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Transport of competing Na and K ions by (222)C_{10}-cryptand, an ionizable mobile carrier: effects of pH and temperature

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Abstract

The kinetics of the electroneutral exchange of competing sodium and potassium with protons across the membrane of large unilamellar vesicles (LUV) were determined at two pH values when transport was induced by the simultaneous presence of (222)C_{10}-cryptand and FCCP (proton carrier) at various temperatures. The aim of the present work was to quantify the pH-dependent enthalpies of an ionizable mobile carrier affinities for competing alkali cations, and to focus on the effects of pH and temperature on the competitive transport selectivity of the carrier for K^+ over Na^+ ions. At any given temperature and pH, the apparent pH-dependent affinity of (222)C_{10} was higher for K^+ than for Na^+. The enthalpy of this affinity for K^+ was significantly lower than that for Na^+, whereas it varied similarly with the pH ($\Delta H(K_{mK}^{pH})$ = 32.8 and 37.0 kJ/mol, and $\Delta H(K_{mNa}^{pH})$ = 47.9 and 52.9 kJ/mol at pH 7.8 and 8.8, respectively). When using a kinetic model, the pH effect on these parameters was discriminated ($\Delta H(K_{mK})$ = 37.9 kJ/mol and $\Delta H(K_{mNa})$ = 53.9 kJ/mol). The pH-dependence of the $\Delta H(K_m^{pH})$ of the cations could therefore theoretically be shown to arise from the temperature-induced changes in the ionization of the buffer dissolved in the aqueous phases and of the amine groups of the binding cavity of the carrier. The K/Na competitive transport selectivity (S_c(K/Na)) of (222)C_{10} increased linearly with the K^+ concentration. It decreased hyperbolically with increasing concentration of Na^+ while being independent of pH at any given temperature. In equimolecular ionic mixtures, S_c(K/Na) varied from 2.2 to 3.0 when temperature rose from 20°C to 35°C ($\Delta H(S_c(K/Na))$ = 15.6 ± 0.5 kJ/mol). The results are discussed in terms of the structural, physico-chemical and electrical characteristics of carriers and complexes.

Keywords: Ionizable mobile carrier; Alkali cation transport kinetics; Competitive transport selectivity; pH; Temperature; 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. However, 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 [11–15] membranes.

To shed light on the predominant parameters modulating the competitive selectivity of ion transport by mobile carriers, and more specifically by those which ionize, it seemed to be of interest to study the transport of K^+ and Na^+ ions by (222)C_{10}-cryptand at various pH values and temperatures when both ions were present simultaneously.

Abbreviations: (222)C_{10}-cryptand, 1,10-diaza-5-decyl-4,7,13,21,24-hexaoxa-bicyclo[8.8.8]hexacosane; S, substrate; C_S, substrate concentration; (′), external media; (″), internal media; k, translocation rate constant of the unprotonated carrier (M); k^+, translocation rate constant of the monoprotonated carrier (MH); k_{MS}, translocation rate constant of the cation-carrier complex (MS); k_a, association rate constant; k_d, dissociation rate constant; K_d, dissociation constant; J_i, initial rate of cation influx; J_{max} and J_{max}^{pH}, pH-independent and pH-dependent maximal initial rates of cation influx; T_{max} and T_{max}^{pH}, pH-independent and pH-dependent maximal turnover rates of cation influx; K_m and K_m^{pH}, pH-independent and pH-dependent Michaelis constants; pK_m and pK_m^{pH}, negative logarithmic values of the pH-independent and pH-dependent Michaelis constants; $\Delta H(K_m)$ and $\Delta H(K_m^{pH})$, enthalpies of the pH-independent and pH-dependent Michaelis constants; $E(T_{max})$ and $E(T_{max}^{pH})$, activation energies of the pH-independent and pH-dependent maximal turnover rates of cation influx; $\Delta H(K_{d1})$ and $\Delta H(K_{d2})$, enthalpies of the first and second protonations of the cryptand; S_c(K/Na), competitive transport selectivity of the cryptand for K^+ over Na^+ ions (J_K / J_{Na}); $\Delta H(S_c(K/Na))$, enthalpy of the competitive transport selectivity of the cryptand.

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Transport of K^+ and Na^+ deserves special attention in view of the very important biological role of these cations. Besides, it has already been shown that $(222)C_{10}$ transports both of them in separated experiments and that the efficiency of cation transport by this carrier depends on pH [16]. Furthermore and owing to the existence of physiological thermal gradients which may reach 20°C in man [17], an appropriate evaluation of the transport selectivity of ionophores, at variable temperature, needs to be studied.

The synthetic macrobicyclic polyaminoether $(222)C_{10}$-cryptand, i.e., the 1,10-diaza-5-decyl-4,7,13,16,21,24-hexaoxa-bicyclo[8.8.8]hexacosane [18], 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 fundamental point of view, cryptands are very interesting examples of mobile carriers [16,19–22]. 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 K^+ and Na^+ transport as unique substrates of $(222)C_{10}$ ('Zero trans influx' experiments), we reported that the ratio between the cations transport rates (J_K/J_{Na}) decreased as the concentrations of K^+ and Na^+ at equal concentrations rose [16]. This finding appeared to be fairly compatible with the fact that the stability of $(222)C_{10}$ complexes is greater when formed with K^+ than with Na^+ [23]. This J_K/J_{Na} ratio also decreased when the 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 is the first to focus on the pH-dependence of the enthalpy for the apparent affinity of an ionizable mobile carrier for alkali cations and on the enthalpy for its ionic transport selectivity. It quantifies the pH-and temperature-induced variations of the kinetic parameters of K^+ and Na^+ transport by the $(222)C_{10}$-cryptand in competition experiments. A theoretical treatment of the effect of the temperature on the apparent affinity of the carrier for K^+ and Na^+, and of its pH-dependence is also presented. The results are discussed in terms of the structural and electrical characteristics of carrier and complex,

