Plant Physiol. (1985) 77, 74–78
0032-0889/85/77/0074/05/$01.00/0

Acclimation to Low Temperature by Microsomal Membranes from Tomato Cell Cultures

Received for publication June 11, 1984 and in revised form September 24, 1984

FRANCES M. DUPONT*[1] AND J. BRIAN MUDD
ARCO Plant Cell Research Institute, 6560 Trinity Court, Dublin, California 94568

ABSTRACT

Sealed vesicles were prepared from microsomal membranes from cell suspension cultures of tomato (*Lycopersicon esculentum* Mill cv VF36). ATP-dependent proton transport activity by the vesicles was measured as quenching of fluorescence of acridine orange. Measurements of proton transport were correlated with the activity of a nitrate-inhibitable ATPase. The initial rate of proton influx into the vesicles was strongly temperature dependent with a Q_{10} of 2 and a maximum rate near 35°C. The data suggest that passive permeability did not increase at chilling temperatures but did increase rapidly with temperatures above 30°C. A comparison was made between membranes from cell cultures grown at 28°C and 9°C. The temperature optimum for proton transport broadened and shifted to a lower temperature range in membranes from cells maintained at 9°C.

Membrane properties have been postulated to play a major role in the ability of plants to tolerate or acclimate to temperature stress (3, 16, 22, 24). Research efforts on chilling injury, temperature tolerance, and acclimation of plants to low and high temperatures have understandably been centered on the membranes that are easy to identify and are well characterized, that is the membranes of chloroplasts and mitochondria, or deal only with lipid extracts from entire tissues. However, Ono *et al.* (19) made a comparison of the effects of chilling temperatures on membranes of *Anacystis nidulans* and discovered that chilling injury was correlated with a phase change in the lipids of the cytoplasmic membrane and not with the phase change in the chloroplast membranes. Their studies illustrate the need to analyze the response of the individual membranes of the plant cell to temperature. Data are scarce on the effect of temperature on plasma and vacuolar membranes in higher plants. While the potential role of the plasma membrane in responses to temperature is recognized (13, 16), little mention has been made of the effect of temperature on the tonoplast. However, the tonoplast may be as important as the plasma membrane. In many plant cells, including the tomato cells described in this paper, the cytoplasm is a thin layer sandwiched between the cell wall and the vacuole. We estimated that the surface area of tonoplast in the tomato cells, including the many cytoplasmic strands, was approximately twice the surface area of the plasma membrane, providing twice the surface area for diffusion and transport. Studies of the effect of temperature on transport in vesicles isolated from the tonoplast will help to determine the specific effects of temperature on the functions of the tonoplast, and determine the upper and lower limits for optimal functioning of the membrane.

Suspension cultures of tomato cells have been used as model systems to study the effects of water stress (12) and chilling (5, 8) on plant cells. Use of suspension cultures may serve to separate cellular from whole plant aspects of acclimation to or tolerance of stress. Suspension cultures provide a source of large quantities of membranes while making it possible to avoid or accurately monitor the effects of age and differentiation on membrane composition. Vesicles, capable of ATP-dependent proton transport, have been isolated from tomato cells and characterized (9). The characteristics of the proton-translocating ATPase resemble those of the tonoplast ATPase of red beet (1, 23). In this paper, we describe the use of an *in vitro* transport assay to test the effect of temperature upon active transport by the anion-sensitive proton translocating ATPase of tomato cells.

MATERIALS AND METHODS

Cell Cultures. Callus of *L. esculentum* Mill cv VF36 was initiated from hypocotyls of aseptic seedlings. Suspension cultures were initiated from hypocotyl callus and were maintained as described previously (8). The suspension cultures were initiated 1 year prior to the experiments shown, and were maintained on a medium containing ammonium as the sole nitrogen source. The cultures were maintained by weekly subculture of 5 ml of cells (early stationary phase) into 175 ml of fresh medium in 500 ml Erlenmeyer flasks, and kept at 28°C on a reciprocal shaker at 60 cycles/min.

Chilling Treatment. Flasks, inoculated as described above, were maintained at 28°C for 4 d, then transferred to a temperature-controlled orbital shaker (New Brunswick Scientific, Edison, NJ) and maintained in a cold room at 9°C and 180 rpm.

