THE KINETICS OF POTASSIUM ION COMPLEXATION BY IONOPHORES

Duncan H. HAYNES*
Johnson Foundation for Medical Research, University of Pennsylvania, Philadelphia, Pennsylvania 19104, USA

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1. Introduction

The mobile carrier theory for ionophore-facilitated cation transport through membranes [1, 2] has been supported strongly by the finding of a correlation between the ability of the ionophores to induce transport and the ability of the molecules to extract ions from an aqueous medium into an organic phase via the mechanism of complexation [1, 3]. Further evidence for the carrier theory was gained from a nuclear magnetic resonance (NMR) study [3, 4] which demonstrated a rapid exchange between valinomycin and its KCNS complex in CH_3OH–$CHCl_3$ (80:20, v/v). It was suggested that the behaviour of the ionophore in this semi-polar solvent may be similar to that at the water–membrane interface [3, 4]. The observation of fast exchange was later extended for the macrolide actin antibiotics in semi-polar solvents [5, 6] but the rate constants were not measured. Subsequently Diebler et al. [7] reported a value of 3×10^6 M^{-1} sec^{-1} for the bimolecular rate constant for complex formation between monactin and Na^+ in methanol. The present study compares the kinetics of K^+ complexation for valinomycin and the 4 macrolide actins.

It has not yet been clearly demonstrated whether the differences in transport rate for valinomycin and the macrolide actins are simply the result of differences in the complex stability constants, or if differences in the complexation kinetics may also play an important role. The comparisons in this study suggest that the rate of decomplexation at the water–membrane interface is not generally the rate-limiting step in the transport reaction sequence.

2. Materials and methods

KCNS was purchased from K and K laboratories, Plainfield, N.Y. CD_3OD and $CDCl_3$ (isotopic contamination ca. 0.1%) were obtained from Brinkmann Instruments, Inc., Westbury, N.Y. The $CDCl_3$ was dried over Na_2SO_4 before use.

Valinomycin was obtained from Eli Lilly and Co., Indianapolis, Ind. Nonactin, monactin, dinactin, and trinactin were obtained from CIBA, Basel.

Proton NMR spectra were taken on either a Varian HA 60 or a JEOLCO C6OH 60 MHz spectrometer. The instruments were operated with an external H_2O lock and the chemical shift values of the resonances were measured relative to the position of tetramethylsilane (TMS) which was added as an internal standard.

3. Results and discussion

The differences in the NMR spectra of valinomycin and its KCNS and CsCNS complexes have been reported previously [3, 4]. The shifts in the lactate methyl resonance [4] are used for the kinetic analysis in this study. The 220 MHz spectra of the macrolide actins and their $KClO_4$ complexes have been reported [5, 6]. Fig. 1 shows the perturbation of the 60 MHz spectrum of nonactin by KCNS complexation. The shift of resonance [6] was used to study the K^+ complexation kinetics of the 4 macrolide actins.

Titration of the ionophores with KCNS in CH_3OH–

* Present address: Max-Planck-Institut für biophysikalische Chemie, 3400 Göttingen-Nikolausberg, West Germany.