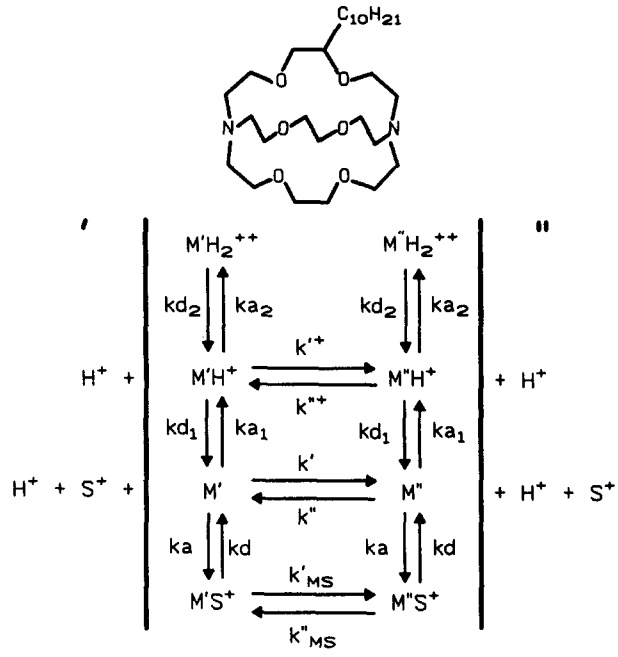

Fig. 1. Reaction scheme of cation transport (S^+) mediated by $(222)C_{10}$-cryptand, a carrier possessing three ionization states at the external (') and internal (") interfaces of the membrane: unprotonated (M), monoprotonated (MH^+) and diprotonated (MH_2^{2+}).

Table 1
Effect of temperature T (in °C) and pH on the apparent Michaelis parameters (J_{max}^{pH}, K_m^{pH}) and K_{mNa}^{pH}/K_{mK}^{pH} ratio for the competitive transport of K^+ and Na^+ ions by $(222)C_{10}$-cryptand

T (°C)	pH	J_{max}^{pH} (nmol/s)	K_{mK}^{pH} (mM)	K_{mNa}^{pH} (mM)	K_{mNa}^{pH}/K_{mK}^{pH}
20	7.91 ± 0.02	0.9 ± 0.0	16.1 ± 1.6	35.5 ± 0.4	2.23 ± 0.25
25	7.80 ± 0.02	1.2 ± 0.0	19.0 ± 0.5	50.5 ± 0.8	2.66 ± 0.03
30	7.79 ± 0.03	1.4 ± 0.0	26.1 ± 0.5	72.6 ± 1.9	2.78 ± 0.02
35	7.72 ± 0.01	1.8 ± 0.0	29.5 ± 1.6	88.9 ± 3.3	3.02 ± 0.06
20	8.81 ± 0.05	1.1 ± 0.0	11.4 ± 0.3	24.7 ± 1.7	2.18 ± 0.11
25	8.84 ± 0.04	1.3 ± 0.0	13.7 ± 0.1	33.9 ± 3.3	2.48 ± 0.25
30	8.75 ± 0.08	1.6 ± 0.1	18.1 ± 0.8	48.6 ± 2.6	2.68 ± 0.04
35	8.68 ± 0.11	2.0 ± 0.0	23.5 ± 2.3	70.1 ± 4.4	3.00 ± 0.11

Competitive transport of K^+ and Na^+ ions (C'_K = 2.9–42.3 mM at C'_{Na} = 0 mM; C'_K = 2.9–24.0 mM at C'_{Na} = 14.2 and 24.2 mM) by 0.5 μM (or 0.31 mmol/mol lipid, i.e., about 0.8 nmol carrier/m^2 surface membrane) $(222)C_{10}$-cryptand through negatively charged LUV membranes at 20, 25, 30 and 35°C (pH 7.8 and 8.8). Means (±S.E.) were determined by the simultaneous fitting of the data obtained C'_{Na} = 0, 14.2 and 24.2 mM from two LUV preparations.

and the interactions occurring between an ionizable cryptand and the membrane.

2. Materials and methods

(222)C_{10}-cryptand was from Merck (Darmstadt, W. Germany). 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 Bistris-propane and 0.67 M D-mannitol ($\omega'' = 0.79$ M) (pH 6.8 or 7.8 at 25°C). Salt solutions were 0.585 M K_2SO_4 and 0.585 M Na_2SO_4 ($\omega = 1.0$ M). FCCP was dissolved in absolute ethanol, and (222)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 [14,15].

Proton outfluxes were measured as follows: 0.5 ml LUV suspension was added to 3.5 ml external solution in the titrating vessel and equilibrated at 20, 25, 30 and 35°C under nitrogen stream. The external pH ($pH' = 6.8$ and 7.8), 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 7.8 and 8.8. Owing to the temperature-dependence of the buffer pH (about -0.01 pH unit/°C) only its values at 25°C are given in the following for simplification (Table 1). 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 (222)C_{10} to a final concentration of 0.5 μM (or 0.31 mmol/mol lipid, i.e., about 0.8 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. At each pH investigated (7.8 and 8.8), the external K^+ concentrations were varied from 2.9 to 42.3 mM in the absence of Na^+ ions, and from 2.9 to 24.0 mM when the external Na^+ concentration was maintained constant at levels of 14.2 and 24.2 mM, respectively. Such experiments were performed on two different LUV preparations at each pH and temperature. 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 1 mM 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 [24], the variations with the external potassium concentration (C'_K) in the proton efflux (J_i) were fitted by the following equation:

$$J_i = \frac{J^{pH}_{maxK} \cdot C'_K + J^{pH}_{maxNa} \cdot C'_{Na}\left(K^{pH}_{mK}/K^{pH}_{mNa}\right)}{K^{pH}_{mK}\left(1 + C'_{Na}/K^{pH}_{mNa}\right) + C'_K} \quad (1)$$

where J^{pH}_{maxK} and J^{pH}_{maxNa} are the apparent pH-dependent maximal velocities with K^+ and Na^+ ions as unique substrates, K^{pH}_{mK} and K^{pH}_{mNa}, the pH-dependent Michaelis constants of (222)C_{10} for K^+ and Na^+, respectively, and C'_{Na}, the external aqueous concentration of Na^+ ions. In the case of cryptand-mediated transport, the maximal initial rate (J^{pH}_{max}) is independent of the nature of the alkali cation transported ($J^{pH}_{maxK} = J^{pH}_{maxNa}$) [16,19]. The Michaelis parameters (J^{pH}_{max}, K^{pH}_{mK} and K^{pH}_{mNa}) were determined by the simultaneous fitting of the J_i vs. C'_K curves obtained at $C'_{Na} = 0$, 14.2 and 24.2 mM from the study of two different LUV preparations.

Regression lines were calculated using the least-squares 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 (222)C_{10}-cryptand (Fig. 1) has already been described in detail elsewhere [16]. This cryptand forms inclusion complexes with K^+ or Na^+ ions in which the cation is contained inside the intramolecular binding cavity. Owing to the radius of the cavity (1.4 Å), the binding of K^+ (ionic radius 1.33 Å) and Na^+ (ionic radius 0.98 Å) ions is exclusive [25]. At the pH values investigated here (from 7.8 to 8.8), the carrier containing two ionizable tertiary amine groups exists in three different states of ionization: unprotonated (M), monoprotonated (MH^+) and diprotonated (MH_2^{2+}). It has also been shown that protonation destroys the cation-carrier complex since protons also bind inside the cavity. Therefore, only unprotonated carrier (M) is able to bind alkali cations (S^+) [25].

