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THE EFFECT OF pH ON THE OsO_4-REVEALED STRUCTURE OF THE PLASMA MEMBRANE OF *CHARA CORALLINA*

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SUMMARY

Statistically average absorbance profiles were obtained of the membrane image in electronmicrographs of OsO_4 stained cells of *Chara corallina*, cultured and fixed at pH 7 and pH 9. At pH 7 the membrane appeared to be symmetrical about a central plane. At pH 9 the membrane showed a marked asymmetry in its structure. At pH 9 the central electron-lucent region on the cytoplasmic side of the centre of the membrane was approx. 35% smaller than at pH 7. The corresponding region in the outer half of the membrane was the same at both pH values. At pH 9 the overall thickness of the membrane was also approx. 10% smaller than at pH 7.

INTRODUCTION

The transport properties, particularly the ion transport properties and the associated electrical characteristics of the plasma membrane are sensitive to the pH of the surrounding extra and intra cellular medium[1–5].

It has been suggested that the effects of pH are likely to arise through pH induced conformal changes in the membrane proteins and or more directly on changes in the density of ionized sites such as COO^- and NH_3^+ on acidic and basic amino acids in the polypeptide chains.

The present communication is concerned with the effect of pH on the structure of the plasma membrane in OsO_4 fixed, "araldite" imbedded, cells of *Chara corallina*. Studies have previously been made of the effect of pH on the electrical characteristics, particularly the punch-through phenomena, of the plasma membrane of the giant cells of this species[2].

EXPERIMENTAL METHODS

Cells for the experiments were cultured in the laboratory in an controlled ionic and temperature environment. The culture medium was, apart from a minor modification, that described by Forsberg[6]. The ionic composition of the solution was controlled by daily measurements and adjustments of pH (adjusted with NaOH or HCl to pH 7 or pH 9) and weekly measurements and adjustments of K^+ and Na^+ (adjusted by addition of NaCl, KCl or replacement by fresh culture solution). The tanks

were kept in a temperature controlled room at 20±2 °C. A summary of the ionic composition of the culture solution is given in Table I.

The cells selected for electron microscopy were generally small nodal cells at the tip of the plant. For ease of manipulation these cells were left attached to the larger, more mature, cells below them.

TABLE I

CULTURE MEDIUM (pH 7) MODIFIED FROM FORSBERG[6]

Compound	*Concentration (μequiv/l)*
$CaCl_2$	970
NH_4Cl	70
$MgSO_4$	820
Na_2CO_3	380
Na_2SiO_3	160
KCl	320
K_2HPO_4	1.63
$FeCl_3$	4.43
$ZnCl_2$	1.47
$MnCl_2$	0.02
$CoCl_2$	0.0168
$CuCl_2$	0.0469
H_3BO_3	20
Na_2MoO_4	80
NTA* (mg/l)	20
Tris (g/l)	0.2

* NTA, Nitrilotriacetic acid.

The cells were fixed for 2 h in 1% OsO_4 buffered to the pH in which the cells were cultured with 0.1 M phosphate buffer. After thorough washing with the buffer they were dehydrated in an ethanol series, transferred to propylene oxide and embedded in "Araldite".

In some cases the cells were prefixed in a 6% glutaraldehyde solution buffered to the required pH with 0.025 M phosphate buffer containing[7] added $CaCl_2$. This fixative was diluted 1:2 before usage and specimens were fixed at 70 °C for 2–16 h.

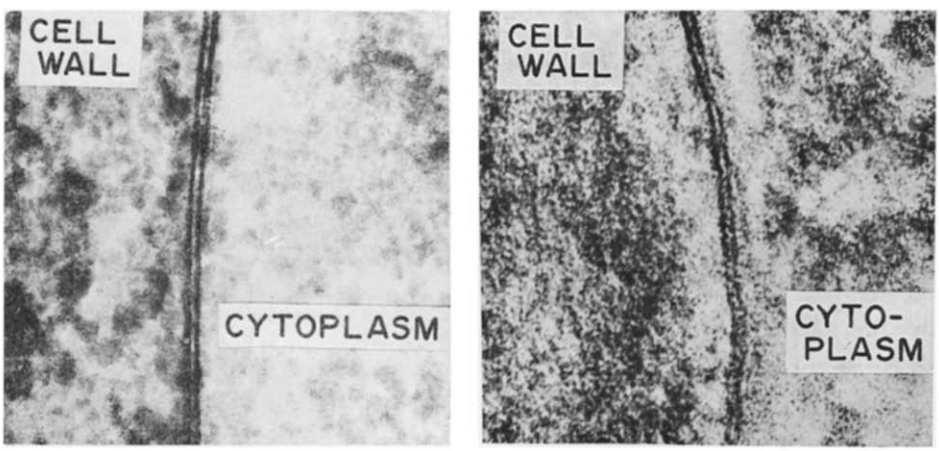
Thin sections (silver and gold approx. 60–150 nm) were cut on glass knives using a "Reichert" ultramicrotome. They were mounted on bare grids (or grids coated with parlodion-carbon), stained with uranium and lead and examined in a "Phillips EM 300" electron microscope at 80 kV. The magnification on the machine was determined by calibrating the instrument, before and after a run, with a diffraction grating.

