion than that for K^+. The Na/K competitive transport selectivity (S_C(Na/K)) of (221)C_{10} increased linearly with the Na^+ concentrations, decreased hyperbolically with increasing those of K^+ and was independent of the pH and of the carrier concentration. In equimolecular ionic mixtures, this competitive selectivity amounted to about 1.5 and when the pH rose, the carrier selectivity for Na^+ over K^+ ions was enhanced by cation competition compared to transport of cations as unique substrates. Equations were established to describe the variations of the competitive transport selectivity (S_C) of cryptands, and for comparison of their noncompetitive selectivity (S_{NC}), with the ionic concentrations, the Michaelis parameters of the cations and the pH. The reaction order in Na^+ (n(Na)) increased significantly with decreasing the pH and the K^+ concentration. The results are discussed in terms of the structural, physico-chemical and electrical characteristics of carriers and complexes.

Keywords: Cryptand; Alkali cation transport kinetics; Competitive transport selectivity; pH; Lipid membrane

1. Introduction

It has by now been clearly established that the quantitative ion specificity of ionophores varies with numerous parameters [1–4] and it has been stressed by Behr et al. [5] that it should be measured only in true competition experiments, i.e., when ions are present simultaneously. Under these conditions, the carrier specificity called here 'competitive transport selectivity' differs from its 'noncompetitive transport selectivity'. The latter is determined when ions are transported as unique substrates of the carrier, i.e., in the absence of competitive inhibition of each cation by the other ('noncompetitive conditions'). Very few data in the literature were in fact obtained from the study of ionic mixtures in the case of ion transport through biological [6–10] and lipid bilayer [11–14] membranes.

To shed light on the predominant parameters modulating the competitive selectivity of ion transport by mobile carriers, we have recently studied the transport of Na^+ and K^+ ions by (221)C_{10}-cryptand, at various temperatures, when both ions were present simultaneously [14]. It has been shown that the Na^+/K^+ competitive transport selectivity of this carrier increased slightly as the temperature rose and that, under physiological conditions, the (221)C_{10}-cryptand would behave as an efficient non-electrogenic Na^+/K^+ exchanger. Since the efficiency of cation transport by (221)C_{10} depends on pH [15], it seemed to be of interest to study this transport at various pH values and carrier concentrations.

The synthetic macrobicyclic polyaminoether (221)C_{10}-cryptand, i.e., the 1,10-diaza-5-decyl-4,7,13,16,21-pentaoxa-bicyclo[8.8.5]tricosane [16], is an amphiphilic molecule composed of a hydrophilic intramolecular binding cavity and a ten-carbon aliphatic side chain allowing its solubilization into lipid membranes (Fig. 1). From the fundamen-

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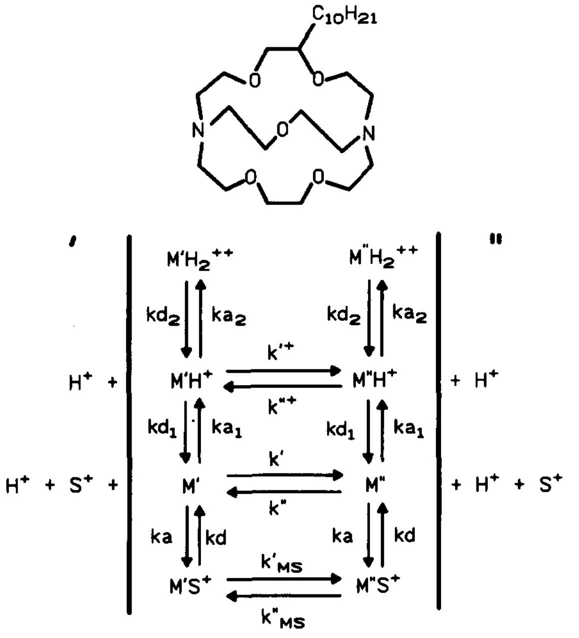

Fig. 1. Reaction scheme of cation transport (S^+) mediated by $(221)C_{10}$-cryptand, a carrier possessing three ionization states: unprotonated (M), monoprotonated (MH^+) and diprotonated (MH_2^{2+}).

tal point of view, cryptands are very interesting examples of mobile carriers [15,17–20]. The scheme of cation transport by these ionophores basically resembles that of valinomycin: a neutral carrier may form positively charged complexes and cross the membrane. It has a higher degree of complexity than valinomycin, however, since the free carrier concentration is pH-dependent.

In a previous study of cation transport by $(221)C_{10}$ ('Zero trans influx' experiments) under noncompetitive conditions (separate experiments for each ion), we reported that the Na^+/K^+ noncompetitive transport selectivity of $(221)C_{10}$, decreased as the concentrations of Na^+ and K^+ at equal concentrations ($C'_{Na} = C'_K$) rose [15]. This finding appeared to be fairly compatible with the fact that the stability of $(221)C_{10}$ complexes is greater when formed with Na^+ than with K^+ [21]. This selectivity also decreased when pH rose. Under these conditions, a decrease in the strength of the H^+/cation competition within the intramolecular binding cavity indeed favoured to a greater extent the transport of the cation forming the least stable complexes with the cryptand.

The present study concerns the pH- and carrier concentration-dependencies of the competitive transport selectivity of a mobile carrier. It quantifies the kinetic parameters of the transport of Na^+ and K^+ ions by the $(221)C_{10}$-cryptand in competition experiments and focusses on their pH- and carrier concentration-induced variations. A theoretical treatment of the effect of the pH on both the competitive and noncompetitive transport selectivities of cryptands for ions is also presented. The results are discussed in terms of the structural and electrical characteristics of the carrier and complex, and the interactions occurring between an ionizable cryptand and the membrane.

2. Materials and methods

All materials and methods have already been described previously [14,15].

The external vesicular solution was 0.11 M choline sulfate and 0.7 M D-mannitol ($\omega' = 1.0$ M). The internal vesicular buffer consisted of 0.05 M bis-Tris propane and 0.67 M D-mannitol ($\omega'' = 0.79$ M) (pH 6.7 or 7.7). Salt solutions were 0.585 M Na_2SO_4 and 0.585 M K_2SO_4 ($\omega = 1.0$ M). FCCP was dissolved in absolute ethanol, and $(221)C_{10}$ in benzene.

The kinetics of cation transport were investigated on large unilamellar vesicles (LUV) containing L-α-phosphatidylcholine, L-α-phosphatidic acid and cholesterol in an 8:1:1 molar ratio [14,17].

Proton effluxes were measured as follows: 0.5 ml LUV suspension were added to 3.5 ml external solution in the titrating vessel and equilibrated at 25° C under nitrogen stream. The external pH (pH' = 6.7 or 7.7), which was the same as the initial internal pH (pH''), was measured, and a one-pH-unit gradient was induced by adding choline base until the external pH (pH'_f) reached 7.7 or 8.7. FCCP was added to a final concentration of 2.4 μM (or 1.5 mmol/mol lipid, i.e., about 3.7 nmol carrier/m^2 surface membrane), and then $(221)C_{10}$ to a final concentration of 0.1, 0.5 and 1.0 μM (or 0.08, 0.31 and 0.61 mmol/mol lipid, i.e., about 0.2, 0.8 and 1.6 nmol carrier/m^2 surface membrane). Transport kinetics were induced by adding various volumes of K_2SO_4, Na_2SO_4 or K_2SO_4/Na_2SO_4 mixtures. This addition was performed after the equilibrium for the carrier partition between the aqueous phases and the membrane had been reached. The external Na^+ concentrations were varied from 2.9 to 42.3 mM in the absence of K^+ ions, and from 2.9 to 23.5 mM when the external K^+ concentration was maintained constant at levels of 14.2 and 23.5 mM, respectively. Such experiments were performed on two different LUV preparations at each pH and carrier concentration. The pH variations were recorded continuously as a function of time. At equilibrium, the buffering power of the sample was measured by adding 50 μl of 0.001 M H_2SO_4 which allowed the magnitude of the proton efflux to be determined at any time during transport. The variations with time in the proton effluxes, and consequently in the alkali cations influxes, fitted monoexponentials. The initial rates of cation transport were determined by drawing the tangent of the recorder trace at the moment at which alkali cations were added to the samples.