In the present study, S^+ represented K^+ or Na^+ ions as competing substrates of the cryptand [16,25]. Kinetic equations have been derived recently according to the model by Devés and Krupka [26] for reversible inhibition of facilitated transport systems [15]. In deriving these equations, 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 [27,28].

(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 K^+ and Na^+ transport rates (on which this study focused) [16]. As a result and owing to the high number of parameters that must be determined from fitting experimental data, the presence of the photonophore 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 buried to a large extent inside the intramolecular binding cavity of the carrier [14,25].

(4) Owing to its highly hydrophilic nature, the diprotonated carrier (MH_2^{2+}) did not cross the lipophilic region of the membrane [23].

The expression for the rate of K^+ transport is given by

$$dC''_K/dt = k'_{MK}[M'K] - k''_{MK}[M''K] \quad (2)$$

A similar equation may be written for Na^+ 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 ($C''_K = C''_{Na} = 0$), the variations of the overall cation transport rate (J_i) with the external K^+ concentration is described by

$$J_i = \frac{(J_{maxK}/Y)C'_K + (J_{maxNa}/Y)C'_{Na}(K_{mK}/K_{mNa})}{K_{mK}(X/Y + C'_{Na}/K_{mNa}) + C'_K} \quad (3)$$

with

$$X = A/\left(1 + k''^{+}C''_H/k''K_{d1}\right)$$

$$A = 1 + [k''/(k' + k'')]\left[C'_H/K_{d1} + C'^2_H/K_{d1}K_{d2}\right] + [k'/(k' + k'')]\left[C''_H/K_{d1} + C''^2_H/K_{d1}K_{d2}\right] + [k''^{+}/(k' + k'')]\left[(C''_H/K_{d1}) \times \left(1 + C'_H/K_{d1} + C'^2_H/K_{d1}K_{d2}\right)\right] + [k'^{+}/(k' + k'')]\left[(C'_H/K_{d1}) \times \left(1 + C''_H/K_{d1} + C''^2_H/K_{d1}K_{d2}\right)\right] \quad (4)$$

$$Y = B/\left(1 + k''^{+}C''_H/k''K_{d1}\right)$$

$$B = 1 + [k'_{MS}/(k'' + k'_{MS})] \times \left(k''^{+}C''_H/k'_{MS}K_{d1} + C''_H/K_{d1} + C''^2_H/K_{d1}K_{d2}\right) \quad (5)$$

$$J_{max} = k'_{MS} \cdot M_t/(1 + k'_{MS}/k'') \quad (6)$$

$$K_{mS} = K'_S[(1 + k'/k'')/(1 + k'_{MS}/k'')] \quad (7)$$

and therefore, the apparent Michaelis parameters for K^+ transport by (222)C_{10}-cryptand are given by

$$J_{maxK}(app) = J_{maxK}/Y = J^{pH}_{maxK} \quad (8)$$

$$K_{mK}(app) = K_{mK}(X/Y + C'_{Na}/K_{mNa}) \quad (9)$$

Let

$$K^{pH}_{mK} = K_{mK}X/Y \quad (10)$$

then

$$K_{mK}(app) = K^{pH}_{mK}\left(1 + C'_{Na}/K^{pH}_{mNa}\right) \quad (11)$$

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. (12) below), and consequently those of J_{max} (Eq. (6)) and J_i (Eq. (3)), fall to zero.

Since K^+ and Na^+ complexes with cryptands are large organic cations of the same size and shape, and apparent electrical charge, then $J_{maxK} = J_{maxNa}$ [16,19]. 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. If a constant field strength is assumed in the membrane [29], then

$$k'_{MS} = k_{MS} \cdot e^{+u/2} \quad (12)$$

$$k''_{MS} = k_{MS} \cdot e^{-u/2} \quad (13)$$

$$k'^{+} = k^{+} \cdot e^{+u/2} \quad (14)$$

$$k''^{+} = k^{+} \cdot e^{-u/2} \quad (15)$$

($u = -E_m \cdot F/R \cdot T$ and E_m, F, R, T are membrane potential, Faraday, gas constant and absolute temperature). Besides, protons like alkali cations are buried 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 hydrophilic 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 potassium (Φ_K) and sodium (Φ_{Na}) ions carried by the cryptand. The proton and cation fluxes were related by

$$\Phi_K + \Phi_{Na} = -\Phi_H = \Phi_{MS} \quad (16)$$

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 $(222)C_{10}$ and FCCP. In the absence of $(222)C_{10}$ 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 temperature. This result was in agreement with the fact that $(222)C_{10}$ has been found to exhibit saturation of the transport rate as a function of K^+ concentration [16].

The initial rates (J_i) of cation translocation were determined at 20, 25, 30 and 35°C and two pH levels (pH 7.8 and 8.8) on two different LUV preparations under each set of experimental conditions. As the pH of the buffer dissolved in the aqueous phases varied by about −0.01 pH unit/°C (Table 1), then only its values at 25°C (7.8 and 8.8) are given in the following for simplification. Owing to the high number of experimental conditions investigated (152 sets), the J_i values determined here are not presented except, for illustration, those obtained on one LUV preparation (Fig. 2). The data given below correspond therefore to fitted or calculated data, and in each case this will be specified.

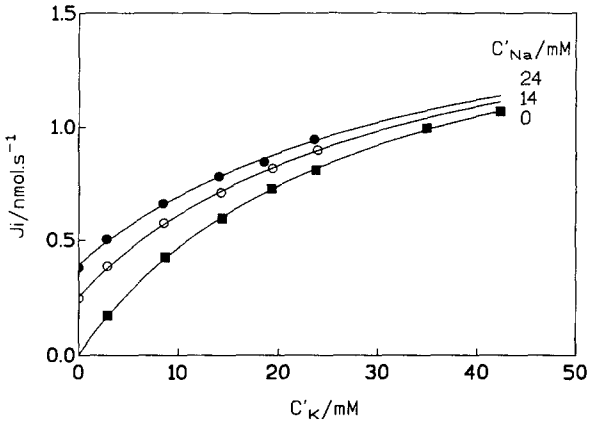

Fig. 2. Dependence of the initial influx (J_i) of competing K^+ and Na^+ ions on the external K^+ concentration (C'_K): competitive transport of K^+ and Na^+ ions (C'_K = 2.9–42.3 mM at C'_{Na} = 0 mM (squares); C'_K = 2.9–24.0 mM at C'_{Na} = 14.2 (open circles) and 24.2 mM (filled circles)) by 0.5 μM (or 0.31 mmol/mol lipid, i.e., about 0.8 nmol carrier/m^2 surface membrane) $(222)C_{10}$-cryptand through negatively charged LUV membranes at 35°C (pH 7.8). The curves drawn in this figure were determined according to Eq. (1) in Materials and methods, by the simultaneous fitting of the experimental data obtained at C'_{Na} = 0, 14.2 and 24.2 mM on one LUV preparation. Each point is the result on one measurement.

