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A STUDY OF GRAMICIDIN USING DEUTERIUM OXIDE

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Summary

The single channel conductivity of the gramicidin channel has been measured for all the alkali ions using both H_2O and ^2H_2O as a medium. Significant changes in conductivity with medium have been observed in all cases except lithium.

The membrane-active antibiotic gramicidin has been the subject of a substantial body of research. It is of particular interest as it has the property of mediating the transport of monovalent cations across membranes and furthermore this property switches on and off randomly with time. This behaviour was first studied by Hladky and Haydon [1,2]. They were able to introduce a very small number of molecules of this material into a black lipid membrane and hence to measure the conductivity of individual channels each consisting of two or possibly four molecules. Subsequently this property has been used to investigate various aspects of the conduction of ions through membranes and work in this field up to late 1976 has been reviewed by one of us [3].

There has been considerable controversy as to whether ions cross membranes in the hydrated or the unhydrated state. In the case of the gramicidin channel Myers and Haydon [4] and Finkelstein [5] have produced convincing evidence that the channel contains water but the actual question of hydration remains unresolved. Two models for this channel have been extensively discussed [6,7]. Both predict a channel having an effective radius of 0.2 nm. Clearly, under these circumstances full hydration would not be possible. One possible method of investigating this question further is to study the single channel conduction of gramicidin using ionic solutions both in water and also in deuterium oxide. With the exception of some otherwise unpublished results to which Finkelstein [5] makes a brief reference, we have been unable to find any description of such work in the literature.

We have accordingly studied the single channel behaviour of gramicidin A

TABLE I

A COMPARISON OF THE EFFECT OF REPLACING H_2O BY ^2H_2O ON THE MOBILITY OF THE ALKALI METAL IONS

Results are shown for ions in bulk solution and also for ions in the gramicidin A channel.

Ion	Pauling radius (nm)	Mobility in ^2H_2O compared with that in H_2O in bulk solution	Channel conductivity in ^2H_2O compared with that in H_2O
Li^+	0.068	0.80	0.97
Na^+	0.097	0.82	0.90
K^+	0.133	0.83	0.89
Rb^+	0.147	—	0.87
Cs^+	0.167	—	0.86

working with 0.5 M solutions of the alkali ions in both H_2O and ^2H_2O using an applied potential of 100 mV. (The same conductivity ratios were observed in all cases at 50 mV with the exception of Li where measurements were only made at 100 mV.) The method of measurement has already been described by us elsewhere [3,8]. The gramicidin A was synthesised in this laboratory [8]. Various minor improvements in measuring technique have reduced the error to a point where we believe that our comparative measurements in H_2O and ^2H_2O on a particular ion are accurate to ±1%. The absolute value of the single channel conductivities observed at 20°C agreed with those reported by Hladky and Haydon [2] at 23°C to within 5%. Our comparative results are given in Table I together with comparative results taken from the literature [9,10] for ions in bulk solution where these are known.

Our results were obtained at approx. 20°C. The ratio of the viscosity of H_2O to that of ^2H_2O at this temperature is 0.82 [11]. It will be seen from the table that the ratio, in bulk solution, of ^2H_2O compared to that of H_2O is 0.83 for K^+. Although values of this ratio are not available for Rb^+ and Cs^+ we would not expect it to differ greatly from the value for K^+. It will be seen that the corresponding ratio of single channel conductivities approaches this value for Cs^+. Thus for the larger ions the mobility both in bulk solution and in the gramicidin channel varies inversely as the viscosity of the medium. It should be pointed out that Läuger [12] has shown that, for the situation envisaged here, the approach of ions to the channel is not a rate limiting process. We thus infer that, for the larger ions, the passage through the channel is largely controlled by the viscosity of the medium or, more correctly, by some microscopic property of the medium linearly related to the macroscopic viscosity. This behaviour is less marked for the smaller ions. In particular Li^+ exhibits a channel conductivity one order of magnitude smaller than that of Cs^+ [2] and this conductivity is little affected by viscosity.

One possible explanation of these results would be that the rate limiting process in conduction is the effect whereby the ion acts as a piston and the water in the channel moves with it. Such a picture would certainly account for the change in conductivity with viscosity in the case of the larger ions. This might also explain the fact that the conductivity using lithium changes hardly at all with viscosity but would not explain the well established result [2] that the conductivity for lithium is much smaller than for the larger ions.

Another possible explanation of these results is that the larger ions pass down the centre of a channel sufficiently wide so that their interaction is mainly with water rather than the side of the channel. The smaller ions, having a higher electric field at their surfaces, presumably become bound for short periods to the negatively charged oxygens which are constituents of the polypeptide which forms the inner surface of the channel. This latter effect will then be the principal rate limiting process. If this latter model is correct it would be hard to reconcile the postulated [6,7] channel radius of 0.2 nm with the fact that caesium (with a Pauling radius of 0.167 nm) interacts hardly at all with the inner surface of the channel.

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