CYTOPLASMIC ION EXCHANGE DURING REST AND EXCITATION IN *CHARA AUSTRALIS**

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Introduction

The ion-exchange properties of the cytoplasm are of fundamental importance in determining the overall ionic relations of the cell.

The presence of fixed or indiffusible ions in a system, apart from a simple Donnan equilibrium, can also lead to selectivity in adsorption amongst the cations. For instance, the existence of selectivity in cation adsorption in the cell wall of *Chara* has been demonstrated by Vorobiev and Kurella (1965).

According to one school of thought the sole origin of cellular potentials can be attributed to selectivity in cation adsorption in the bulk cytoplasm (Nasonov and Aleksandrov 1943; Ling 1952). This school of thought also sees the action potential as arising from a transient change in ion selectivity of the cytoplasm (Ling 1962). This is in sharp contrast to the more widely accepted interpretation in terms of membrane excitability (e.g. Hodgkin and Huxley 1952*a*, 1952*b*).

This communication deals with experiments done with cells of *C. australis* aimed at:

(1) Direct measurement of ion exchange of the cytoplasm.

(2) Detecting transients in ion-exchange properties of the cytoplasm during excitation.

Methods

The method is based on recording the potential difference between two microelectrodes, filled with different concentrations of the same monovalent salt, inserted into the cytoplasm. The total potential difference between two such solutions in contact with the cytoplasm is made up of two components: (1) a diffusion potential due to differences in the mobility of the anions and cations, and (2) the sum of two Donnan potentials at the solution–fixed-charge phase boundary. A detailed analysis of such a system has been given by Teorell (1953).

For the electrical measurements standard electrophysiological techniques were used. A schematic diagram of the set-up is shown in Figure 1. The cell could be stimulated by the application of depolarizing pulses supplied from a current generator via a 2·5N KCl-filled microelectrode inserted into the vacuole and a Ag/AgCl grid in the external solution.

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The microelectrodes, designated C_1 and C_2 and filled with 0·2N KCl (C_1) and 2·0N KCl (C_2), were inserted directly into the cytoplasm. These electrodes had a tip diameter $\lesssim 1$ μ and were spaced about 1 mm apart at the tip.

The potential differences between these cytoplasmic electrodes and between electrode C_2 and a similar reference electrode in the external solution (R) were measured with electrometer amplifiers whose outputs were recorded on a chart recorder.

The majority of the experiments were done with internodal cells, about 1·5 mm in diameter and $\simeq 2$ cm long. The external solution used in all the experiments was artificial pond water (0·1 mN KCl, 1·0 mN NaCl, plus 0·2 mN $CaCl_2$).

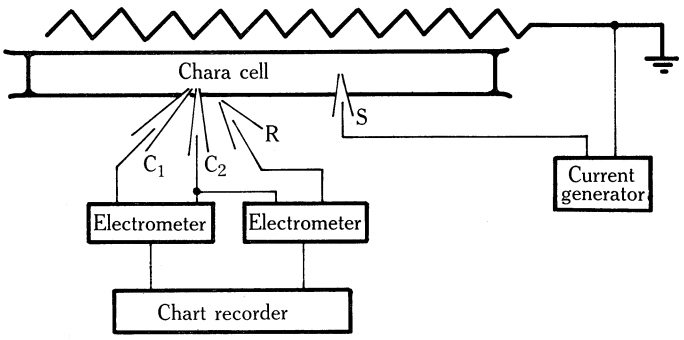

Fig. 1.—Schematic diagram of the experimental apparatus. C_1 and C_2, microelectrodes filled with 0·2N KCl and either 2·0N KCl or 0·2N NaCl respectively, and inserted into the cytoplasm; R, 2·0N KCl-filled external reference electrode. The cell could be stimulated by the application of depolarizing current pulses via a 2·5N KCl-filled microelectrode (S) inserted into the vacuole. The cell was bathed in a flowing solution of artificial pond water.