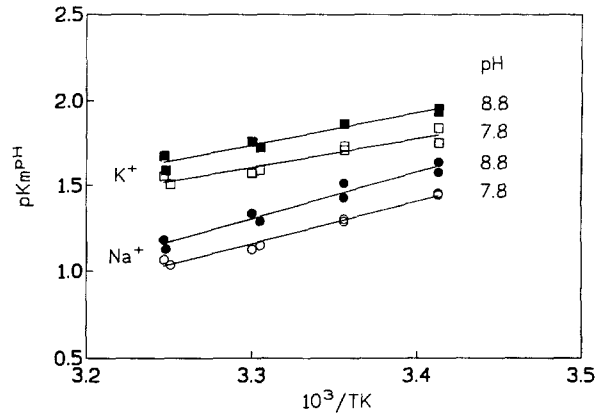

Fig. 3. Temperature dependence of the apparent Michaelis constants (K_m^{pH}) of K^+ and Na^+ ions at variable pH: Vant'Hoff plot of pK_m^{pH} for transport of competing K^+ (squares) and Na^+ (circles) ions (C'_K = 2.9–42.3 mM at C'_{Na} = 0 mM; C'_K = 2.9–24.0 mM at C'_{Na} = 14.2 and 24.2 mM) by 0.5 μM (or 0.31 mmol/mol lipid, i.e., about 0.8 nmol carrier/m^2 surface membrane) $(222)C_{10}$-cryptand through negatively charged LUV membranes at 20, 25, 30 and 35°C (pH 7.8 (open symbols) and 8.8 (filled symbols)). Each point corresponds to the K_m^{pH} value obtained from the study of one LUV preparation. $\Delta H(K_{mK}^{pH}) = 32.8 \pm 4.1$ and 37.0 ± 3.5 kJ/mol, and $\Delta H(K_{mNa}^{pH}) = 47.9 \pm 2.7$ and 52.9 ± 4.4 kJ/mol at pH 7.8 and 8.8, respectively.

4.1. Michaelis parameters and their temperature dependence

Fitted K_m^{pH}, J_{max}^{pH}, $E(T_{max}^{pH})$ and $\Delta H(K_m^{pH})$

The apparent pH-dependent Michaelis parameters were determined by the simultaneous fitting of the J_i vs. C'_K plots of the experimental data obtained from two LUV preparations at each pH and temperature according to Eq. (1) in Materials and methods. The values of the apparent pH-dependent K_m^{pH}, J_{max}^{pH} and K_{mNa}^{pH}/K_{mK}^{pH} of $(222)C_{10}$-cryptand increased with temperature at any given pH (Table 1). Under each set of experimental conditions, the K_{mK}^{pH} values were lower than the K_{mNa}^{pH} ones, whereas the K_{mNa}^{pH}/K_{mK}^{pH} values almost did not vary with pH (Table 1).

Under rapid equilibrium conditions, the thermodynamic parameters of cation transport could be estimated using the K_m^{pH} and J_{max}^{pH} values reported in Table 1. In the 20 to 35°C temperature range, the pK_m^{pH} of $(222)C_{10}$ when transporting K^+ and Na^+ ions, i.e., $-\log K_m^{pH}$ and $\log J_{max}^{pH}$, varied linearly with the reciprocal of the absolute temperature ($1/T$(K)). The enthalpy for the apparent pH-dependent affinity of the cryptand for K^+ ($\Delta H(K_{mK}^{pH})$) and Na^+ ($\Delta H(K_{mNa}^{pH})$) ions, and the activation energy for cation transport by the carrier ($E(T_{max}^{pH})$) were, respectively, estimated from the slopes of the Vant'Hoff plots of pK_{mK}^{pH} and pK_{mNa}^{pH} (Fig. 3), and of the Arrhenius plot of $\log T_{max}^{pH}$ (Fig. 4). Their values were equal to $\Delta H(K_{mK}^{pH}) =$

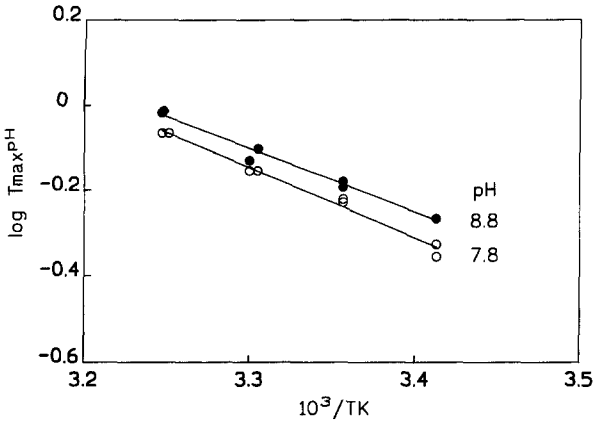

Fig. 4. Temperature dependence of the maximum turnover rate (T_{max}^{pH}) at variable pH: Arrhenius plot of T_{max}^{pH} (ions per carrier molecule per s) for the competitive transport of K^+ and Na^+ ions ($C'_K = 2.9–42.3$ mM at $C'_{Na} = 0$ mM; $C'_K = 2.9–24.0$ mM at $C'_{Na} = 14.2$ and 24.2 mM) by 0.5 μM (or 0.31 mmol/mol lipid, i.e., about 0.8 nmol carrier/m^2 surface membrane) by $(222)C_{10}$-cryptand through negatively charged LUV membranes at 20, 25, 30 and 35°C (pH 7.8 (open circles) and 8.8 (filled circles)). Each point corresponds to the T_{max}^{pH} value obtained from the study of one LUV preparation. $E(T_{max}^{pH}) = 31.8 \pm 1.5$ and 28.9 ± 1.6 kJ/mol at pH 7.8 and 8.8, respectively.

