KINETICS OF COMPLEXATION OF SODIUM IONS WITH VALINOMYCIN IN METHANOL BY ^{23}Na NMR SPECTROSCOPY

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1. Introduction

The study of ionophores, which are low molecular weight, naturally occurring compounds capable of binding alkali ions, has recently gained considerable interest. It was demonstrated that these compounds increase the solubility of alkali ions in lipids and they are believed to play an important role in alkali ion transport through membranes [1]. The complexation processes which occurs at the membrane surfaces are very likely the rate determining steps in the overall process of the ion transport through the membranes [2]. It is, therefore, of interest to study the kinetics of these complexation reactions. The rates involved are, however, very fast and fall in the range accessible only by the relaxation or the NMR techniques. Complexation of alkali ions with valinomycin was studied in methanol by Funck, Eggers and Grell [3] using the temperature jump and ultrasonic dispersion methods, and the complexation of K^+ with a series of ionophores in methanol-chloroform mixtures was studied by Haynes [4] using proton NMR. The effect of complexation on the proton NMR is, however, quite small. A much stronger effect is expected on the alkali ion resonances due to the quadrupole interaction in the complexed form [5, 6]. In the present work we report a study of the complexation of valinomycin with sodium ions in methanol using ^{23}Na NMR. The approach is similar to that used previously by us in the study of the complexation reaction of dibenzocrown (DBC) derivatives with sodium ions [6]. It is based on the fact that there is a significant difference in the nuclear relaxation rates of ^{23}Na between the solvated and the complexed forms. The lower symmetry of the complex results in short ^{23}Na relaxation times relative to their values in the more symmetric solvated form. Chemical exchange of sodium between the two environments affects the relaxation rate of the observed NMR signal from which the kinetic parameters can then be obtained.

2. Materials and methods

Spectroscopic grade methanol was dried by refluxing over Mg for several hours. NaSCN (analytical grade) was dried in a vacuum oven at 100°C overnight. Valinomycin (A grade) was obtained from Eli Lilly and Co., and was used without further purification. T_1 measurements of ^{23}Na were made by the 180–90° pulse sequence using a Brucker 322S pulse spectrometer at 15.8 MHz. The signal-to-noise ratio of the free induction decay signal was enhanced using a HP 5480A signal analyzer. Usually several thousand repetitions were required to obtain a satisfactory signal-to-noise ratio.

3. Kinetic measurements

Longitudinal relaxation times of ^{23}Na were measured over a wide temperature range (−100°C to +30°C) in methanol solutions containing various concentrations of NaSCN (50 to 200 mM) and Valinomycin (7 to 22 mM). A typical run is shown in fig. 1. Also shown in the figure are results of T_1 measurements in a solution containing NaSCN without valinomycin. The results demonstrate clearly the occurrence of an exchange process: in the valinomycin solution the sodium ions occur in two forms, (i) solvated

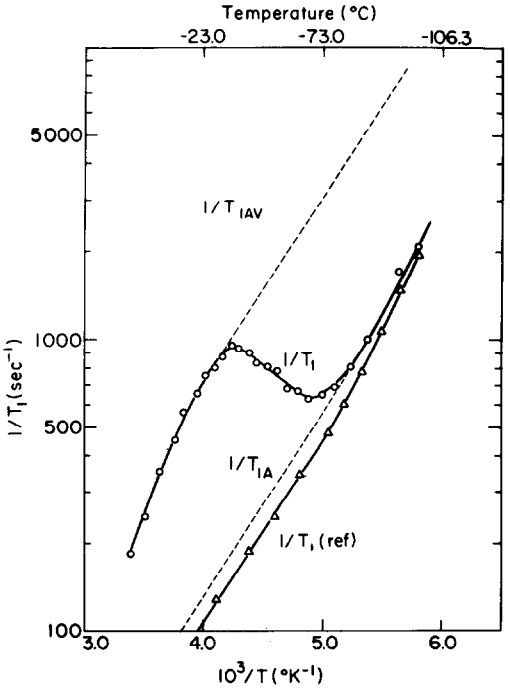

Fig. 1. Semilog plots of $1/T_1$ for ^{23}Na in methanol solutions of NaSCN and valinomycin vers. reciprocal absolute temperature. The circles corresponds to solution number 9 in table 1 and the triangles corresponds to a (reference) solution containing the same concentration of NaSCN, but no valinomycin. The dashed curves were obtained as described in the text.

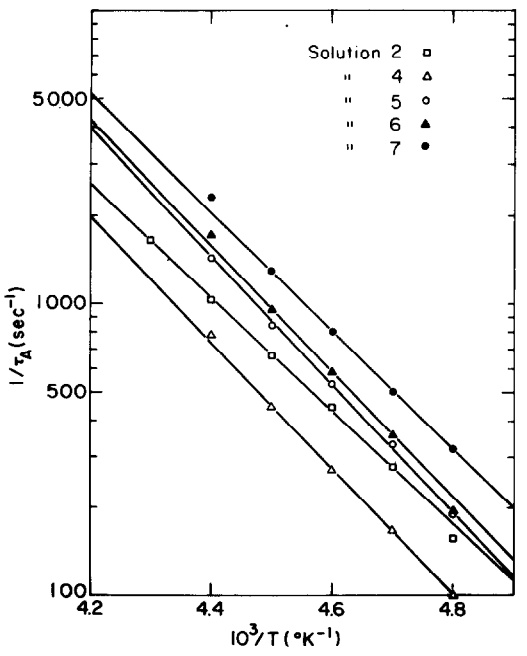

Fig. 2. Arrhenius plots $1/\tau_A$ in methanol solutions of NaSCN and valinomycin. The composition of the solutions are as given in table 1.