According to the kinetic model for competitive inhibition with mixed alternative substrates under steady-state conditions [22], the variations with the external sodium concentration (C'_{Na}) in the proton efflux (J_{i}) were fitted by the following equation [14]:

$$J_{\mathrm{i}} = \frac{J^{\mathrm{pH}}_{\mathrm{maxNa}} \cdot C'_{\mathrm{Na}} + J^{\mathrm{pH}}_{\mathrm{maxK}} \cdot C'_{\mathrm{K}}\left(K^{\mathrm{pH}}_{\mathrm{mNa}}/K^{\mathrm{pH}}_{\mathrm{mK}}\right)}{K^{\mathrm{pH}}_{\mathrm{mNa}}\left(1 + C'_{\mathrm{K}}/K^{\mathrm{pH}}_{\mathrm{mK}}\right) + C'_{\mathrm{Na}}} \tag{1}$$

where $J^{\mathrm{pH}}_{\mathrm{maxNa}}$ and $J^{\mathrm{pH}}_{\mathrm{maxK}}$ are the apparent pH-dependent maximal velocities with Na^+ or K^+ ions as unique substrates, $K^{\mathrm{pH}}_{\mathrm{mNa}}$ and $K^{\mathrm{pH}}_{\mathrm{mK}}$, the pH-dependent Michaelis constants of (221)C_{10} for Na^+ and K^+, respectively, and C'_{K}, the external aqueous concentration of K^+ ions. In the case of cryptand-mediated transport, the maximal initial rate ($J^{\mathrm{pH}}_{\mathrm{max}}$) is independent of the nature of the alkali cation transported ($J^{\mathrm{pH}}_{\mathrm{maxNa}} = J^{\mathrm{pH}}_{\mathrm{maxK}}$) [15,17]. The Michaelis parameters ($J^{\mathrm{pH}}_{\mathrm{max}}$, $K^{\mathrm{pH}}_{\mathrm{mNa}}$ and $K^{\mathrm{pH}}_{\mathrm{mK}}$) were determined by the simultaneous fitting of the J_{i} vs. C'_{Na} curves obtained at $C'_{\mathrm{K}} = 0$, 14.2 and 23.5 mM from the study of two different LUV preparations.

Regression lines were calculated using the least-square method and compared by performing covariance analysis. Differences were taken to be significant at $P < 0.05$.

3. Kinetic theory

The model for cation transport by (221)C_{10}-cryptand (Fig. 1) has already been described in detail elsewhere [15,18–20]. It assumes that at the pH investigated, a carrier containing two ionizable tertiary amine groups exists in three different states of ionization: unprotonated (M), monoprotonated (MH^+) and diprotonated (MH_2^{2+}), and that only unprotonated carrier (M) is able to bind alkali cations (S^+) [23].

In the present study, S^+ represented Na^+ or K^+ ions as competing substrates of the cryptand. Kinetic equations were derived according to the model by Devés and Krupka [24] for reversible inhibition of facilitated transport systems. In this model, the binding constants for addition of substrates and protons to unprotonated carrier, and of protons to monoprotonated carrier on either the outer (′) or inner (″) membrane/solution interface, K_{Na}, K_{K}, K_1 etc. are defined as dissociation constants, having units of concentration. When the substrates and protons move down their electrochemical potential gradient to achieve equilibrium, the principle of detailed equilibrium dictates the following relationship among the constants in Fig. 1:

$$a = k'' \cdot k'_{\mathrm{MNa}} \cdot K''_{\mathrm{Na}} / k' \cdot k''_{\mathrm{MNa}} \cdot K'_{\mathrm{Na}} \tag{2}$$

$$b = k'' \cdot k'_{\mathrm{MK}} \cdot K''_{\mathrm{K}} / k' \cdot k''_{\mathrm{MK}} \cdot K'_{\mathrm{K}} \tag{3}$$

$$c = k'' \cdot k'^{+} \cdot K''_1 / k' \cdot k''^{+} \cdot K'_1 \tag{4}$$

where $a = \mathrm{e}^{+u(\mathrm{Na})}$, $b = \mathrm{e}^{+u(\mathrm{K})}$, $c = \mathrm{e}^{+u(\mathrm{H})}$, $u(\mathrm{Na}) = -E_{\mathrm{mNa}} \cdot F/R \cdot T$, $u(\mathrm{K}) = -E_{\mathrm{mK}} \cdot F/R \cdot T$, $u(\mathrm{H}) = -E_{\mathrm{mH}} \cdot F/R \cdot T$ and E_{mNa}, E_{mK}, E_{mH} = membrane potentials for Na^+, K^+ and H^+ ions (F, R, T = Faraday, gas constant and absolute temperature). At equilibrium, constants a, b, and c are equal to the ratio of the final concentrations of substrate and proton inside and outside the liposome [24]: $a = (C''_{\mathrm{Na}}/C'_{\mathrm{Na}})_{\mathrm{final}}$, $b = (C''_{\mathrm{K}}/C'_{\mathrm{K}})_{\mathrm{final}}$ and $c = (C''_{\mathrm{H}}/C'_{\mathrm{H}})_{\mathrm{final}}$. It must be underlined that equilibrium of the system under study is reached when the electrochemical potential difference of cations across the membrane equals that of protons. Therefore, constants a, b and c are different from unity at equilibrium [17].

In deriving the rate equation it was assumed that:

(1) The rate-limiting step of the transport process was the translocation of the cation-carrier complexes through the membrane rather than diffusion up to the carrier binding cavity or formation and dissociation of the cation-carrier complexes and protonated carrier species (rapid equilibrium conditions). This assumption is reasonable in view of the high speed of interfacial processes [25,26].

(2) The steady-state condition of electroneutral flow applied since at the protonophore concentration used here, the rates of cation/H^+ exchanges through LUV membranes were under the sole control of Na^+ and K^+ transport rates (on which this study focused) [15,18–20]. As a result and owing to the high number of parameters that must be determined from experimental data fitting, the presence of the protonophore was not taken into account in the equations derived below. In this case, a comparison of the relative variations of the parameters of the system was entirely valid although the values of these parameters were apparent ones.

(3) No interaction existed between the anionic form of the protonophore (FCCP^-) and the positively charged cryptand-cation complex since the electrical charge of the cation is burried to a large extent inside the intramolecular binding cavity of the carrier [14,23].

(4) Owing to its highly hydrophilic nature, the diprotonated carrier (MH_2^{2+}) did not cross the lipophilic region of the membrane [21].

An equation is first written expressing the steady-state condition that the sum of movements of all forms of carrier in the inward direction must equal that in the outward:

$$\begin{aligned} &k'[\mathrm{M}'] + k'_{\mathrm{MNa}}[\mathrm{M}'\mathrm{Na}] + k'_{\mathrm{MK}}[\mathrm{M}'\mathrm{K}] + k'^{+}[\mathrm{M}'\mathrm{H}] \\ &\quad = k''[\mathrm{M}''] + k''_{\mathrm{MNa}}[\mathrm{M}''\mathrm{Na}] + k''_{\mathrm{MK}}[\mathrm{M}''\mathrm{K}] + k''^{+}[\mathrm{M}''\mathrm{H}] \end{aligned} \tag{5}$$

Next, the total concentration of carrier in all forms is a constant, M_{t}:

$$\begin{aligned} M_{\mathrm{t}} = {} & [\mathrm{M}'] + [\mathrm{M}'\mathrm{Na}] + [\mathrm{M}'\mathrm{K}] + [\mathrm{M}'\mathrm{H}] + [\mathrm{M}'\mathrm{H}_2] \\ & + [\mathrm{M}''] + [\mathrm{M}''\mathrm{Na}] + [\mathrm{M}''\mathrm{K}] + [\mathrm{M}''\mathrm{H}] + [\mathrm{M}''\mathrm{H}_2] \end{aligned} \tag{6}$$

These equations, together with the dissociation constants for the different carrier species, $C'_{\mathrm{Na}} \cdot [\mathrm{M}']/[\mathrm{M}'\mathrm{Na}] = K'_{\mathrm{Na}}$ etc., enable to write down an expression for the rate of Na^+ transport:

$$\mathrm{d}C''_{\mathrm{Na}}/\mathrm{d}t = k'_{\mathrm{MNa}}[\mathrm{M}'\mathrm{Na}] - k''_{\mathrm{MNa}}[\mathrm{M}''\mathrm{Na}] \tag{7}$$