32.8 ± 4.1 and 37.0 ± 3.5 kJ/mol, $\Delta H(K_{mNa}^{pH}) = 47.9 \pm 2.7$ and 52.9 ± 4.4 kJ/mol, and $E(T_{max}^{pH}) = 31.8 \pm 1.5$ and 28.9 ± 1.6 kJ/mol, at pH 7.8 and 8.8, respectively. Covariance analysis of the data showed that: (i) $\Delta H(K_{mNa}^{pH})$ was significantly higher than $\Delta H(K_{mK}^{pH})$ at any given pH, i.e., whatever the pH, the slope value of the pK_{mNa}^{pH} vs. $1/T$(K) regression line was higher than that of the pK_{mK}^{pH} vs. $1/T$(K) one; (ii) raising the pH from 7.8 to 8.8 induced a significant increase in the pK_{mK}^{pH} and pK_{mNa}^{pH} values, and a nonsignificant increase in the $\Delta H(K_{mK}^{pH})$ (4.2 kJ/mol) and $\Delta H(K_{mNa}^{pH})$ (5.0 kJ/mol) ones (see below), i.e., whatever the cation type, the y-intercept value of the $pK_m^{pH\ 8.8}$ vs. $1/T$(K) regression line was significantly higher than that of the $pK_m^{pH\ 7.8}$ vs. $1/T$(K) one, whereas the slope values of these lines did not differ significantly; and (iii) raising the pH from 7.8 to 8.8 increased significantly the log T_{max}^{pH} values, and therefore those for T_{max}^{pH}, whereas the $E(T_{max}^{pH})$ value was not modified significantly, i.e., the value for the y-intercept of the log $T_{max}^{pH\ 8.8}$ vs. $1/T$(K) regression line was significantly higher than that of the log $T_{max}^{pH\ 7.8}$ vs. $1/T$(K) one, whereas the slope values of these lines did not differ significantly.

Fitted K_m, J_{max}, $E(T_{max})$ and $\Delta H(K_m)$

In order to discriminate the effect of pH on the apparent Michaelis parameters of the cryptand, the J_i vs. C'_K plots of the experimental data obtained at each temperature (pH 7.8 and 8.8) were fitted simultaneously according to Eq. (3) in Kinetic theory. The experimental pH-induced variations of the Michaelis parameters ($K_m^{pH\ 7.8}/K_m^{pH\ 8.8}$ about 1.4 and $J_{max}^{pH\ 7.8}/J_{max}^{pH\ 8.8}$ about 0.9) of the cryptand were fairly accounted for by setting in this equation $pK_2 = 5.11$, 5.00, 4.89 and 4.79 ($\Delta H(K_{d2}) = 36.4$ kJ/mol) at 20, 25, 30 and 35°C, respectively. Table 2 reports the values obtained for all the apparent parameters of cation transport by $(222)C_{10}$ when occurring through membranes having a 60 mV membrane potential (negative inside) as was the case here.

As above, the thermodynamic parameters of cation transport and of the first protonation of the cryptand could be estimated using the K_m, T_{max} and pK_1 values reported in Table 2. In the 20 to 35°C temperature range, the values obtained were $\Delta H(K_{mK}) = 37.9 \pm 3.2$ kJ/mol, significantly different from $\Delta H(K_{mNa}) = 53.5 \pm 2.0$ kJ/mol, $E(T_{max}) = 27.4 \pm 2.3$ kJ/mol, and $\Delta H(K_{d1}) = 45.0 \pm 13.2$ kJ/mol.

pH-dependence of $\Delta H(K_m^{pH})$

Whatever the cation type, the $\Delta H(K_m^{pH})$ of $(222)C_{10}$ was found here to increase by 4–5 kJ/mol when raising the pH from 7.8 to 8.8, i.e., the higher the pH, the steeper the slope of the pK_m^{pH} vs. $1/T$(K) regression line (Fig. 3). When the temperature was raised, the dissociation of the cation-carrier complexes was enhanced. This intrinsic effect of temperature ('T-Effect' or Effect 1) on the carrier affinity induced an increase in the K_m^{pH} values (decrease in pK_m^{pH}). Concomitantly, the ionization of the buffer (pH decreased) and of the amine groups of the carrier binding cavity (pK_1 and pK_2 decreased) was favoured. In turn, the

Table 2
Effect of temperature T (in °C) on Michaelis parameters (J_{max}, K_m), ionization pK values (pK_1 and pK_2) of the cryptand and translocation rate constants ($k_{MS}/k = k^+/k$) for the competitive transport of K^+ and Na^+ ions by $(222)C_{10}$-cryptand

T (°C)	J_{max} (nmol/s)	K_{mK} (mM)	K_{mNa} (mM)	pK_1	pK_2	$k_{MS}/k = k^+/k$
20	1.1 ± 0.0	10.5 ± 0.5	23.0 ± 1.1	7.10 ± 0.04	5.11	0.052 ± 0.017
25	1.3 ± 0.0	12.6 ± 0.7	32.2 ± 1.6	6.90 ± 0.05	5.00	0.023 ± 0.014
30	1.6 ± 0.0	16.5 ± 0.8	45.4 ± 1.9	6.92 ± 0.04	4.89	0.025 ± 0.013
35	2.0 ± 0.0	22.2 ± 0.7	66.9 ± 1.8	6.66 ± 0.03	4.79	0.050 ± 0.017

Competitive transport of K^+ and Na^+ ions ($C'_K = 2.9–42.3$ mM at $C'_{Na} = 0$ mM; $C'_K = 2.9–24.0$ mM at $C'_{Na} = 14.2$ and 24.2 mM) by 0.5 μM (or 0.31 mmol/mol lipid, i.e., about 0.8 nmol carrier/m^2 surface membrane) $(222)C_{10}$-cryptand through negatively charged LUV membranes at 20, 25, 30 and 35°C (pH 7.8 and 8.8). Means ($\pm$S.E.) were determined according to Eq. (3) in Kinetic theory by the simultaneous fitting of the data obtained at each temperature (two LUV preparations at each pH). In this equation, pK_2 was set equal to 5.00 at 25°C with $\Delta H(K_{d2}) = 36.4$ kJ/mol.