sodium ions, Na^+, and (ii) complexed sodium ions, $Val–Na^+$. At low temperatures (below −80°C) exchange of sodium between the two forms is slow, but only the resonance of the uncomplexed ions is detected – the relaxation time of the complexed form is too short to contribute to the observed signal. At the other extreme (above −20°C) exchange is so rapid as to average out the relaxation rate in the two environments. Finally, in the intermediate temperature range, the 'kinetic region' (−30°C to −70°C), the exchange rate is of the order of nuclear relaxation rates, and the observed T_1 depends on the chemical exchange rate. It is in this region that kinetic data can be obtained from the experimental results. In references 6 expressions were derived relating the observed relaxation rates $1/T_1$ (or $1/T_2$) with the kinetic parameters of the exchange reaction. The following equation was obtained

$$\frac{1}{\tau_A} = \frac{(1/T_{1B} - 1/T_1)(1/T_1 - 1/T_{1A})}{(1/T_{1av} - 1/T_1)} P_B \qquad (1)$$

where

$$1/T_{1av} = P_A/T_{1A} + P_B/T_{1B} \qquad (2)$$

P_A and P_B are the fractions of uncomplexed sodium ions. T_{1A} and T_{1B}, the longitudinal relaxation times of ^{23}Na in the corresponding species, and τ_A is the mean lifetime of solvated sodium between successive complexations.

In the quantiative analysis of the experimental results, $1/T_{1A}$ was obtained by extrapolating the low temperature $1/T_1$ values parallel to those of the reference solution (lower dashed line in fig. 1). $1/T_{1B}$ at the high temperature range can be obtained from $1/T_{1av}$ using Eq. 2, if P_A and P_B are known. It appeared that for most solutions complexation of valinomycin in the high temperature range was not complete, and therefore the equilibrium constant must be known for the calculation of P_A and P_B. However, in the

Table 1
Composition and kinetic parameters for the various solutions studied

	NaSCN (mM)	Valinomycin (mM)	$1/\tau_A$ (sec^{-1} at −55°C)	$k_{off} \times 10^{-3}$ (sec^{-1} at −55°C)	ΔE (kcal/mol)
1	59	9.1	490	2.7	11.4
2	56	15.0	450	1.2	8.8
3	59	18.0	1140	2.6	9.2
4	110	7.6	270	3.7	9.8
5	110	13.0	530	4.1	9.9
6	110	13.0	590	4.5	9.7
7	110	19.0	800	4.0	9.3
8	90	21.0	820	2.7	8.9
9	190	8.8	320	6.8	11.3
10	190	14.0	700	9.1	9.5
11	190	22.0	620	4.8	9.1

solutions containing a large access of NaSCN it could be expected that most of the valinomycin was complexed and therefore P_B = [total valinomycin]/[total NaSCN]. Thus from Eq. 2 $1/T_{1B}$ could be calculated, and by extrapolation its value in the kinetic region was obtained (upper dashed curve in fig. 1). Finally for the calculation of $1/\tau_A$ it was assumed that in the kinetic region (i.e. below −30°C) complexation was complete in all solutions. A few plots of calculated $1/\tau_A$ vers. reciprocal absolute temperature are shown in fig. 2. The results for the kinetic parameters in the various solutions studied are summarized in table 1.

4. Discussion

The complexation kinetics of sodium ions with valinomycin was studied previously by Funck et al. [3] using relaxation methods. They analyzed their results in terms of a two step model; a diffusion controlled formation of an intermediate, followed by the formation of the stable complex. The overall reaction can be formulated as

$$Na^+ + \text{Valinomycin} \underset{k_{off}}{\overset{k_{on}}{\rightleftarrows}} \text{Val–}Na^+ \qquad (3)$$

If we assume that sodium exchange occurs only via this reaction then k_{off} is related to $1/\tau_A$ by

$$k_{off} = \frac{1}{\tau_A} \frac{[Na^+]}{[\text{Val–}Na^+]} \qquad (4)$$

Using the results of Table 1 with an average activation energy of 9.5 kcal/mol, values ranging from 10^6 to 3×10^6 sec^{-1} for k_{off} (at 25°C) are obtained. The results are in agreement with those of reference 3 (2×10^6 sec^{-1}), but there is a considerable scatter. This scatter is partly due to experimental inaccuracy, especially in the more dilute solutions, but is probably mainly due to the difficulties in estimating $1/T_{1B}$; e.g. the assumption of a single complexed form with a single relaxation time and the assumption of complete complexation in the kinetic region may not be quite right.

It is interesting to note that the activation energy for the decomplexation reaction is quite significant, and of the order found for the analogous reaction with DBC [6] and other ionophors [7]. These high values must, therefore, be identified with the energy associated with the configuration changes occurring during decomplexation.

More accurate measurements may yield information on the occurrence of other exchange reactions, such as

$$(Na^+)^* + \text{Val–}Na^+ \rightleftarrows Na^+ + \text{Val–}(Na^+)^* \qquad (5)$$

which might be important in ion transport phenomena. The sensitivity can greatly be increased by working at higher frequencies using superconducting magnets. This might also allow the extension of this method to the study of ^{39}K.

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