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Na^+–K^+-DEPENDENT CONFORMATION CHANGE OF PROTEINS OF EXCITABLE MEMBRANES

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SUMMARY

The K^+, Na^+ and Ca^{2+} form of excitable membranes of rat brain were investigated by infrared, ORD and CD spectroscopy. It is shown that with the K^+ form the conformation of relatively large parts of the membrane proteins occurs as an antiparallel β structure. No β structure is found with the Na^+ and Ca^{2+} form. In the presence of these ions the proteins are largely helical. This suggests that during the action potential, membrane proteins change their conformation depending on the cations shifted.

INTRODUCTION

The postulation has been made by numerous authors[1-9] to the effect that on excitation a conformation change of the proteins in the axon membrane occurs. Tasaki and co-workers[1,11-13] have collected comprehensive experimental results which suggest that an ion-dependent conformation change occurs on excitation in the membrane. They found, for instance, that the fluorescence of molecules adsorbed at the axons changes during conduction, and conclude from this that the physicochemical properties of the macromolecules around the dye molecules in the axon membrane change drastically during the process of nerve conduction[12]. Cohen *et al.*[14] found corresponding changes by means of light scattering and birefringence experiments. Shrager *et al.*[15] came to the same conclusion after experiments with protein cross-linking aldehydes. Clark and Strickholm[16] studied the influence of K^+ concentration and pH on the membrane resistance. Hsia *et al.*[17] demonstrate ion-dependent changes to the protein conformation using the cell membranes of *Halobacterium salinarium* by means of spin label experiments.

The conformation of a protein can be determined directly using infrared, ORD and CD spectroscopy. Whether β structure occurs, or helix or coil structure, can be particularly well demonstrated by the infrared spectra, whilst ORD and CD spectra enable a distinction to be made between the helix and coil state. Wallach and Zahler[18] have shown that with plasma membranes the greater part of the membrane proteins certainly does not occur in β conformation, contrary to the concept suggested by

the Danielli–Davson[19] model. These authors found proteins with β structure only under extreme conditions, namely with lipid-extracted membranes studied at pH $<$ 2. Similar results, especially with erythrocyte and *Micrococcus lysodeikticus* membranes, were reported by numerous authors[20–22]. Graham and Wallach[23] further showed that in the presence of ATP and Mg^{2+} the conformation of membrane proteins of erythrocyte membranes changes to an antiparallel β structure.

MATERIALS AND METHODS

A fraction enriched with non-myelinated axons was prepared from the whole brain of 5–8-weeks-old rats[24]. The fraction P_2B was diluted to 0.6 M sucrose and placed on a second sucrose-density gradient consisting of a 20-ml sample, 10 ml 0.8 M and 30 ml 1.0 M sucrose and was centrifuged for 5 h in the SW 25.2 rotor of the Spinco Model L preparative ultracentrifuge at 22500 rev./min. The axon fraction at the 0.8, 1.0 M sucrose interface was collected, diluted to 0.32 M sucrose and centrifuged at 105000 $\times$ *g* for 75 min. The pellet was osmotically shocked by homogenizing with 20 vol. distilled water and sedimented again. When starting from 30 g fresh brain, the final suspension of the purified axon membranes in 1.5 ml contained 8.7 mg protein using the method of Lowry *et al.*[25], and acetylcholinesterase (EC 3.1.1.7) of the specific activity 14.9 μmoles/min per mg using the method of Ellman *et al.*[26].

The membrane suspension was dialysed at 4 °C for 30 h against 1 mM Tris–HCl buffer of pH 7.4 containing 1 mM EDTA. The ions under investigation were then exchanged through dialysis lasting 20 h for 100 mM KCl, NaCl or 50 mM $CaCl_2$ solution. This solution was buffered with the corresponding cacodylate salt to pH 7.4 (5 mM). Finally the samples were dialysed for 4 h against 0.1 mM salt solutions (pH 7.0) prepared by neutralisation of bidistilled water. Subsequent production of the films was effected under complete exclusion of CO_2. Further portions of the non-dialysed membrane suspension were dialysed against 200 nM tetrodotoxin in the bidistilled water neutralised by 0.1 mM NaOH.

Sample preparation and plotting of the infrared spectra

100 μl of the membrane suspension containing 0.6 mg proteins were used for plotting the infrared spectra. They were dried on a Ge disc at 15 °C according to the centrifuging process described in ref. 27 at approx. 58 % air humidity (hydrous saturated NaBr solution). This process[27] guarantees more than 2 % reproducibility of the layer thickness. Drying the films at approx. 58 % air humidity should prevent the secondary or tertiary structure of the membrane from changing due to dehydration. The samples were subsequently placed in the cells described in ref. 28 and hydrated for 20 h at 90 % air humidity (saturated hydrous $BaCl_2$ solution) at 25 °C prior to plotting the spectra. On the basis of the final water content of the membrane sample of about 50 % at 90 % air humidity, a final salt concentration of about 20 mM was estimated. Minor differences in the final water content of the sample which are caused by the different ionic species are not critical, since no conformation change was observed down to 50 % air humidity. Deuteration was effected *via* the vapour phase with a saturated $BaCl_2$ ^2H_2O solution[28].