Results and Discussion

In the resting state there was a potential difference of about 40 mV between electrodes C_1 and C_2 (C_1 positive with respect to C_2). To check whether this potential was due to electrode errors the electrodes were inserted further into the cell, i.e. into the vacuole, after measurements in the cytoplasm had been made. The potential between the electrodes was then less than 5 mV. A zero check was also made with the electrodes in artificial pond water.

When the cell was stimulated the potential between the electrodes in the cytoplasm showed a transient response, though not of the same form as the action potential.

Again the electrodes were inserted into the vacuole after measurements in the cytoplasm had been completed. In each case then (1) a change in the resting potential of about 10 mV (less negative) was observed on going into the vacuole from the cytoplasm; (2) there was no transient response between the formerly cytoplasmic electrodes; and (3) the action potential measured with electrode C_2 with respect to electrode R changed from a typical "plasmalemma" action potential to a "vacuolar" action potential (cf. Findlay and Hope 1964*a*).

Control experiments were also performed using two electrodes both filled with 0·2N KCl inserted into the cytoplasm. A null result was obtained in these experiments, indicating that ohmic potential differences induced by current loops were negligible.

Figure 2 shows the results of a typical experiment obtained with electrodes C_1 and C_2. The peak change in the potential between these electrodes during excitation was about 70 mV, C_1 becoming negative with respect to C_2. Since the potential is reversed it appears that during excitation the effective net fixed charge changes from a negative to a positive value. The time course of the potential, however, is different from that of the action potential itself. The peak of the action potential appears to coincide with the point of maximum slope of the K^+/Cl^- selectivity–time curve. This potential curve is also decidedly flattened at the peak compared with the action potential proper.

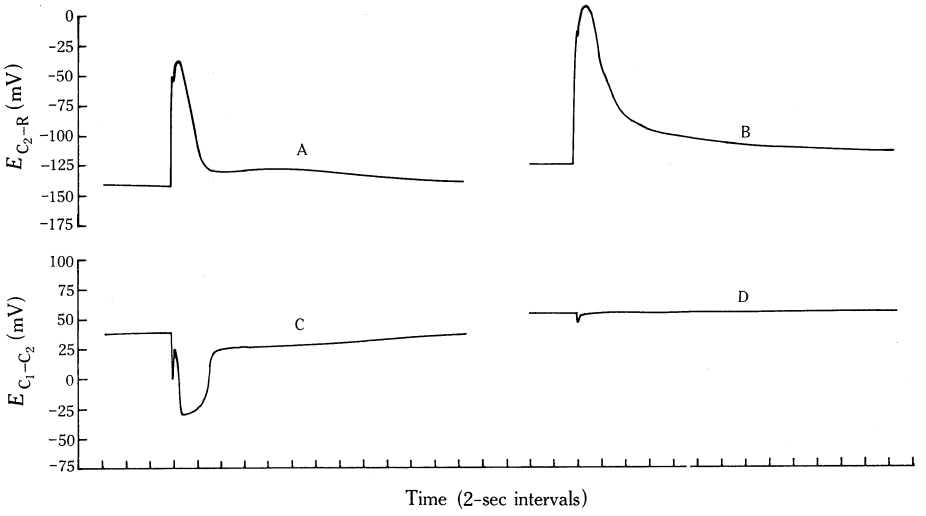

Fig. 2.—*A–D*, transients in the potassium/chloride selectivity in the cytoplasm of *C. australis* during excitation. *C* and *D*, potentials recorded between electrodes filled with 0·2N KCl (C_1) and 2·0N KCl (C_2). *A* and *B*, corresponding potential recorded between electrode C_2 and the similar electrode in the external solution (R). *A* and *C*, both electrodes in cytoplasm. It can be seen from *C* that during the action potential, the cytoplasm changes from a cation-selective system to an anion-selective system. Note that the time course of this potential is different in form from the action potential itself. *B* and *D*, both electrodes pushed on into the vacuole. No response is now recorded (apart from the small artifact) between the two intracellular electrodes.