Profiles of absorbance of the electron micrographs of the membranes were obtained using a "Joyce" optical scanning microdensitometer. Individual scans were made of intact sections of the membrane image corresponding to a segment 100–200Å in length of the membrane. The slit width in the microdensitometer (which determines the resolution) was set to correspond to approx. 2 Å layers of the membrane.

The individual absorbance profiles obtained were converted into numerical form, matched at the central point of minimum absorbance, and the statistically average absorbances (and standard errors) at 5 Å intervals determined. From this the numerically average profiles were constructed.

RESULTS

The appearance of the plasmalemma of *C. corallina* was like the classical tri-layer membranes usually seen in electromicrographs of cells. It was found, however, that prefixing with gluteraldehyde, in the majority of cases, destroyed or disrupted the membrane structure either completely or to a very major extent. Examples of the electronmicrographs showing the plasmalemma revealed with OsO_4 fixation, in cells cultured and fixed, at pH 7 and pH 9 are shown in Plates I and II, respectively. Generally the membranes seen in the electronmicrographs appeared more granular at pH 9 than at pH 7. However, the differences in granularity at the two pH values were usually much less than that seen in Plates I and II. The results obtained for the average absorbance profiles of the plasmalemma image would not be affected by such differences in granularity.



Plates I and II. Examples of electronmicrographs showing the plasmalemma of OsO_4 fixed cells of *C. corallina*, cultured and fixed at pH 7 (Plate I) and pH 9 (Plate II). Magnification $\times 200\,000$.

The tonoplast, the presence of which is inferred from various electrical experiments, was not seen in any of the approx. 400 cell sections examined.

An example of the profile of absorbance for the plasmalemma image in the electronmicrographs (for a cell cultured and fixed at pH 9) is shown in Fig. 1.

Fig. 2 gives a comparison of the numerically averaged absorbance profiles for the plasmalemma at pH 7 and pH 9. It is evident that the average profile of the membrane at pH 7 has a high degree of symmetry, despite the fact that individual profiles of 100–200 Å segments often appeared to be asymmetrical. The profile at pH 9 shows a distinct decrease in the width of the central (electron lucent) region on the cytoplasmic side of the membrane.

Each individual scan produces a profile which is a convolution of the actual profile (averaged over 100–200 Å long segments of the membrane) and the aperture of the slit. In view of the fact that the final profiles are statistically averaged over individual scans it seemed unwarranted to deconvolute these final profiles. In any

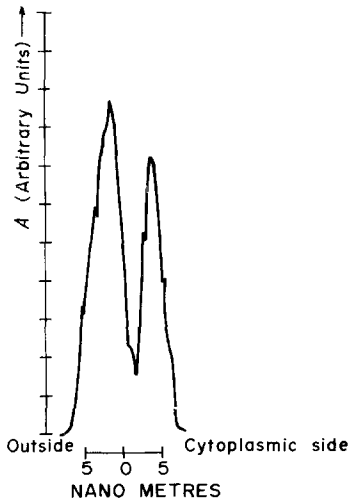


Fig. 1. An absorbance profile for the image of the plasmalemma in an electronmicrograph of an OsO_4 fixed cell of *C. corallina*. In this case the cell was cultured and fixed at pH 9.

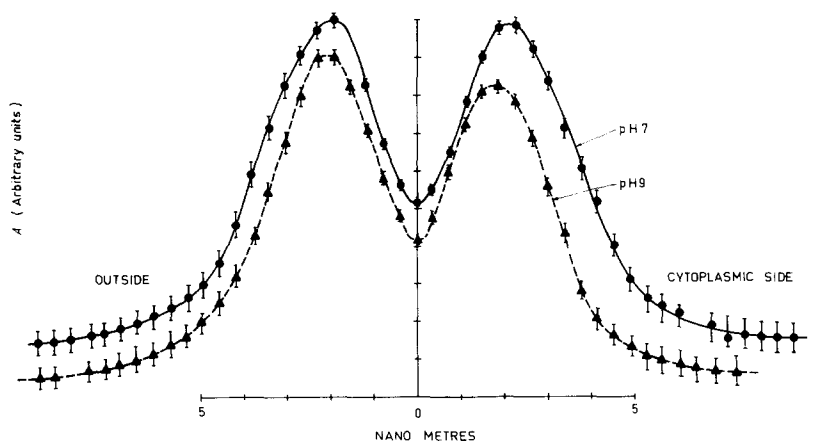


Fig. 2. The numerically averaged absorbance profiles for the plasmalemma images in electronmicrographs of cells of *C. corallina* cultured and fixed at pH 7 and pH 9. To obtain the average profiles at each pH individual absorbance profiles for 26 sections at each pH were matched at the central point of minimum absorbance. The individual profiles, converted into numerical form were then used to obtain the statistically average absorbance at 5-Å intervals. The error bars shown on the two curves are the standard errors.