The infrared spectra were plotted with the photospectrometer model 325 made by Perkin–Elmer Bodenseewerk, Ueberlingen (gain 3.1, program 6.5, response 3).

RESULTS

Fig. 1 shows infrared spectra of the K^+, Na^+ and Ca^{2+} form of the axon membranes. With the K^+ form an intensive band at 1630 cm^{-1} is observed in the range of the amide band (CO stretching vibration in the peptide group) at H_2O hydration, one at 1648 cm^{-1} and a rather weak shoulder at 1695 cm^{-1}. According to refs 29–32, the band at 1630 cm^{-1} is caused by membrane proteins present in β structure. This is at least in part an antiparallel β structure, as indicated by the weak shoulder at 1695 cm^{-1}. The band at 1648 cm^{-1} is caused by helical or coiled membrane proteins. Accordingly, with the K^+ form a large portion of the membrane proteins has an at least partially antiparallel β structure. If the samples are stored over a longer period of time at 4 °C, the β structure disappears. After approx. 14 days only the band at 1648 cm^{-1} is still to be observed. The question to which extent the samples reflect the native membrane structure is open. However, the ionic-specific differences became independent of artifacts by starting from the same preparation and running the samples in parallel.

Contrary to the K^+ form, in the case of the Na^+ and the Ca^{2+} membrane form, likewise after addition of tetrodotoxin, only one band at 1652 cm^{-1} is observed. This band indicates that the membrane proteins occur in the presence of these ions either

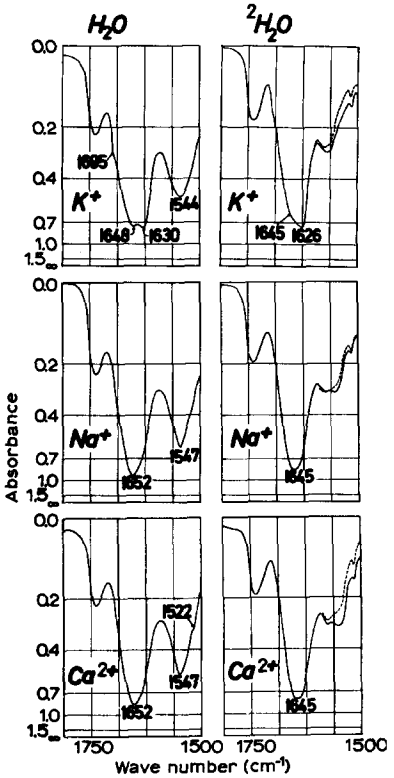

Fig. 1. Infrared spectra of K^+, Na^+ and Ca^{2+} form of axon membranes, left H_2O and right ^2H_2O hydrated. ^2H_2O hydrated sample: ———, 15 h exchange; ·····, 93 h exchange.

as an α helix or in disordered conformation. ORD and CD spectra show that the membrane proteins occur in the presence of the Na^+ and Ca^{2+} largely in helical conformation. This is shown, for instance, by the typical trough in the ORD spectra at 2330 Å and by the crossing-over with the zero line at 2200 Å (*cf* Yang[29], p. 239). An exact quantitative evolution of the spectra of membranes is problematic[34].

The amide II band (CN stretching and NH in-plane bending) is observed in the case of the Na^+ and Ca^{2+} form at 1547 cm^{-1}. With the K^+ form the amide II band is observed at 1544 cm^{-1}. This band, considering the large β structure portion, lies with the K^+ form at a relatively high wave number value. The portion of this band due to the β structure is superposed by that of the helical portions to such an extent that only one band is observed, broadened to smaller wave numbers. Variations of the humidity of the films have shown that the contribution of the H_2O scissor vibration to the absorption at about 1640 cm^{-1} is masked by the intensive amide I bands and do not influence the results.

In the case of the ^2H_2O-hydrated membranes, the amide I bands are shifted somewhat toward smaller wave numbers, conforming to details given in publications[35]. With the K^+ form the weak shoulder at 1695 cm^{-1} has disappeared. This is understandable, since this shoulder shifts on deuteration extremely markedly—as for instance with β-lactoglobulin from 1690 to 1675 cm^{-1}—that is, this shoulder shifts on deuteration towards smaller wave numbers so as to become masked by the band caused by the helical portions. The amide II band shifts on deuteration on account of its NH portion from 1545 to 1450 cm^{-1}.

In summary it can be established that with the K^+ form large portions of the membrane proteins have at least a partially antiparallel β structure. In contrast, no β structure is found with the Na^+ and Ca^{2+} form. In the presence of these ions the membrane proteins are largely helical. No corresponding effects could be observed under comparable conditions for erythrocyte membranes.

DISCUSSION

These findings show that K^+, Na^+ and Ca^{2+} may cause specific conformations of proteins of the axon membrane. The kinetic model by Jain *et al.*[9] for the action cycle of the excitable membrane exists in a time sequence of five different conformations. The high degree of biological safety of this cycle is probably regulated not only by changes of the membrane potential but also by the influence of the various shifted ions on the conformation in the different states of the cycle.

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