A similar equation may be written for K^+ transport. Assuming that the dissociation constants for the complexed and protonated species are equal on both sides of the membrane then, in the case of zero-trans influx experiments ($C''_{Na} = C''_K = 0$), the variations of the overall cation transport rate (J_i) with the external Na^+ concentration is described by

$$J_i = \frac{(J_{maxNa}/Y)C'_{Na} + (J_{maxK}/Y)C'_K(K_{mNa}/K_{mK})}{K_{mNa}(X/Y + C'_K/K_{mK}) + C'_{Na}} \tag{8}$$

with

$$X = A/(1 + k''^{+}C''_H/k''K_1)$$

$$\begin{aligned} A = 1 &+ [k''/(k'+k'')][C'_H/K_1 + C'^2_H/K_1K_2] \\ &+ [k'/(k'+k'')][C''_H/K_1 + C''^2_H/K_1K_2] \\ &+ [k''^{+}/(k'+k'')] \\ &\times[(C''_H/K_1)(1 + C'_H/K_1 + C'^2_H/K_1K_2] \\ &+ [k'^{+}/(k'+k'')] \\ &\times[(C'_H/K_1)(1 + C''_H/K_1 + C''^2_H/K_1K_2)] \end{aligned} \tag{9}$$

$$Y = B/(1 + k''^{+}C''_H/k''K_1)$$

$$\begin{aligned} B = 1 &+ [k'_{MS}/(k'' + k'_{MS})] \\ &\times[k''^{+}C''_H/k'_{MS}K_1 + C''_H/K_1 + C''^2_H/K_1K_2] \end{aligned} \tag{10}$$

$$J_{max} = k'_{MS} \cdot M_t/(1 + k'_{MS}/k'') \tag{11}$$

$$K_{mS} = K'_S[(1 + k'/k'')/(1 + k'_{MS}/k'')] \tag{12}$$

and therefore, the apparent Michaelis parameters for Na^+ transport by (221)C_{10}-cryptand are given by

$$J_{maxNa}(\text{app}) = J_{maxNa}/Y = J^{pH}_{maxNa} \tag{13}$$

$$K_{mNa}(\text{app}) = K_{mNa}(X/Y + C'_K/K_{mK}) \tag{14}$$

Let

$$K^{pH}_{mNa} = K_{mNa}X/Y \tag{15}$$

then

$$K_{mNa}(\text{app}) = K^{pH}_{mNa}(1 + C'_K/K^{pH}_{mK}) \tag{16}$$

It must be stressed that the electroneutrality of the system under investigation here was maintained during transport by an efflux of protons in exchange with cations. When the internal proton concentration C''_H falls to zero, the membrane potential tends towards the infinite (positive inside). As a result, the value of the translocation rate constant of the cation-carrier complex k'_{MS} (Eq. (17) below), and consequently those of J_{max} (Eq. (11)) and J_i (Eq. (8)), fall to zero.

Since Na^+ and K^+ complexes with cryptands are large organic cations of the same size and shape, and apparent electrical charge, then $J_{maxNa} = J_{maxK}$ [15,17]. For the neutral carrier (M), the rate constants k' and k'' are the same (k) when the transport is not limited by steric obstruction in the membrane (high membrane saturation level in carriers). The rate constants of the charged carriers depend on the membrane potential (negative inside). If a constant field strength is assumed in the membrane [27], then

$$k'_{MS} = k_{MS} \cdot e^{+u/2} \tag{17}$$

$$k''_{MS} = k_{MS} \cdot e^{-u/2} \tag{18}$$

$$k'^{+} = k^{+} \cdot e^{+u/2} \tag{19}$$

$$k''^{+} = k^{+} \cdot e^{-u/2} \tag{20}$$

Besides, protons like alkali cations are burried inside the intramolecular cavity. It was therefore likely that the rate constants for the translocation through the membrane of the cation-carrier complexes (k_{MS}) and the monoprotonated carriers (k^+) had the same value. The highly hydrophillic nature of the diprotonated carriers (MH_2^{2+}) was assumed to prevent it from crossing the membrane (see above).

The cation transport driving force was the reverse proton ($C''_H = 10\ C'_H$) and cation concentration gradients. Their dissipation induced an efflux of protons (Φ_H) carried by the protonophore FCCP, coupled to an influx of sodium (Φ_{Na}) and potassium (Φ_K) ions carried by the cryptand. The proton and cation fluxes were related by

$$\Phi_{Na} + \Phi_K = -\Phi_H = \Phi_{MS} \tag{21}$$

In terms of free energy, the influx of alkali cations was favoured by both the ion concentration gradients and the electric field in the membrane.

4. Results

The electroneutral exchange of sodium and potassium ions with protons across LUV membranes was induced by the simultaneous presence of (221)C_{10} and FCCP. In the absence of (221)C_{10} and FCCP, no transport occurred. To ensure that the rates of cation/H^+ exchanges through LUV membranes were under the sole control of cation transport rates (on which this study focused), a FCCP concentration of 2.4 μM was used. At this concentration, proton transport was not the rate-limiting step for the cation/H^+ exchanges occurring through LUV membranes, whatever the pH and the carrier concentration. This result was in agreement with the fact that (221)C_{10} has been found to exhibit saturation of the transport rate as a function of Na^+ concentration [15].

The initial rates (J_i) of cation translocation by 0.1, 0.5 and 1.0 μM (or 0.08, 0.31 and 0.61 mmol/mol lipid, i.e., about 0.2, 0.8 and 1.6 nmol carrier/m^2 surface membrane) (221)C_{10} through negatively charged LUV membranes (pH 7.7 and 8.7) were determined at Na^+ concentrations varying from 2.9 to 42.3 mM in the absence of K^+ ions, and from 2.9 to 23.5 mM in the presence of competing K^+ ions ($C'_K = 14.2$ and 23.5 mM). The experiments were performed on two different LUV preparations under each set of experimental conditions. Owing to the high number of experimental conditions investigated (114 sets), the J_i values determined here are not presented except, for illustration, those obtained at 1.0 μM (or 0.61 mmol/mol lipid, i.e., about 1.6 nmol carrier/m^2 surface membrane) (221)C_{10} (Fig. 2). The data given below correspond therefore to fitted or calculated data, and in each case this will be specified.

4.1. Michaelis parameters

Fitted J^{pH}_{max} and K^{pH}_m

The apparent pH-dependent Michaelis parameters were determined by the simultaneous fitting of the J_i vs. C'_{Na} plots of the experimental data obtained from two LUV preparations at each pH and carrier concentration according to Eq. (1) in Materials and methods. The values of the apparent pH-dependent K^{pH}_m, J^{pH}_{max}, maximum turnover rates (T^{pH}_{max}) and specificity constants (T^{pH}_{max}/K^{pH}_m) of (221)C_{10}-cryptand are reported in Table 1.

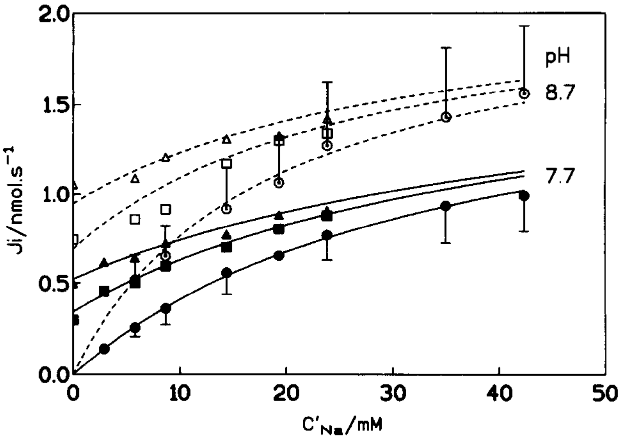

Fig. 2. pH-dependence of the initial influx (J_i) of competing Na^+ and K^+ ions on the external Na^+ concentration (C'_{Na}): competitive transport of Na^+ and K^+ ions ($C'_{Na} = 2.9$–42.3 mM at $C'_K = 0$ mM (circles); $C'_{Na} = 2.9$–23.5 mM at $C'_K = 14.2$ (squares) and 23.5 mM (triangles)) by 1.0 mM (or 0.61 mmol/mol lipid, i.e., about 1.6 nmol carrier/m^2 surface membrane) (221)C_{10}-cryptand through negatively charged LUV membranes (pH 7.7 and 8.7). Points are means ($n = 2$) of J_i values determined under each set of experimental conditions on two LUV preparations. For clarity, error bars (± S.E.) are only indicated at $C'_K = 0$ mM. The curves drawn in this figure were determined according to Eq. (8) in Kinetic theory, by the simultaneous fitting of the experimental data obtained at $C'_K = 0$, 14.2 and 23.5 mM (pH 7.7 and 8.7) on four LUV preparations (two at each pH).