The observation of a potential difference between electrodes C_1 and C_2 in the cytoplasm confirms the earlier work of Walker (1955) which indicated that the cytoplasm is a negative fixed-charge Donnan phase. It can be shown that for such a system the solution–phase potential approaches zero as the concentration of ions in the solution becomes large compared with the fixed-charge concentration. For the cytoplasm of *Chara* this occurs at a concentration of $\simeq$1N (Walker 1955), indicating that the fixed-charge concentration is approximately 0·1N. This value was also suggested from measurements of cytoplasmic ion concentrations (Coster 1966).

It is likely that the cytoplasm contains both fixed cationic as well as fixed anionic sites. During excitation there appears to be a change in the effective sign of the fixed charge in the cytoplasm; the cytoplasm changes from a predominantly cation-exchange system to an anion-exchange system.

It is thus obvious from the present results that excitation is intimately linked with a bulk-phase phenomenon associated with changes in cytoplasmic fixed charge. It has previously been shown that the action potential in *Chara* is associated with a transient efflux of chloride ions, and a transient efflux of potassium ions was also inferred (Mullins 1962; Findlay and Hope 1964*a*, 1964*b*; Hope and Findlay 1964; Mailman and Mullins 1966). This was attributed to a transient increase in the membrane permeability to chloride ions. This would cause the intracellular potential to shift from the resting potential, which is close to the potassium equilibrium potential, towards the chloride equilibrium potential.

The transient fluxes (and electrical currents) and changes in potentials are also expected if during excitation the cytoplasm undergoes a transient loss of potassium/chloride selectivity. Thus previous experimental work is completely consistent with the present findings.

If there is a substantial electronic rearrangement of fixed-charge sites in the cytoplasm during excitation then this could easily give rise to changes in its rheological properties. For instance, substantial long-range ordering of the water structure could arise, tending to "freeze" the cytoplasm. This could provide a plausible explanation for the cessation of cytoplasmic streaming during excitation in characean cells.

Some measurements were also made of potential differences between electrodes filled with 0·2N KCl (C_1) and 0·2N NaCl (C_2'), inserted into the cytoplasm. It was found that the potential between these electrodes was $\simeq$23 mV (electrode C_1 negative with respect to electrode C_2'). This potential difference undergoes a transient during excitation (Fig. 3).

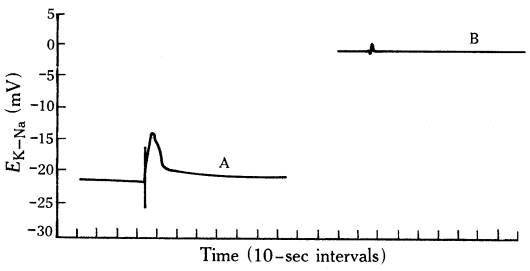

Fig. 3.—Transients in the potential between 0·2N KCl- and 0·2N NaCl-filled electrodes in the cytoplasm of *C. australis* during excitation. Other details of the procedure as for Figure 2. *A*, electrodes in cytoplasm; *B*, electrodes in vacuole. The transient in this potential is not nearly as large as the change in cation/anion selectivity during excitation.

The observation of this potential can be interpreted in two ways: (1) the potential is just a diffusion potential and is due to the differences in the mobilities of K^+ and Na^+ ions. The ratio of these mobilities, U_{K+}/U_{Na+}, in the cytoplasm would have to be different from that in a corresponding aqueous solution (a ratio U_{K+}/U_{Na+} of approximately 4–5 is required); or (2) the potential is due to selectivity in cation adsorption. It is difficult to see how these potentials can be calculated for the present experiments.

The small transient observed in these potentials ($\simeq$10 mV) during excitation could easily be due to changes in the mobility ratio during excitation, since it appears that the cytoplasmic properties in general undergo changes during excitation.

At this stage, therefore, it appears that the results obtained in these experiments can be explained purely in terms of Donnan potentials and transients in the effective

fixed-charge concentration. While the experimental results with the 0·2N KCl *v.* the 0·2N NaCl electrodes could be attributed to selectivity in cation adsorption, there is no compelling reason to do so at present, since they could equally well be explained in terms of a diffusion process.

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