strength of the cation/proton competition within the binding cavity was modified, and therefore, the K_m^{pH} values were expected to increase because of the decrease in pH ('*T*-pH-Effect' or Effect 2), and to decrease because of that of pK_1 ('*T*-pK_1-Effect' or Effect 3) and pK_2 ('*T*-pK_2-Effect' or Effect 4). As a result, the overall temperature-induced change in the pK_m^{pH} values of (222)C_{10} may be described at any given pH (7.8 and 8.8) by

$$\begin{aligned} &d(pK_m^{pH})/d(1/T) \\ &= \partial pK_m^{pH}/\partial(1/T) \\ &\quad + (\partial pK_m^{pH}/\partial pH)(d(pH)/d(1/T)) \\ &\quad + (\partial pK_m^{pH}/\partial pK_1)(d(pK_1)/d(1/T)) \\ &\quad + (\partial pK_m^{pH}/\partial pK_2)(d(pK_2)/d(1/T)) \end{aligned} \quad (17)$$

In order to quantify the respective influence of these four effects, the $d(pK_m^{pH})/d(1/T)$ values for each cation were calculated at pH 7.8 and 8.8 using Eqs. (4), (5), (10) and (17), and the enthalpies estimated from the data reported in Table 1 and Table 2. In these calculations, the intrinsic effect of temperature on pK_m^{pH}, i.e., $\partial pK_m^{pH}/\partial(1/T)$, was approximated by $d(pK_m/d(1/T)$, i.e., $\Delta H(K_m)$. Table 3 shows that the calculated $\Delta H(K_m^{pH})$ values were in fair agreement with the experimental ones. It also shows that the decreased value of $\Delta H(K_m^{pH})$ compared to that of $\Delta H(K_m)$ (Effect 1) was almost entirely accounted for by the opposing effects on $\Delta H(K_m^{pH})$ of the temperature-induced changes of the pH (Effect 2) and pK_1 (Effect 3). Since the latter two effects decreased in magnitude at pH 8.8, then the $\Delta H(K_m^{pH})$ values were expected to be higher at pH 8.8 than at pH 7.8, in agreement with the results found here.

4.2. K / Na competitive transport selectivity (S_c(K / Na)) of (222)C_{10}: J_K / J_{Na}

Calculated S_c(K / Na) and fitted $\Delta H(S_c(K/Na))$

When rearranging Eq. (3) in Kinetic theory in order to evidence the individual initial influxes of K^+ and Na^+ ions, and applying it to cation transport by cryptands ($J_{maxK} = J_{maxNa}$), then the variation of the K/Na competitive transport selectivity (S_c(K/Na)) of (222)C_{10}-cryptand with the external cation concentrations (C'_S) and the Michaelis constant (K_m) of the alkali cations is described by

$$S_c(K/Na) = (C'_K/K_{mK})/(C'_{Na}/K_{mNa}) \quad (18)$$

This transport selectivity increases therefore linearly with the external K^+ concentration, decreases hyperbolically with increasing that of Na^+ and, according to Eq. (10) in Kinetic theory, it does not depend on pH since $K_{mNa}^{pH}/K_{mK}^{pH} = K_{mNa}/K_{mK}$. This equation also shows that when $C'_K = C'_{Na}$ under rapid equilibrium conditions, then S_c(K/Na) is equal to K_{mNa}/K_{mK}. The S_c(K/Na) of (222)C_{10} was calculated under each set of experimental conditions according to Eq. (18) and using the values of the apparent K_m values reported in Table 2. In the temperature (20 to 35°C) and external K^+ concentration (0 to 24 mM) ranges investigated here, the values for S_c(K/Na) varied from 0 to 3.69 at C'_{Na} = 14.2 mM and 20°C (5.07 at 35°C), and from 0 to 2.14 at C'_{Na} = 24.2 mM and 20°C (2.94 at 35°C). The slopes of the S_c(K/Na) vs. C'_K regression lines were respectively equal to 1.53 mM^{-1} at C'_{Na} = 14.2 mM and 20°C (2.11 mM^{-1} at 35°C), and to 0.89 mM^{-1} at C'_{Na} = 24.2 mM and 20°C (1.23 mM^{-1} at 35°C). Moreover, at identical external cation concentrations ($C'_K = C'_{Na}$ = 14.2 or 24.2 mM), the competitive transport se-

Table 3
Influence of cation type and pH on the enthalpy for the apparent affinity ($\Delta H(K_m^{pH})$) of (222)C_{10}-cryptand for K^+ and Na^+ ions

	pH	Temperature effects (kJ/mol)				$\Delta H(K_m^{pH})$	$\Delta H(K_m^{pH})$
		'*T*' (Effect 1)	'*T*-pH' (Effect 2)	'*T*-pK_1' (Effect 3)	'*T*-pK_2' (Effect 4)	calculated (kJ/mol)	experimental (kJ/mol)
K^+	7.8	37.9 ± 3.2 (100%)	6.0 (16.0%)	−13.3 (−35.0%)	−0.125 (−0.3%)	30.4 ± 0.7	32.8 ± 4.1
	8.8	37.9 ± 3.2 (100%)	1.0 (2.5%)	−2.5 (−6.6%)	−0.003 (−0.0%)	36.4 ± 0.2	37.0 ± 3.5
Na^+	7.8	53.5 ± 2.0 (100%)	6.0 (11.2%)	−13.3 (−24.9%)	−0.125 (−0.3%)	46.0 ± 0.7	47.9 ± 2.7
	8.8	53.5 ± 2.0 (100%)	1.0 (1.8%)	−2.5 (−4.6%)	−0.003 (−0.0%)	52.0 ± 0.2	52.9 ± 4.4

Competitive transport of K^+ and Na^+ ions (C'_K = 2.9–42.3 mM at C'_{Na} = 0 mM; C'_K = 2.9–24.0 mM at C'_{Na} = 14.2 and 24.2 mM) by 0.5 μM (or 0.31 mmol/mol lipid, i.e., about 0.8 nmol carrier/m^2 surface membrane) (222)C_{10}-cryptand through negatively charged LUV membranes at 20, 25, 30 and 35°C (pH 7.8 and 8.8). According to Eq. (17) in Results, the variation of pK_m^{pH} with the reciprocal absolute temperature ($1/T$(K)) is equal to the sum of the intrinsic effect of the temperature on pK_m^{pH}, i.e., '*T*-effect' (Effect 1, set equal to 100.0%), and of effects on pK_m^{pH} of the temperature-induced changes of the pH, i.e., '*T*-pH-effect' (Effect 2), pK_1, i.e., '*T*-pK_1-effect' (Effect 3) and pK_2, i.e., '*T*-pK_2-effect' (Effect 4). The data reported in this table were calculated at pH 7.8 and 8.8 using Eqs. (4), (5) and (10) in Kinetic theory, Eq. (17) in Results, and the enthalpies estimated from the data reported in Tables 1 and 2.

lectivity of the cryptand was equal to about 2.1 at 20°C and 3.0 at 35°C, i.e., the initial rate of K^+ transport (J_K) by (222)C_{10} was about 2 to 3 times higher that of Na^+ (J_{Na}) depending on the temperature (20 to 35°C).