Table 1
Effect of pH and carrier concentration (C'_M) on the apparent Michaelis parameters (J^{pH}_{max}, K^{pH}_m), maximum turnover rate (T^{pH}_{max}) and specific constant (T^{pH}_{max}/K^{pH}_m) for the competitive transport of Na^+ and K^+ ions by (221)C_{10}-cryptand

pH	C'_M (μM)	J^{pH}_{max} (nmol s^{-1})	K^{pH}_{mNa} (mM)	K^{pH}_{mK} (mM)	T^{pH}_{max} (s^{-1})	$T^{pH}_{max}/K^{pH}_{mNa}$ (s^{-1} mM^{-1})	T^{pH}_{max}/K^{pH}_{mK} (s^{-1} mM^{-1})
7.7	0.1	0.6 ± 0.1	37.7 ± 3.4	56.0 ± 4.5	1.1	0.029	0.020
	0.5	1.2 ± 0.1	34.5 ± 3.0	57.1 ± 4.7	0.6	0.017	0.010
	1.0	1.8 ± 0.1	31.5 ± 1.7	60.6 ± 2.7	0.4	0.013	0.007
8.7	0.1	0.6 ± 0.1	18.7 ± 0.8	26.7 ± 1.5	1.2	0.063	0.044
	0.5	1.5 ± 0.1	22.0 ± 1.8	27.3 ± 2.1	0.7	0.031	0.025
	1.0	2.2 ± 0.1	19.9 ± 1.3	28.3 ± 2.0	0.5	0.027	0.019

Competitive transport of Na^+ and K^+ ions ($C'_{Na} = 2.9$–42.3 mM at $C'_K = 0$ mM; $C'_{Na} = 2.9$–23.5 mM at $C'_K = 14.2$ and 23.5 mM) by 0.1, 0.5 and 1.0 μM (or 0.08, 0.31 and 0.61 mmol/mol lipid, i.e., about 0.2, 0.8 and 1.6 nmol carrier/m^2 surface membrane) (221)C_{10}-cryptand through negatively charged LUV membranes (pH 7.7 and 8.7). Means (± S.E.) were determined by the simultaneous fitting of the data obtained $C'_K = 0$, 14.2 and 23.5 mM from two LUV preparations.

Table 2
Effect of carrier concentration (C'_M) on Michaelis parameters (J_{max}, K_m), maximum turnover rate (T_{max}), specific constant (T_{max}/K_m), ionization pK's (p$K_1$and pK_2) of the cryptand and translocation rate constants ($k_{MS}/k = k^+/k$) for the competitive transport of Na^+ and K^+ ions by (221)C_{10}-cryptand

C'_M (μM)	J_{max} (nmol s^{-1})	K_{mNa} (mM)	K_{mK} (mM)	T_{max} (s^{-1})	T_{max}/K_{mNa} (s^{-1} mM^{-1})	T_{max}/K_{mK} (s^{-1} mM^{-1})	pK_1	pK_2	$k_{MS}/k = k^+/k$
0.1	0.6 ± 0.1	17.7 ± 2.3	26.1 ± 3.4	1.24	0.070	0.048	7.10 ± 0.07	5.0	0.016
0.5	1.5 ± 0.1	18.2 ± 2.3	24.7 ± 3.0	0.69	0.038	0.028	7.14 ± 0.09	5.0	0.019
1.0	2.2 ± 0.1	16.2 ± 1.6	26.8 ± 2.7	0.53	0.033	0.020	7.19 ± 0.07	5.0	0.018

Competitive transport of Na^+ and K^+ ions ($C'_{Na} = 2.9$–42.3 mM at $C'_K = 0$ mM; $C'_{Na} = 2.9$–23.5 mM at $C'_K = 14.2$ and 23.5 mM) by 0.1, 0.5 and 1.0 μM (or 0.08, 0.31 and 0.61 mmol/mol lipid, i.e., about 0.2, 0.8 and 1.6 nmol carrier/m^2 surface membrane) (221)C_{10}-cryptand through negatively charged LUV membranes (pH 7.7 and 8.7). Means (± S.E.) were determined according to Eq. (8) in Kinetic theory by the simultaneous fitting of the data obtained at each carrier concentration (two LUV preparations at each pH). In this equation, pK_2 was set equal to 5.0 pH units.

Table 3
Effects of competing K^+ ions on the apparent K_m for Na^+ (K_{mNa}(app)) and of competing Na^+ ions on the apparent K_m for K^+ (K_{mK}(app))

pH	C'_M (μM)	K_{mNa}(app) (mM) at C'_K (mM)			K_{mK}(app) (mM) at C'_{Na} (mM)		
		0	14.2	23.5	0	14.2	23.5
7.7	0.1	37.7	47.3	53.5	56.0	77.1	90.9
	0.5	34.5	43.1	48.7	57.1	80.6	96.0
	1.0	31.5	38.9	43.7	60.6	87.9	105.8
8.7	0.1	18.7	28.6	35.2	26.7	47.0	60.3
	0.5	22.0	33.4	40.9	27.3	44.9	56.5
	1.0	19.9	29.9	36.4	28.3	48.5	61.7

Competitive transport of Na^+ and K^+ ions (C'_{Na} = 2.9–42.3 mM at C'_K = 0 mM; C'_{Na} = 2.9–23.5 mM at C'_K = 14.2 and 23.5 mM) by 0.1, 0.5 and 1.0 μM (or 0.08, 0.31 and 0.61 mmol/mol lipid, i.e., about 0.2, 0.8 and 1.6 nmol carrier/m^2 surface membrane) (221)C_{10}-cryptand through negatively charged LUV membranes (pH 7.7 and 8.7). The values of K_m(app) at C'_S = 0 mM correspond to means of the K_{mNa}^{pH} and K_{mK}^{pH} values determined at all the cryptand concentrations used in each set of experimental conditions (Table 1) while those at C'_S = 14.2 and 23.5 mM were calculated according to Eq. (16) in Kinetic theory and using the K_m^{pH} values reported in Table 1.

Fitted J_{max} and K_m

In order to discriminate the effect of pH on the apparent Michaelis parameters of the cryptand, the J_i vs. C'_{Na} plots of the experimental data obtained at each carrier concentration (pH 7.7 and 8.7) were fitted simultaneously according to Eq. (8) in Kinetic theory. The experimental pH-induced variations of the Michaelis parameters ($K_m^{pH\ 7.7}/K_m^{pH\ 8.7}$ about 2 and $J_{max}^{pH\ 7.7}/J_{max}^{pH\ 8.7}$ about 0.8) of the cryptand were fairly accounted for by setting $pK_2 = 5.0$ in this equation. Table 2 reports the values obtained for all the apparent parameters of cation transport by (221)C_{10} when occurring through membranes having a 60 mV membrane potential (negative inside) as was the case here.

Calculated K_m(app)

When Na^+ and K^+ ions were transported as alternative substrates of (221)C_{10} then each cation was competitively inhibited by the other. The apparent affinity of the cryptand for each ion decreased (higher K_m(app)) to an extent depending on the concentration of the other as shown by Eq. (16) in Kinetic theory. Using this equation and the apparent K_m^{pH} values reported in Table 1, the values of K_{mNa}(app) and K_{mK}(app) were calculated under each set of experimental conditions (Table 3). It was found that, when raising the external K^+ concentration (C'_K) from 0 to 23.5 mM, the K_{mNa}(app) of the cryptand would have been increased by about 14 mM at pH 7.7, and by 17 mM at pH