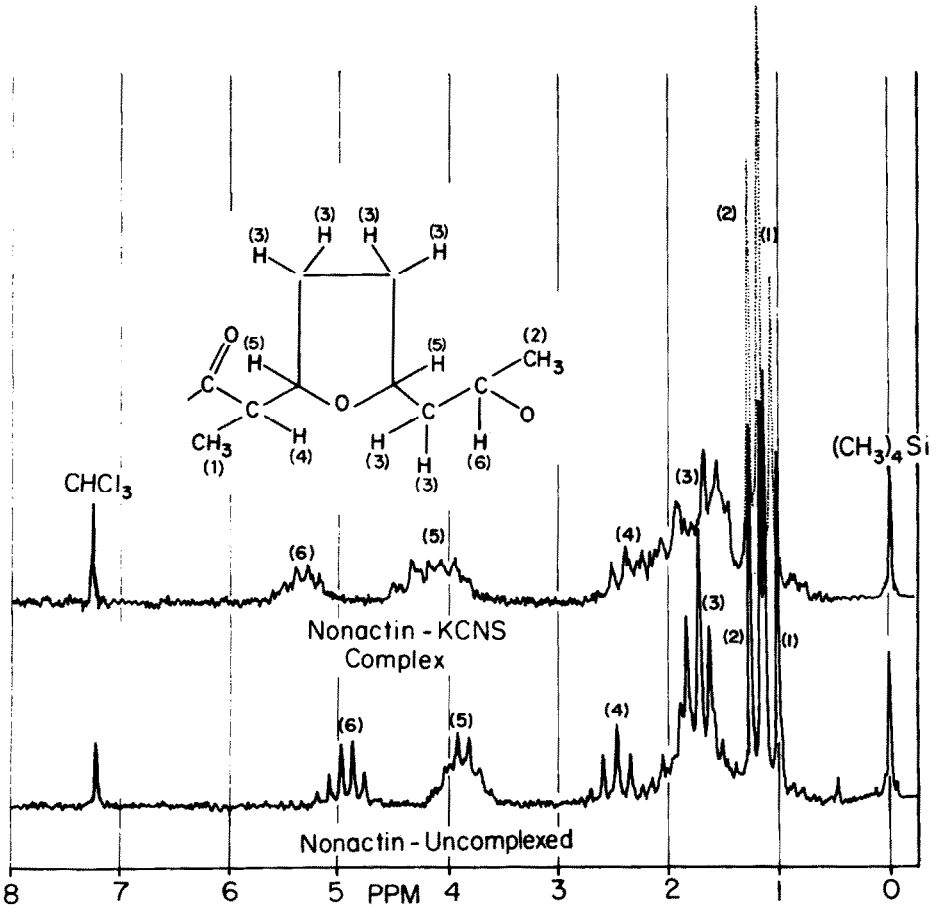

Fig. 1. The 60 MHz spectrum of nonactin and its KCNS complex in $CDCl_3$. Spectra were taken on the Varian instrument. [Nonactin] = 34 mM. Chemical shift values are given in ppm (Hz/60).

$CDCl_3$ (80:20, v/v) lead to the observation that the positions of the resonance behaved as the weighted averages of those of the ionophore and the ionophore–K^+ complex. This indicates that the exchange between these 2 species is fast on the NMR time scale. The exchange reaction could occur by 2 possible mechanisms:

$$(1)\quad I+K^+ \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} I{-}K^+$$

or

$$(2)\quad I^* + I{-}K^+ \xrightarrow{k_1} I + I^*{-}K^+$$

where I and $I{-}K^+$ represent the ionophore and its K^+ complex, respectively. The 2 mechanisms can be distinguished by a study of the dependence of the exchange rate upon the total ionophore concentration.

The exchange rate was measured from the "initial broadening" of the resonances using equations given by Johnson [8]. The dependence of the full width at half height, $\Delta\nu_{\frac{1}{2}}$(exch.) (in Hz) of a given resonance of I in the presence of exchange with a much smaller fraction of $I{-}K^+$ in given by:

$$(3)\ \Delta\nu_{\frac{1}{2}}(\text{exch.}) = \Delta\nu^0_{\frac{1}{2}} + \frac{P_j 4\pi(\Delta\delta)^2\tau}{1+4\pi^2(\Delta\delta)^2\tau^2}$$

where P_j is the fraction of the total ionophore in the $I{-}K^+$ form (where $P_j < 0.15$) and where $\Delta\delta$ is the dif-

Table
The rate constants for exchange of ionophores and K^+ in $CH_3OH-CDCl_3$ (80:20, v/v).

Ionophore	$K_S(=k_{on}/k_{off})(M^{-1})$*	$k_{on}(M^{-1}sec^{-1})$**	$k_{off}(sec^{-1})$***	$k_1(M^{-1}sec^{-1})$***
Valinomycin	$> 5 \times 10^3$	$> 1.1 \times 10^5$	21 ± 5	< 200
Nonactin	$> 5 \times 10^3$	$> 1.6 \times 10^5$	32.3 ± 0.6	< 70
Monactin	$> 5 \times 10^3$	$> 1.1 \times 10^5$	22.9 ± 1.6	
Dinactin	$> 5 \times 10^3$	$> 1.1 \times 10^5$	21 ± 6	
Trinactin	$> 5 \times 10^3$	$> 0.9 \times 10^5$	18 ± 5	

* Lower limit was evaluated from the sharpness of the end point of the titration of ionophore with KCNS at 21°. The titrations of the macrolide actins and valinomycin were made with deuterated and normal methanol, respectively.
** Calculated from K_S and k_{off}.
*** Calculated from eq. (5). Valinomycin experiments were carried out for $[I]_t$ = 23 and 46 mM. Nonactin experiments were carried out for $[I]_t$ = 20 and 34 mM. Experiments for the other macrolide actions were for $[I]_t$ = 34 mM and k_{off} was calculated under the assumption of a negligible contribution of the process of eq. (2).

ference in the resonance positions (Hz) for the 2 species in absence of exchange. The term τ is the reciprocal of the exchange rate given by:

$$(4) \quad 1/\tau = 1/\tau_I + 1/\tau_{IK}$$

where τ_I and τ_{IK} are the respective lifetimes for the nucleus in the 2 states. Eq. (3) predicts that as $1/\tau$ is increased, the width of the dominant resonance will first increase (initial broadening) from, and then decrease to, the original value of the width at half height, $\Delta\nu_{\frac{1}{2}}^0$. Such behaviour was observed for valinomycin and the macrolide actins in polar solvents. The initial broadening of the lactate methyl resonance of valinomycin in $CH_3OH-CDCl_3$ (80:20, v/v) upon titration with KCNS is shown in fig. 2.