The enthalpy for the competitive transport selectivity of (222)C_{10} was estimated from the slope of the log S_c(K/Na) vs. $1/T$(K) regressions lines established under each set of experimental conditions. The values obtained for $\Delta H(S_c(K/Na))$ ranged between 16.2 ± 1.4 and 14.4 ± 1.6 kJ/mol, whether the external cation concentrations were the same or not. They did not differ significantly from the theoretical value of 15.6 kJ/mol expected here for $\Delta H(S_c(K/Na))$, independent of the cation concentrations ($\Delta H(S_c(K/Na)) = \Delta H(K_{mNa}) - \Delta H(K_{mK})$ [14]).

5. Discussion

When the temperature was raised from 20 to 35°C, the maximal initial rate (J_{max}^{pH}) for the competitive transport potassium and sodium ions by (222)C_{10}, and the maximum turnover rate (T_{max}^{pH}) increased. As predicted by the Arrhenius equation, the true rate constants for the molecular processes of cation transport by cryptand (Fig. 1) increased exponentially with the temperature. In addition, the membrane fluidity and lateral mobility of carriers and complexes also increased with the temperature [21,22,30].

At any given pH, the apparent K_m^{pH} of the cryptand for potassium and sodium ions also increased with the temperature (20–35°C), i.e., the higher the temperature, the lower the affinity of the cryptand for the alkali cations, and indeed, increasing the temperature favoured to a high extent the endothermic process of cation-carrier complexes dissociation (Table 1). The apparent affinity of (222)C_{10} was higher for K^+ than for Na^+. A similar result has been found in a previous study on K^+ and Na^+ transport as unique substrates of the cryptand [16]. This result was certainly due to the greater stability of the complexes formed with K^+ than with Na^+ [25], since the radius of the (222)C_{10} cavity (1.4 Å) is better adapted to the binding of K^+ (ionic radius 1.33 Å) than of Na^+ (ionic radius 0.98 Å) [25,31]. The values determined here at 25°C for K_{mK}^{pH} (19.0 mM at pH 7.8 and 13.7 mM at pH 8.8) and K_{mNa}^{pH} (50.5 mM at pH 7.8 and 33.9 mM at pH 8.8) (Table 1) were higher than those obtained previously at the same temperature, i.e., 3.2 mM at pH 7.6 and 2.7 mM at pH 8.6 for K_{mK}^{pH}, and 35.3 mM at pH 7.6 and 19.1 mM at pH 8.6 for K_{mNa}^{pH} [16]. The discrepancy existing between the two sets of data, specially in the case of K^+ transport may have arisen from: (i) a difference in the methods used to determine the Michaelis parameters, i.e., curvilinear regressions here and Lineweaver-Burk plots previously, the magnitude of the error associated to the latter method being higher in the case of K^+ transport (low slope value) than in that of Na^+; (ii) a difference in the methods used to quantify proton outfluxes, i.e., pH measurements here and pH-Stat titrations previously; and (iii) a difference in the ionic strength within the external membrane/solution interface since the cation concentration ranges investigated (2.9–42.3 mM here and 1.3–17.0 mM previously) in the two studies were very different. An increase in the ionic strength may have induce a decrease in the ionization constants of the amine groups of the binding cavity of the cryptand (increase in pK_1 and pK_2), thus favouring proton binding inside the intramolecular cavities and the release of Na^+ and K^+ ions (increase in K_m^{pH}) [20].

Raising the pH from 7.8 to 8.8 in the 20 to 35°C temperature range, slightly enhanced J_{max}^{pH} and reduced the apparent K_m^{pH} for potassium and sodium ions by nearly 5–8 and 11–24 mM, respectively (Table 1). Concomitantly, the pH-induced increments in the apparent pK_m^{pH} for K^+ (0.15 at 20°C and 0.10 at 35°C) and Na^+ (0.16 at 20°C and 0.10 at 35°C) ions were very similar at any given temperature, and indeed theoretically these increments were expected to be the same whatever the strength of alkali cation binding to the carrier. The variations in J_{max}^{pH} and K_m^{pH} were the result of a decrease in the strength of cation transport inhibition by internal and external protons (see below).

"According to the familiar rules of enzyme kinetics, when a substrate and inhibitor compete for the active site, inhibition is overcome by high substrate concentrations and is called competitive; when they are bound at different sites, inhibition persists at all substrate concentrations and is called non-competitive. This is not necessarily true of transport systems, where inhibition often persists despite addition of substrate and inhibitor to the same site. The reason is that the carrier operates between two aqueous compartments, outside and inside the cell. The substrate is normally present, for experimental purposes, in only one compartment initially. If the inhibitor is present in the other, the substrate may be unable to displace it from the carrier, in which case the behaviour will be non-competitive" [26]. Based on these observations, Devés and Krupka [26] have stressed that a sharp distinction must be made between competitive or non-competitive mechanisms and competitive or non-competitive kinetics in the case of transport systems. They have analyzed the kinetics of inhibition in various kinds of experiments and shown that, when the substrate is present outside ('Zero trans influx' experiments) and the competitive inhibitor on both sides of the membrane (as was the case here for protons), then both J_{max} and K_m vary, i.e., the kinetic form of the inhibition is a mixed-type inhibition, although the mechanism is a simple competitive inhibition. One of their main conclusions was that "a competitive inhibition mechanism frequently gives rise to non-competitive or mixed-type inhibition kinetics, depending on the experimental design, whereas a non-competitive inhibition mechanism never produces competitive kinetics" [26]. 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 accounted satisfactorily for the pH-dependence of the Michaelis parameters (J_{max}^{pH} and K_m^{pH}) observed here when setting $pK_2 = 5.0$ at 25°C, and assuming for this second protonation an enthalpy of 36.4 kJ/mol. This $\Delta H(K_{d2})$ value was chosen as being in the middle range of those determined for the (222)-cryptand homologue in water and in methanol [32]. It was therefore likely that the pK_1 values obtained here (Table 2) had an enthalpy ($\Delta H(K_{d1})$ 45.0 kJ/mol) which was also in the middle range of those for the (222)-cryptand homologue in the same two solvents [32]. The values reported in Table 2 for the ionization constants of the amine groups of the cryptand were about 2.3–2.7 pH units lower than those determined for these groups in water at 25°C [25]. This decrease may have mainly arisen from the variation of the dielectric constant within the membrane/solution interface [33–36], 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 [37].