Table 4
Influence of pH and external cation concentrations (C'_S) on the Na/K competitive transport selectivity (S_C(Na/K)) of (221)C_{10}-cryptand

pH	S(Na/K)	C'_K (mM)											
		14.2						23.5					
		C'_{Na} (mM)						C'_{Na} (mM)					
		0	5.8	8.6	14.3	19.1	23.5	0	5.8	8.6	14.3	19.1	23.5
	S_C	0	0.61 (0.03)	0.90 (0.05)	1.51 (0.09)	2.00 (0.12)	2.47 (0.14)	0	0.37 (0.02)	0.55 (0.04)	0.91 (0.05)	1.20 (0.08)	1.48 (0.09)
7.7	S_{NC}	0	0.65 (0.03)	0.92 (0.05)	1.37 (0.06)	1.67 (0.08)	1.91 (0.09)	0	0.45 (0.02)	0.63 (0.03)	0.94 (0.04)	1.13 (0.05)	1.29 (0.05)
8.7	S_{NC}	0	0.70 (0.03)	0.93 (0.04)	1.29 (0.05)	1.51 (0.05)	1.67 (0.06)	0	0.51 (0.02)	0.69 (0.02)	0.95 (0.04)	1.10 (0.04)	1.22 (0.04)
7.7	S_C/S_{NC}		0.92 (0.01)	0.98 (0.01)	1.10 (0.01)	1.20 (0.02)	1.29 (0.02)		0.82 (0.01)	0.87 (0.01)	0.97 (0.02)	1.06 (0.02)	1.14 (0.02)
8.7	S_C/S_{NC}		0.87 (0.02)	0.97 (0.02)	1.16 (0.03)	1.33 (0.04)	1.48 (0.04)		0.72 (0.01)	0.80 (0.02)	0.96 (0.03)	1.09 (0.03)	1.21 (0.04)

Competitive transport of Na^+ and K^+ ions (C'_{Na} = 2.9–42.3 mM at C'_K = 0 mM; C'_{Na} = 2.9–23.5 mM at C'_K = 14.2 and 23.5 mM) by 0.1, 0.5 and 1.0 μM (or 0.08, 0.31 and 0.61 mmol/mol lipid, i.e., about 0.2, 0.8 and 1.6 nmol carrier/m^2 surface membrane) (221)C_{10}-cryptand through negatively charged LUV membranes (pH 7.7 and 8.7). S_C(Na/K) values were means of the selectivities calculated at all the cryptand concentrations used according to Eq. (A-2) in Appendix A and using K_m values reported in Table 2. For comparison, the noncompetitive transport selectivities (S_{NC}(Na/K)) of the cryptand are also given; their values were means of the selectivities calculated at all the cryptand concentrations used according to Eq. (A-6) in Appendix A and using K_m values reported in Table 2. The values of S_C(Na/K)/S_{NC}(Na/K) are also reported and the errors (±S.E.) are given in brackets.

8.7. A similar increase in the external Na^+ concentration (0 to 23.5 mM) would have induce however a much higher variation in the value of the K_{mK}(app) of (221)C_{10}, i.e., about 40 mM at pH 7.7, and 32 mM at pH 8.7.

4.2. Na / K competitive (S_C(Na / K)) and noncompetitive (S_{NC}(Na / K)) transport selectivities of (221)C_{10}: J_{Na} / J_K

The Na/K transport selectivity of (221)C_{10} is equal to the ratio of the individual initial influxes of sodium (J_{Na}) and potassium (J_K) ions. It is called here 'competitive transport selectivity' when both ions are present simultaneously, i.e., when each cation is competitively inhibited by the other (true competition experiments) or 'noncompetitive transport selectivity' when ions are transported as unique substrates of the carrier, i.e., in the absence of the competitive inhibition of each cation by the other (separate experiments).

Calculated S_C(Na / K)

The values of the Na/K competitive transport selectivity (S_C(Na/K)) of (221)C_{10} were calculated using Eq. (A-2) of Appendix A and the values of the apparent K_m's reported in Table 2 (Table 4). According to this equation, the transport selectivity increased linearly with the external Na^+ concentration, and decreased hyperbolically with increasing that of K^+. Besides, as underlined in Appendix A, it did not depend on pH. When the external Na^+ concentration varied from 0 to 23.5 mM, the competitive transport selectivity increased from 0 to about 2.5 at $C'_K = 14.2$ mM, and from 0 to about 1.5 at $C'_K = 23.5$ mM (Table 4). Moreover, at identical external cation concentrations ($C'_{Na} = C'_K = 14.2$ or 23.5 mM), the competitive transport selectivity of the cryptand was equal to about 1.5, i.e., the initial rate of Na^+ transport (J_{Na}) by (221)C_{10} was 1.5-times higher that of K^+ (J_K) (Table 4).

Calculated S_{NC}(Na / K)

For comparison, the noncompetitive transport selectivity (S_{NC}Na/K) of (221)C_{10} was calculated under each set of experimental conditions using Eq. (A-6) of Appendix A and the values of the apparent K_m's reported in Table 2 (Table 4). According to this equation, the variations in the noncompetitive transport selectivity with the external concentrations of either Na^+ or K^+ ions were described by rectangular hyperbolic functions. This noncompetitive selectivity also varied with the pH.

Table 4 shows that in equimolecular ionic mixtures ($C'_{Na} = C'_K = 14.2$ or 23.5 mM), S_{NC}(Na/K) decreased with increasing the cation concentration at any given pH, and that in these equimolecular ionic mixtures, the noncompetitive transport selectivity of the cryptand decreased with raising the pH from 7.7 to 8.7.

Table 4 also shows that, at any given pH and external K^+ concentration (C'_K), the competitive transport selectivity (S_C(Na/K)) of the cryptand varied to a greater extent with the external Na^+ concentration (C'_{Na}) than its noncompetitive selectivity (S_{NC}(Na/K)). As an example, when the external Na^+ concentration increased from 0 to 23.5 mM and $C'_K = 14.2$ mM (pH 8.7), the magnitude of the variation of the competitive transport selectivity was 1.5-times higher than that of the noncompetitive (S_{NC}(Na/K)) one.

Calculated S_C(Na / K) / S_{NC}(Na / K)

The ratio between the Na/K competitive (S_C(Na/K)) and noncompetitive (S_{NC}(Na/K)) transport selectivities of cryptands is described by Eq. (A-9) in Appendix A. According to this equation, when $C'_{Na}/K_{mNa} = C'_K/K_{mK}$, the transport selectivity of the carrier at any given pH does not depend on whether Na^+ and K^+ ions are transported as unique or competing substrates (S_C(Na/K)/S_{NC}(Na/K) = 1) (Table 4). On the reverse, when $C'_{Na}/K_{mNa} > C'_K/K_{mK}$, the transport selectivity of (221)C_{10} for Na^+ over K^+ ions is favoured by cation competition compared to transport of these cations as unique substrates (S_C(Na/K)/S_{NC}(Na/K) > 1), whereas it is unfavoured by such a competition (S_C(Na/K)/S_{NC}(Na/K) < 1) when $C'_{Na}/K_{mNa} < C'_K/K_{mK}$.

Table 5

Effects of pH and carrier concentration (C'_M) on fitted n(Na) and calculated theoretical n_{th}(Na) reaction orders in Na

pH	C'_M (μM)	n(Na) at C'_K (mM)			n_{th}(Na) at C'_K (mM)		
		0	14.2	23.5	0	14.2	23.5
7.7	0.1	0.81 ± 0.01	0.34 ± 0.04	0.26 ± 0.06	0.73	0.35	0.25
	0.5	0.68 ± 0.01	0.40 ± 0.03	0.24 ± 0.06	0.75	0.33	0.23
	1.0	0.79 ± 0.01	0.34 ± 0.08	0.20 ± 0.06	0.75	0.33	0.24
8.7	0.1	0.59 ± 0.06	0.30 ± 0.07	0.22 ± 0.06	0.60	0.25	0.17
	0.5	0.58 ± 0.04	0.27 ± 0.06	0.13 ± 0.04	0.61	0.24	0.17
	1.0	0.63 ± 0.01	0.32 ± 0.03	0.19 ± 0.01	0.59	0.26	0.18

Competitive transport of Na^+ and K^+ ions ($C'_{Na} = 2.9$–42.3 mM at $C'_K = 0$ mM; $C'_{Na} = 2.9$–23.5 mM at $C'_K = 14.2$ and 23.5 mM) by 0.1, 0.5 and 1.0 μM (or 0.08, 0.31 and 0.61 mmol/mol lipid, i.e., about 0.2, 0.8 and 1.6 nmol carrier/m^2 surface membrane) (221)C_{10}-cryptand through negatively charged LUV membranes (pH 7.7 and 8.7). Fitted n(Na) values (± S.E.) were means of the slope values of the individual linear log J_i vs. log C'_{Na} regressions established under each set of experimental conditions. n_{th}(Na) values were means of the theoretical reaction orders calculated, according to Eq. (B-5) in Appendix B and using the K_m values reported in Table 2, at all the external Na^+ concentrations (C'_{Na}) used in each set of experimental conditions.