case the slit width used corresponded to only 2 Å strips of the original membrane material and hence the average delimiting boundaries of the three layers in the electronmicrographs of the membrane can simply (though perhaps a little arbitrarily) be set at the points of inflection of the absorbance profiles.

Using this criterion the statistically average trilayer structures at pH 7 and 9 are shown in Fig. 3.

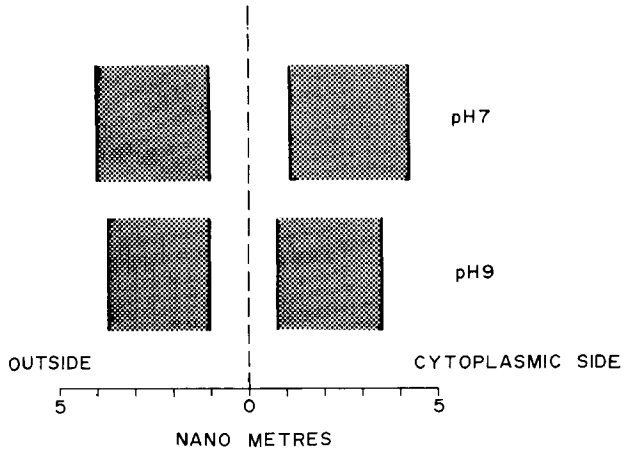


Fig. 3. The structure of the plasmalemma of *C. corallina* deduced from the averaged absorbance profiles shown in Fig. 2. For this reconstruction the boundaries of the apparent trilaminar structure were taken at the points of inflection of the averaged profiles.

DISCUSSION

It is clear from the density profiles shown in Fig. 2 that pH has an effect on the structure of the plasmalemma of *C. corallina* revealed after OsO_4 fixation, dehydration and araldite imbedding. Further, the effect of pH is more pronounced in the half of the membrane facing the cytoplasm than the outer half facing the cell wall.

It is, of course, entirely possible that the pH does not affect the membrane structure *in vivo* but that the effects observed are simply artifacts due to the effect of pH on the OsO_4 fixation and dehydration process. However, the fact that the pH effects are not uniform throughout the membrane but are largely confined to the inner half of the membrane facing the cytoplasm tends to support the notion that the effects are not simply pH dependent artifacts due to fixation and embedding.

From the profiles shown in Fig. 2 it appears that the total width of the half of the membrane facing the cytoplasm is significantly greater at pH 7 than at pH 9. This is in part due to a decrease of the sharpness of the profile at pH 7 (perhaps due to greater globularity of the inner electron dense layer of the membrane). Using the point of inflection to delimit the boundaries of the electron dense layers however, (see Fig. 3) it is seen that the overall width of the membrane is approx. 10% smaller at pH 9 than at pH 7 but the greater part of this change occurs in the part to the cytoplasmic side of the centre.

Further, at pH 7 the central electron-lucent region to the cytoplasmic side of the centre of the membrane is some 35% larger than at pH 9. On the other hand the electron-lucent regions to the outside of the centre of the membrane are almost identical at pH 7 and pH 9.

If the central electron-lucent layer in OsO_4 fixed membranes is identified with the ionic depletion layer in the double fixed charge model of cell membranes[8], then the present findings are qualitatively in agreement with those inferred on the basis of this model by Coster[2] from the variation of the punch-through potential in these cells with external pH. It was suggested on the basis of these results that the ratio of the widths of depletion layer in the cytoplasmic and outer halves of the membrane should decrease with increasing external pH.

It is not immediately obvious how these results can be reconciled with the protein coated lipid bilayer model of the membrane. Apart from being the result of a pH sensitive effect of the fixation and dehydration processes it is also not clear how the overall thickness of the membrane could be pH dependent other than through a pH dependence in the conformation of membrane proteins.

It is also interesting to note that at extremes of pH (<5.5 and >9.5) the trilaminar structure of the membrane was not seen. These cells cultered at these extremes of pH, however, not unexpectedly, were barely viable. It would appear that the membrane structure is unstable at such extremes of pH. This finding is also consistent with the observations of Carstensen and Smearing[9] on OsO_4 fixed erythrocytes.

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