The rate constants for the ionization and protonation of the amine groups of the cryptand, for the alkali cation complexation in its cavity and also for Na^+-(222) decomplexation were shown to be very high [27,28]. Conversely, the rate constant for K^+-(222) decomplexation in water was rather low, i.e., 7.5 s^{-1} at 25°C [27]. Consequently, only the activation energy for the decomplexation process of K^+ ions might have contributed significantly to the energy involved in the overall transport process by $(222)C_{10}$. As a result, the overall activation energy for alkali cation transport by cryptands is probably supplied by the energy of the four following molecular processes [19]: (i) entry of the complexed intramolecular binding cavity into the membrane; (ii) translocation of the complex through the membrane; (iii) decomplexation at the internal interface; and (iv) back-diffusion of the free carriers. Of the four processes, translocation of the charged complexes (k'_{MS}) might be the slowest, owing to the electrostatic force opposing the translocation of cation-carrier complexes across the membrane [38]. The partition equilibrium of valinomycin between water and membrane has been found to have a negative temperature coefficient, i.e., the total number of carrier molecules in the membrane decreases with increasing temperatures [39,40]. It seems likely that this also holds in the case of cryptands. However, since the partition coefficient of the cryptands is very high, the interfacial $(222)C_{10}$ concentration almost did not vary in the temperature range investigated here.

The $E(T_{max}^{pH})$ values obtained here for the competitive transport of K^+ and Na^+ ions by $(222)C_{10}$ were lower than that previously found in the case of cation transport by $(221)C_{10}$ [14]. This difference was probably mainly due to the fact that cations are buried to a larger extent in the binding cavity of $(222)C_{10}$ than in that of $(221)C_{10}$. There are considerable differences in the values reported in the literature for the temperature dependence of alkali cation transport by macrocyclic antibiotics [38–43]. Except for the negative value reported by Benz et al. [42], these values ranged between 63 and 230 kJ/mol, so that the $E(T_{max}^{pH})$ values obtained here for $(222)C_{10}$ were slightly lower than those previously reported since, as underlined above, the electrical charge of alkali cations is buried to a large extent inside its intramolecular cavity.

The values determined here for the enthalpy of the apparent K_m^{pH} of $(222)C_{10}$ for K^+ were 1.5-times lower than those for Na^+. On the basis of the data reported by Kauffmann et al. [44], the reverse would be expected since these authors found a smaller enthalpy for K^+ (−47.7 kJ/mol) than for Na^+ (−30.9 kJ/mol) complexation to the (222)-cryptand homologue. In both studies however, Na^+ and K^+ complexes were enthalpy stabilized and entropy destabilized, and it was likely that the discrepancy may have been accounted for by a difference in the systems investigated, i.e., (222)-cryptand in basic aqueous solutions on the one hand, and $(222)C_{10}$ solubilized in membranes on the other.

The present study also showed that the $\Delta H(K_m^{pH})$ for each cation increased by nearly the same amount (4–5 kJ/mol) when raising the pH from 7.8 to 8.8 (Fig. 3). Even if non statistically significant, this increment amounted to 8–10% the $\Delta H(K_m^{pH})$ values determined at pH 7.8. It was likely that this pH-dependence of the $\Delta H(K_m^{pH})$ was found to be independent of the cation type since, as underlined above, the pH-induced increment in the apparent pK_m^{pH} for K^+ and Na^+ does not theoretically vary with the strength of alkali cation binding to the carrier. It has also be shown that the overall variation of pK_m^{pH} with the temperature ($1/T$(K)) was described relatively well by the sum of the intrinsic effect of this parameter on the carrier affinity, and of the effects of the temperature-induced changes in the pH, pK_1 and pK_2 values (Eq. (17) in Results). Indeed, the calculated $\Delta H(K_m^{pH})$ values were close to the experimental ones (Table 3). The effect on pK_m^{pH} of the temperature-induced change of pK_2 was found to be negligible whatever the cation type and the pH (Table 3). This was certainly due to the fact that the two pH investigated here (7.8 and 8.8) were far from the range of pK_2 values (4.79–5.11) (Table 2). For a similar reason, the effect on pK_m^{pH} of the temperature-induced change of pK_1 was 5-times lower at pH 8.8 than at pH 7.8; at any given pH the latter effect on pK_m^{pH} was partially counterbalanced by that of the temperature-induced change of the pH. As a result, the increase of $\Delta H(K_m^{pH})$ observed here, when the pH was raised from

7.8 to 8.8, was almost entirely explained by the opposing effects on pK_m^{pH} of the changes induced by temperature in the pH and pK_1 values.

The competitive transport selectivity (S_c(K/Na)) of (222)C_{10} for K^+ over Na^+ ions was found to reach a maximum value of about 5 in the temperature and cation concentrations ranges investigated here. This value is slightly higher than that found previously for the (221)C_{10} in the favour of Na^+ over K^+ [14], whereas it 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].

As underlined in Results, the K/Na competitive transport selectivity (S_c(K/Na)) of (222)C_{10} did not depend on pH since $K_{mNa}^{pH}/K_{mK}^{pH} = K_{mNa}/K_{mK}$. It was shown however to increase with temperature, e.g., from about 2 at 20°C to about 3 at 35°C in equimolecular mixtures of K^+ and Na^+ ions. According to Mulliert et al. [14], the enthalpy of this transport selectivity ($\Delta H(S_c(K/Na)) = \Delta H(K_{mNa}) - \Delta H(K_{mK})$) would have reached a value of 15.6 kJ/mol, whether the external cation concentrations were equal or not. This value is in fair agreement with those ranging here between 14.4 and 16.2 kJ/mol. It is also interesting to note that the existence of a temperature-dependence for the S_c(K/Na) of (222)C_{10} is not a general rule for cryptands. Indeed, it has been shown recently that the S_c(Na/K) of (221)C_{10} was nearly independent of temperature, due to the similarity between the enthalpies for the affinity of this carrier for Na^+ and K^+ ions [14].

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 (222)C_{10} in the extracellular and intracellular media. The Na/K competitive transport selectivity (S_c(Na/K)) of (222)C_{10} would reach values ranging between 13 at 20°C and 9 at 35°C in the extracellular medium while its K/Na competitive selectivity would have values of about 60 at 20°C and 85 at 35°C in the intracellular medium. Such potentially high values for the competitive transport selectivity of (222)C_{10} in physiological media suggest that this carrier would behave in a cell like an efficient and non-electrogenic ion gradient-dissipating Na/K exchanger exhibiting a temperature-dependent ionic selectivity.

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