4.3. Reaction order in cation n(S)

Fitted n(Na)

Eq. (B-2) in Appendix B describes the variations in the initial rate (J_i) of Na^+ and K^+ transport when these were transported in competition by $(221)C_{10}$, as a function of the specific concentrations in Na^+ ($\alpha = C'_{Na}/K_{mNa}$) and K^+ ($\beta = C'_K/K_{mK}$). The logarithmic form of this equation is the following:

$$\log J_i/J_{max} = \log k(\beta,X,Y) - n(Na) \cdot \log K_{mNa} + n(Na) \cdot \log C'_{Na} \quad (23)$$

where $k(\beta,X,Y)$ is a constant depending on β, X and Y and $n(Na)$ the reaction order in Na^+, depending on α, β, X and Y. When the Na^+ concentration range was narrow, $\log J_i$ vs. $\log C'_{Na}$ regression was linear.

Table 5 reports the values of the reaction order in Na^+ at various carrier concentrations (0.1, 0.5 and 1.0 μM), external K^+ concentrations (0, 14.2 and 23.5 mM) and pH's (7.7 and 8.7). These fitted $n(Na)$ values were means of the slope values of the individual $\log J_i$ vs. $\log C'_{Na}$ regressions established under each set of experimental conditions. Covariance analysis of the data showed that the slope of the $\log J_i$ vs. $\log C'_{Na}$ regressions decreased significantly with increasing the external K^+ concentration (0 to 23.5 mM) at any given pH and carrier concentration, i.e., the reaction order in Na^+ ($n(Na)$) and therefore the dependence of J_i on the external Na^+ concentration, was influenced to a significant extent by the presence of competing K^+ ions at any given pH and carrier concentration. Statistical analysis also showed that the reaction order in Na^+ ($n(Na)$) decreased significantly with increasing the pH at any given external K^+ and carrier concentration, i.e., the effect of the pH on the dependence of J_i on the external Na^+ concentration was significant at any given K^+ and carrier concentration. Besides, whatever the pH and the external K^+ concentration, the reaction order in Na^+ did not vary significantly with the concentration of $(221)C_{10}$.

Calculated $n_{th}(Na)$

The theoretical reaction orders $n_{th}(Na)$ were means of the reaction orders calculated, using Eq. (B-5) of Appendix B and the apparent K_m values reported in Table 2, at all the external Na^+ concentrations used in each set of experimental conditions (Table 5). In the 5.8 to 23.5 mM Na^+ concentration range the values of these theoretical reaction orders $n_{th}(Na)$ did not differ significantly from those of the fitted reaction orders $n(Na)$.

5. Discussion

At any given pH, the maximal initial rate (J^{pH}_{max}) for the competitive transport of sodium and potassium ions increased nonlinearly with the $(221)C_{10}$ concentration and the maximum turnover rate (T^{pH}_{max}) decreased in the same manner. Concomitantly, the apparent affinity of the cryptand for these cations almost did not vary (similar K^{pH}_m), and therefore the specificity constants T^{pH}_{max}/K^{pH}_m also decreased nonlinearly (Table 1). This nonlinear variation of J^{pH}_{max}, T^{pH}_{max} and T^{pH}_{max}/K^{pH}_m with the carrier concentration was attributed to the effect of electrical repulsion among the positively charged complexes in the lipophilic hydrocarbon region of the membrane [15,17].

Raising the pH from 7.7 to 8.7 slightly enhanced J^{pH}_{max} and T^{pH}_{max}, and reduced the apparent K^{pH}_m for sodium and potassium ions by nearly 14 and 31 mM, respectively (Table 1). Concomitantly, the pH-induced increments in the apparent pK^{pH}_m for Na^+ (0.23) and K^+ (0.32) ions differed slightly although theoretically these increments were expected to be the same whatever the strength of alkali cation binding to the carrier.

According to Devés and Krupka [24], a sharp distinction must be made between competitive or non-competitive mechanisms and competitive or non-competitive kinetics. Cryptands possess two ionizable amine groups inside the intramolecular binding cavity, and therefore protons and alkali cations have the same binding site. At the molecular level, protons are competitive inhibitors of cations, whereas the kinetic form of the inhibition was found here to be a mixed-type inhibition (change in J_{max} and K_m). The kinetic model presented above fairly accounted for the pH-dependence of the Michaelis parameters (J^{pH}_{max} and K^{pH}_m) observed here and the values obtained for the apparent K_{mNa}, K_{mK}, pK_1, k_{MS}/k and k^+/k almost did not vary with the carrier concentration (Table 2). The values reported in Table 2 for the ionization constants of the amine groups of the cryptand were about 3 pH units lower than those determined for these groups in water [23]. This decrement may have mainly arisen from the variation of the dielectric constant within the membrane/solution interface [28–31], and probably also from the fact that the presence of the protonophore was not taken into account in the kinetic model used to fit the data. Besides, the values of the ratio between the rate constants for the translocation of the complexed and free carriers (k_{MS}/k), and of the monoprotonated and free carriers (k^+/k) (Table 2) were of the same order of magnitude than those determined in the case of cation transport by nonactin [32].

The present work showed that the apparent K_m of $(221)C_{10}$ for each cation (K_m(app)) varied with the external concentration of the other (Table 3). Indeed, when present simultaneously on the same side of the membrane, each cation competitively inhibited the binding of the other inside the intramolecular cavity of the cryptand. As Eq. (16) in Kinetic theory shows, the slope of the K_{mNa}(app) vs. C'_K regression is equal to K^{pH}_{mNa}/K^{pH}_{mK} and that of K_{mK}(app) vs. C'_{Na} one, to K^{pH}_{mK}/K^{pH}_{mNa}. Since K^{pH}_{mNa} had lower values than K^{pH}_{mK} at any given pH and carrier concentration, then the strength of K^+ transport inhibition by Na^+ ions was higher than that of Na^+ transport by K^+ ions, i.e., the presence of competing Na^+ ions enhanced to a higher extent the rate-limiting character of interfacial processes of K^+ transport than did competing K^+ ions on that of Na^+ transport.

The competitive transport selectivity (S_C(Na/K)) of $(221)C_{10}$ for Na^+ over K^+ ions was found to reach a maximum value of about 2.5 in the cation and carrier concentration ranges investigated here. This value is lower than those estimated from the experimental data available from published literature [6,9–11]. However, many parameters are known to modulate the transport selectivity of carriers [1–5].

The present results showed that, in equimolecular mixtures of Na^+ and K^+ ions, the Na/K competitive transport selectivity (S_C(Na/K)) of $(221)C_{10}$ reached a value of about 1.5 in the carrier concentration range investigated here. This value was equal to K_{mK}/K_{mNa} (Eq. (A-4) in Appendix A) and did not depend on pH since $K_{mK}^{pH}/K_{mNa}^{pH} = K_{mK}/K_{mNa}$ (Eq. (15) in Kinetic theory).

Due to the existence of reverse physiological K^+ and Na^+ concentration gradients across cellular membranes, an estimation was done of the competitive transport selectivity of $(221)C_{10}$ in the extracellular and intracellular media. The Na/K competitive transport selectivity (S_C(Na/K)) of $(221)C_{10}$ would reach a value of about 40 in the extracellular medium while its K/Na competitive selectivity would have a value of about 20 in the intracellular medium. Such potentially high values for the competitive transport selectivity of $(221)C_{10}$ in physiological media suggest that this carrier would behave in a cell like an efficient non-electrogenic ion gradient-dissipating Na/K exchanger.

The present work also compared the competitive (S_C(Na/K)) and noncompetitive (S_{NC}(Na/K)) transport selectivities of $(221)C_{10}$ under the same experimental conditions. Due to the fact that, when present simultaneously, each ion behaved as a competitive inhibitor with respect to the other, the equations describing the variations of these selectivities with the cation concentrations, the carrier affinities for the ions (K_m) and the pH, were different. An important conclusion was that, depending on the specific concentrations of Na^+ (C'_{Na}/K_{mNa}) and K^+ (C'_K/K_{mK}) ions in the samples, the Na/K transport selectivity of the cryptand at any given pH was enhanced, independent or lowered by cation competition compared to transport of cations as unique substrates of the carrier.