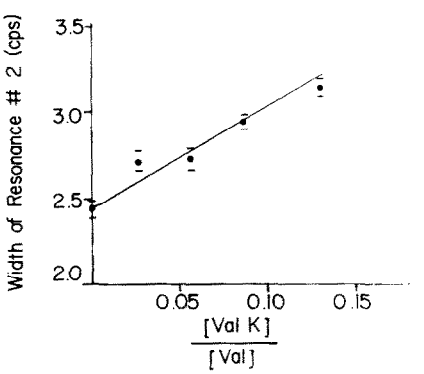

Fig. 2. Exchange broadening of the lactate methyl resonance of valinomycin upon complexation with K^+. The line broadening experiment was carried out for 22.7 mM valinomycin in $CH_3OH-CDCl_3$ (80:20, v/v) at 21°. Under these conditions the concentration of the complex is given by the concentration of KCNS added. Each width value was the average of the widths obtained from 12 separate scans using a 10 fold expansion of the frequency scale. Field homogeneity was tested continuously by scanning the TMS peak.

The slope of fig. 2 was used to evaluate the exchange kinetics. Formulating the exchange reaction as the sum of the contributions from eq. (1) and (2) and by combining eq. (3) and (4) obtains:

$$(5) \quad \frac{d(\pi\Delta\nu_{\frac{1}{2}}(\text{exch.}))}{d\,\frac{[I-K]}{[I]}} = k_{off} + k_1[I]_t$$

giving the dependence of the slope of fig. 2 upon the total ionophore concentration $[I]_t$ and the kinetic constants k_1 and k_{off}. The slope was independent of $[I]_t$ within an experimental error of ca. 10%, indicating that the exchange for valinomycin occurs primarily by the mechanism of eq. (1). Titrations of the macrolide actins gave results similar to the above. The initial broadening of resonance [6] was used for analysis by eq. (3). The extent of the broadening observed in this complex resonance was at least 10 times as great as that expected from the averaging of the resonance envelopes for I and $I-K^+$ due to infinitely fast exchange. The values of k_{off} and the lower limits for k_{on} are given in the table.

The results of this study can be applied to the problem of identifying the rate-limiting step in the carrier mechanism for these ionophores under the following assumptions: 1) the organic solvent used here

approximates the semi-polar environment of the water–membrane interface and 2) that a proportionality exists between the decomplexation rate constants for the ionophores in the organic solvent and at the water–membrane interface. The order of increasing k_{off} for I–K^+ in the CH_3OH–$CDCl_3$ system is valinomycin ⩽ trinactin ⩽ dinactin ⩽ monactin < nonactin. This is exactly the opposite of the order of effectiveness for transport in membranes [9, 10]. Since there is good evidence that the complexes studied here are identical to the complexes which traverse the hydrophobic interior of the membrane, [4, 5, 11] it would seem that the breakdown rate of the complexes on the *trans* side of the membrane is not the rate-limiting step.

Stark and Benz [12] have given preliminary evidence from lipid bilayer experiments that the rate of dissociation of valinomycin–K^+ and monactin–K^+ complexes at the water–membrane interface is between 2.5 and 20 times as great as the rate of transport of the complex across the membrane. The calculated ratio depends upon the choice of antibiotic and lipid composition of the membrane. These data agree with the conclusion of the present study that the decomplexation reaction is probably not the rate-limiting step in the transport sequence.

Jung and Schmid [13] have reported that the rate of valinomycin-facilitated alkali cation transport through thylakoid membranes demonstrates saturation kinetics with respect to the cation concentration in the medium†. Similar behaviour has been observed for the ionophores with rat liver mitochondria [10], and it will be shown in a future communication that the saturation can be described in terms of conversion of the ionophore to a complexed form at the interface. The rates of valinomycin- and macrolide actin-induced transport in most membrane systems would thus seem to depend upon the stability constant for formation of a hydrophobic complex [10], with the rate limiting step represented by the process of transport of the complex or the free ionophore molecule across the membrane.

† These authors concluded that the complexation of cations with the ionophore was the rate-limiting step in the transport sequence in the thylakoid membrane. The model used to fit the data was, however, not internally consistent. The model was apparently derived for the contradictory conditions that 1) the complexation reactions at the interface are at equilibrium and 2) the complex is transported across the membrane faster than it can be formed at the interface. (cf. [13], eq. (9)).

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