The reaction order in substrate n(S) is a parameter characterizing the strength of the dependence of the reaction rate J_i on the substrate concentration (C'_S). Its experimental value is equal to that of the slope of the log J_i vs. log C'_S regression, the latter being usually linear when the substrate concentration range investigated is narrow ($n(S) = d(\log J_i)/d(\log C'_S)$). The knowledge of n(S), and of its possible variations with some parameters of the system, may offer an approach to the understanding of the molecular mechanisms underlying the functioning of biological systems. In the case of saturation processes however, it is well known that the variation of log J_i vs. log C'_S is curvilinear. The value for n(S) therefore varies with the substrate concentration, and it appears of interest to derive the equation describing this relationship. This can be done rather simply when the equation relating J_i to C'_S is known and can be approximated by a power function (Appendix B). The equation of the theoretical reaction order in substrate n_{th}(S) allows thus the calculation of its exact value at any given substrate concentration.

The values determined here for the reaction order in Na^+ depended on pH and on the specific concentrations of Na^+ ($\alpha = C'_{Na}/K_{mNa}$) and K^+ ($\beta = C'_K/K_{mK}$) ions, whereas they did not vary with the carrier concentration (Table 5).

The reaction order in Na^+ was shown here to decrease with increasing the external K^+ concentration, whereas it decreased with increasing that of protons (see below). Although similar variations could have been expected since both entities bind to the cryptand and are carried across the membrane, such a finding suggested that K^+ ions behaved here like alternative substrates, whereas external protons behaved like competitive inhibitors (due to the existence of a pH-gradient inducing an efflux of protons carried by the protonophore FCCP at a non-limiting rate).

When the external K^+ concentration increased, the reaction order in Na^+ decreased at any given pH and carrier concentration, i.e., whatever the pH and the carrier concentration, the higher the external K^+ concentration, the lower the variation of the initial rate (J_i) of cation influxes into LUV's with the external Na^+ concentration (Table 5). Two factors, acting in opposite directions, may have contributed to this result: (i) when the external K^+ concentration increased, the quantity of free carriers available at the external interface to bind Na^+ ions decreased; thus, for a given variation in the external Na^+ concentration, the variation induced in the quantity of Na^+-$(221)C_{10}$ complexes and therefore the reaction order in Na^+, decreased with increasing the external K^+ concentration; and (ii) the apparent K_m of $(221)C_{10}$ for Na^+ ions increased with the external K^+ concentration (Table 3); consequently, the rate-limiting character of the interface processes and therefore the reaction order in Na^+, increased with the external K^+ concentration. The variation in n(Na) with the external K^+ concentration must therefore mainly have been due to the K^+-induced change in the quantity of free carriers at the external membrane/solution interface.

When the external H^+ concentration increased (pH′ decreased from 8.7 to 7.7, and pH″ from 7.7 to 6.7), the reaction in Na^+ increased at any given external K^+ and carrier concentrations, i.e., whatever the carrier and external K^+ concentration, the higher the external H^+ concentration, the higher the dependence of the initial rate (J_i) of cation influxes into LUV's on the external Na^+ concentration (Table 5). Again, the above two factors may have contributed to this result. In this case however, since protons in the external aqueous solution essentially behaved like competitive inhibitors (and not alternative substrates; see above) of Na^+ transport, their effect on the rate-limiting character of interfacial processes was predominant. Consequently, the variation in n(Na) with the pH must mainly have been due to the pH-induced change in the apparent K_{mNa} of $(221)C_{10}$. This was confirmed by the fact that, whatever the pH, the calculated theoretical values for the reaction orders in Na^+ (n_{th}(Na)), were in fair agreement with the fitted n(Na) values.

Whatever the pH and the external K^+ concentration, the reaction order in Na^+ was independent of the carrier concentration, although it might have been expected to decrease at high carrier concentrations, due to the electrical repulsion effect. This result suggested that as the carrier concentration increased, almost no change was induced in the variation of the electrical repulsion effect with the cation concentration.

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Appendix

A. Na / K competitive (S_C(Na / K)) and noncompetitive (S_{NC}(Na / K)) transport selectivity of $(221)C_{10}$-cryptand: J_{Na} / J_K

The Na/K transport selectivity of $(221)C_{10}$ is equal to the ratio of the individual initial influxes of sodium (J_{Na}) and potassium (J_K) ions. It is called here ‘competitive transport selectivity’ when both ions are present simultaneously, i.e., when each cation is competitively inhibited by the other (true competition experiments) or ‘noncompetitive transport selectivity’ when ions are transported as unique substrates of the carrier, i.e., in the absence of the competitive inhibition of each cation by the other (separate experiments).

Na / K competitive transport selectivity (S_C(Na / K))

Eq. (8) in Kinetic theory can be rearranged in order to evidence the individual initial influxes of competing sodium (J_{Na}) and potassium (J_K) ions as follows:

$$J_i = \frac{J_{maxNa} \cdot C'_{Na}/K_{mNa}}{X + Y \cdot C'_{Na}/K_{mNa} + Y \cdot C'_K/K_{mK}} + \frac{J_{maxK} \cdot C'_K/K_{mK}}{X + Y \cdot C'_{Na}/K_{mNa} + Y \cdot C'_K/K_{mK}} \qquad \text{(A-1)}$$

where J_{maxNa}, K_{mNa} and C'_{Na} are the maximum value of the initial reaction rate with Na^+ as unique substrate of the cryptand, the Michaelis constant of Na^+ and the concentration of Na^+, respectively, and the same for K^+, and where X and Y are defined by Eqs. (9) and (10) in Kinetic theory.

Therefore, since $J_{maxNa} = J_{maxK}$ [15,17], the competitive Na/K transport selectivity of the cryptand, i.e., J_{Na}/J_K is given by

$$S_C(\text{Na/K}) = (C'_{Na}/K_{mNa})/(C'_K/K_{mK}) \qquad \text{(A-2)}$$

and, according to Eq. (15) in Kinetic theory, it does not depend on pH since $K^{pH}_{mNa}/K^{pH}_{mK} = K_{mNa}/K_{mK}$.

Let the specific concentrations of Na^+ and K^+ be $\alpha = C'_{Na}/K_{mNa}$ and $\beta = C'_K/K_{mK}$, respectively, thus when $C'_{Na} \neq C'_K$

$$S_C(\text{Na/K}) = \alpha/\beta \qquad \text{(A-3)}$$

and when $C'_{Na} = C'_K$, then

$$S_C(\text{Na/K}) = K_{mK}/K_{mNa} = a \qquad \text{(A-4)}$$

Na / K noncompetitive transport selectivity (S_{NC}(Na / K))

When only one substrate is present, Eq. (A-1) reduces to:

$$J_{Na} = J_{maxNa} \cdot C'_{Na}/(X \cdot K_{mNa} + Y \cdot C'_{Na}) \qquad \text{(A-5)}$$

and the same for K^+.

The noncompetitive Na/K transport selectivity of the cryptand, i.e., J_{Na}/J_K, is given by

$$S_{NC}(\text{Na/K}) = C'_{Na}(K_{mK} + C'_K Y/X) / C'_K(K_{mNa} + C'_{Na} Y/X) \qquad \text{(A-6)}$$

When $C'_{Na} \neq C'_K$, the variations of S_{NC}(Na/K) with the specific concentrations of Na^+ and K^+ are described by

$$S_{NC}(\text{Na/K}) = \alpha(1 + \beta Y/X)/\beta(1 + \alpha Y/X) \qquad \text{(A-7)}$$

and when $C'_{Na} = C'_K$, then

$$S_{NC}(\text{Na/K}) = (a + \alpha Y/X)/(1 + \alpha Y/X) \qquad \text{(A-8)}$$

Ratio between the Na / K competitive and noncompetitive transport selectivities: S_C(Na / K) / S_{NC}(Na / K)

Combination of Eqs. (A-2) and (A-6) gives that expressing the ratio between the Na/K competitive and noncompetitive transport selectivities of the cryptand:

$$S_C(\text{Na/K})/S_{NC}(\text{Na/K}) = (1 + \alpha Y/X)/(1 + \beta Y/X) \qquad \text{(A-9)}$$

When $\alpha = \beta$, the Na/K transport selectivity of the carrier does not depend on whether Na^+ and K^+ ions are transported as unique or competing substrates of the cryptand. On the reverse, when $\alpha > \beta$, its selectivity for Na^+ over K^+ ions is enhanced by cation competition compared to transport of the cations as unique substrates, i.e., $S_C(\mathrm{Na/K})/S_{NC}(\mathrm{Na/K}) > 1$, whereas when $\beta > \alpha$ the reverse occurs.

When $C'_{\mathrm{Na}} = C'_{\mathrm{K}}$, Eq. (A-9) may be transformed as follows:

$$S_C(\mathrm{Na/K})/S_{NC}(\mathrm{Na/K}) = a\cdot(1+\alpha Y/X)/(a+\alpha Y/X) \qquad \text{(A-10)}$$

B. Reaction order in cation : *n*(S)

As Eq. (A-1) in Appendix A shows, the competitive transport of $\mathrm{Na^+}$ and $\mathrm{K^+}$ ions by $(221)\mathrm{C_{10}}$ may be described by

$$J_i/J_{\max} = (\alpha+\beta)/(X+\alpha Y+\beta Y) \qquad \text{(B-1)}$$

where $\alpha = C'_{\mathrm{Na}}/K_{\mathrm{mNa}}$ and $\beta = C'_{\mathrm{K}}/K_{\mathrm{mK}}$.

When varying in a limited range the external $\mathrm{Na^+}$ concentration (C'_{Na}), this equation may be approximated by

$$J_i/J_{\max} = k(\beta,X,Y)\cdot\alpha^{n(\mathrm{Na})} \qquad \text{(B-2)}$$

where $k(\beta,X,Y)$ is a constant depending on β, X and Y and $n(\mathrm{Na})$ the reaction order in $\mathrm{Na^+}$ depending on α, β, X and Y values. It must be underlined that Eq. (A-2) is valid only when $\alpha > 0$.

Combination of Eqs. (B-1) and (B-2) gives

$$J_i/J_{\max} = (\alpha+\beta)/(X+\alpha Y+\beta Y) = k(\beta,X,Y)\cdot\alpha^{n(\mathrm{Na})} \qquad \text{(B-3)}$$

The value of $k(\beta,X,Y)$ may be calculated by setting $\alpha = 1$ in Eq. (B-3), and therefore

$$k(\beta,X,Y) = (1+\beta)/(X+Y+\beta Y) \qquad \text{(B-4)}$$

The value of the reaction order in Na, i.e., $n(\mathrm{Na})$, is equal to the slope of the $\log(\alpha+\beta)/(X+\alpha Y+\beta Y)$ vs. $\log\alpha$ curve, for a given value of α. The value of this derivative is the following:

$$n(\mathrm{Na}) = \alpha/[(\alpha+\beta)\cdot(1+\alpha Y/X+\beta Y/X)] \qquad \text{(B-5)}$$

Consequently, Eq. (B-2) may be expressed as follows:

$$J_i/J_{\max} = [(1+\beta)/(X+Y+\beta Y)] \cdot\alpha^{\alpha/[(\alpha+\beta)\cdot(1+\alpha Y/X+\beta Y/X)]} \qquad \text{(B-6)}$$

In the absence of competing $\mathrm{K^+}$ ions ($\beta = 0$), Eq. (B-6) reduces to Eq. (B-7) which is valid in the entire range of α values, from 0 to ∞:

$$J_i/J_{\max} = [1/(X+Y)]\cdot\alpha^{1/(1+\alpha Y/X)} \qquad \text{(B-7)}$$

and then, the pH-dependence of $n(\mathrm{Na})$ is described by

$$n(\mathrm{Na}) = 1/(1+\alpha Y/X) \qquad \text{(B-8)}$$

Eq. (B-8) shows that when $C'_{\mathrm{Na}} \ll K_{\mathrm{mNa}}$, $C'_{\mathrm{Na}} = K_{\mathrm{mNa}}$ and $C'_{\mathrm{Na}} \gg K_{\mathrm{mNa}}$, then $n(\mathrm{Na}) = 1$, $1/(1+Y/X)$ and 0, respectively.

In the absence of protons ($X = Y = 1$), Eq. (B-6) reduces to Eq. (B-9) which is valid only when $\alpha > 0$.

$$J_i/J_{\max} = [(1+\beta)/(2+\beta)]\cdot\alpha^{\alpha/[(\alpha+\beta)\cdot(1+\alpha+\beta)]} \qquad \text{(B-9)}$$

and then, the $\mathrm{K^+}$-dependence of $n(\mathrm{Na})$ is described by

$$n(\mathrm{Na}) = \alpha/[(\alpha+\beta)\cdot(1+\alpha+\beta)] \qquad \text{(B-10)}$$

References

[1] Dobler, M. (1981) in Ionophores and Their Structures (Wiley-Interscience Publication, ed.), John Wiley and Sons, New York.

[2] Haynes, D.H. and Pressman, B.C. (1974) J. Membr. Biol. 18, 1–21.

[3] Haynes, D.H., Wiens, T. and Pressman, B.C. (1974) J. Membr. Biol. 18, 23–38.

[4] Mueller, P. and Rudin, D.O. (1967) Biochem. Biophys. Res. Commun. 26, 398–404.

[5] Behr, J.P., Kirch, M. and Lehn, J.M. (1985) J. Am. Chem. Soc. 107, 241–246.

[6] Hackette, S.L., Skye, G.E., Burton, C. and Segel, I.H. (1970) J. Biol. Chem. 245, 4241–4250.

[7] Debono, M., Molloy, R.M., Dorman, D.E., Paschal, J.W., Babcock, D.F., Deber, C.M. and Pfeiffer, D.R. (1981) Biochemistry 20, 6865–6872.

[8] Aickin, C.C. and Brading, A.F. (1985) J. Physiol. 366, 267–280.

[9] Simchowitz, L., Ratzlaff, R. and De Weer, P. (1986) J. Gen. Physiol. 88, 195–217.

[10] Simchowitz, L. (1988) J. Gen. Physiol. 91, 835–860.

[11] Zeevi, A. and Margalit, R. (1985) J. Membr. Biol. 86, 61–67.

[12] Riddell, F.G., Arumugam, S., Brophy, P.J., Cox, B.G., Payne, M.C.H. and Southon, T.E. (1988) J. Am. Chem. Soc. 110, 734–738.

[13] Prabhananda, B.S. and Kombrabail, M.H. (1992) Biochim. Biophys. Acta 1106, 171–177.

[14] Mulliert, G., Hill, M., Loiseau, A. and Castaing, M. (1994) Biochim. Biophys. Acta 1193, 263–275.

[15] Castaing, M. and Lehn, J.M. (1987) J. Membr. Biol. 97, 79–95.

[16] Clement, D., Damm, F. and Lehn, J.M. (1976) Heterocycles 5, 477–484.

[17] Castaing, M., Morel, F. and Lehn, J.M. (1986) J. Membr. Biol. 89, 251–267.

[18] Castaing, M., Kraus, J.L., Beaufils, P. and Ricard, J. (1991) Biophys. Chem. 41, 203–215.

[19] Wehrli, S., Ramirez, C., Kraus, J.L. and Castaing, M. (1992) Biochim. Biophys. Acta 1107, 319–330.

[20] Vareille, G., Marion, P., Kraus, J.L. and Castaing, M. (1993) Biochim. Biophys. Acta 1146, 25–37.

[21] Kirch, M. (1980) in Thèse de Doctorat ès Sciences Physiques, pp. 38–95. Strasbourg, France.

[22] Segel, I.H. (1975) in Enzymes Kinetics (Wiley-Interscience Publication, ed.) pp. 113–118, John Wiley and Sons, New York.

[23] Lehn, J.M. and Sauvage, J.P. (1975) J. Am. Chem. Soc. 97, 6700–6707.

[24] Devés, R. and Krupka, R.M. (1978) Biochim. Biophys. Acta 510, 186–200.

[25] Cox, B.G., Garcia-Rosas, J. and Schneider, H. (1981) J. Am. Chem. Soc. 103, 1054–1059.

[26] Pizer, R. (1978) J. Am. Chem. Soc. 100, 4239–4241.

[27] Läuger, P. and Stark, G. (1970) Biochim. Biophys. Acta 211, 458–466.

[28] Ptak, M., Egret-Charlier, M., Sanson, A. and Bouloussa, O. (1980) Biochim. Biophys. Acta 600, 387–397.

[29] Bouloussa, O., Michel, J. and Dupeyrat, M. (1982) in Physical Chemistry of Transmembrane Ion Motions (Spach, G., ed.), pp. 87–95, Elsevier, Amsterdam.

[30] Tocanne, J. and Tessié, J. (1990) Biochim. Biophys. Acta 1031, 111–142.

[31] Bjerrum, P.J. (1992) J. Gen. Physiol. 100, 301–339.

[32] Laprade, R., Grenier, F., Lapointe, J. and Asselin, S. (1982) J. Membr. Biol. 68, 191–206.