Membrane Preparations. Cells were collected by vacuum filtration onto Whatman No. 4 filter paper on a Büchner funnel, and washed with an equal volume of distilled H_2O. Cells grown at 28°C were harvested and washed at room temperature; cells grown at 9°C were harvested and washed in a cold room at 4°C. All other operations were carried out at 4°C. Cells (10–20 g) were immediately weighed, placed in 250 ml of homogenization buffer with 0.5 mm diameter glass beads in a Bead Beater Cell Homogenizer (Biospec Products, Bartlesville, OK). The homogenate was filtered through two layers of Miracloth (Calbiochem), centrifuged at 3,000*g* for 5 min, the pellet discarded, centrifuged at 10,000*g* for 20 min to obtain the mitochondrial pellet, and at 100,000*g* for 35 min to obtain the microsomal pellet. The microsomal and mitochondrial pellets were resuspended in 20 to 40 ml of suspension buffer consisting of 0.25 M sucrose and either 5 mM Tris-HCl (pH 7.5) or 5 mM Pipes-Tris (pH 7.0) as indicated, placed in one or two centrifuge tubes of 38 ml capacity, underlaid with 10 ml of 10% Dextran (70 kD) in suspension buffer, and centrifuged at 80,000*g* for 1 h. The membranes from the dextran interface were collected and frozen at −70°C for up

[1] Present address: USDA Western Regional Laboratory, 800 Buchannan Street, Albany, CA 94710.

to 3 weeks before assay; freezing had no effect on initial rate of quench.

Assays. Proton transport was assayed as ATP-dependent quench of fluorescence of acridine orange (9). The reaction buffer, consisting of 3 ml of 0.25 M sucrose, 50 mM KCl, 1 mM $MgSO_4$, 1 μM acridine orange, and either 10 mM Tris-HCl (pH 7.3) or 10 mM Pipes KOH (pH 7.0) as indicated, was brought to the indicated temperature in a stirred, temperature-controlled cuvette. Temperature of the cuvette was controlled by a Peltier thermostat aided by a Neslab (Portsmouth, NH) circulating water bath. Temperatures given are accurate to the nearest degree. Membrane vesicles were kept on ice, aliquots equivalent to 30 to 100 μg protein were added to the reaction buffer, at the indicated temperature, and 2 to 5 min later quench was initiated by addition of ATP to achieve a final concentration of 1 mM.

ATPase activity was assayed in a total reaction volume of 3.5 ml including 1 mM Tris-ATP, 1 mM $MgSO_4$, 100 μM ammonium molybdate, 100 μM sodium orthovanadate, 1 mM sodium azide, 10 mM Pipes KOH (pH 7.0), and 50 mM KCl or 50 mM KNO_3. The reaction was started by adding 100 μg of enzyme protein. Inorganic phosphate was assayed using a modification of the method of Sanui (27). Aliquots (0.5 ml) of the reaction mixture were removed at timed intervals, immediately placed in a mixture of 1.5 ml of 12% TCA plus 0.5 ml of 3.75% ammonium molybdate in 3 N H_2SO_4. Within 5 min, 2.0 ml of butyl acetate was added to the TCA mixture, vortexed 15 to 20 s, and allowed to settle for 5 min. The upper butyl acetate layer, containing the extracted phosphomolybdate complex, was removed with a pasteur pipette, and placed in a clean test tube. A_{310} was read within 3 h.

RESULTS AND DISCUSSION

In the following discussion, two parameters are used to describe the quench of acridine orange fluorescence. The first parameter is the initial rate of quench (7, 25) (Fig. 1A). It represents the maximum slope measured upon addition of ATP, and is taken to reflect the initial rate of proton influx into the vesicles (2, 7, 30). The second parameter is the maximum quench (Fig. 1B) which describes the maximum decrease in fluorescence during the time course of quench. The maximum quench is assumed to represent the steady state where rate of proton influx, via the H^+-ATPase, equals the rate of passive proton efflux (7, 25). Thus, the effect of temperature on the activity of the enzyme and on membrane permeability to protons can be evaluated by these parameters. It has been suggested, however, that the rate of H^+ influx and efflux may actually be limited in sealed vesicles of this type by the slower fluxes of accompanying anions or cations (25) since permeability of a lipid bilayer to cations and anions is much lower than that for protons and water (10). Therefore, the measurements of initial rate of quench and of maximum quench as a function of temperature are probably indicators not only of the proton fluxes but also of the effect of temperature upon the permeability to anions or cations. We therefore view the measurements as very general indicators of membrane properties, and not as specific indicators of the permeability to protons.

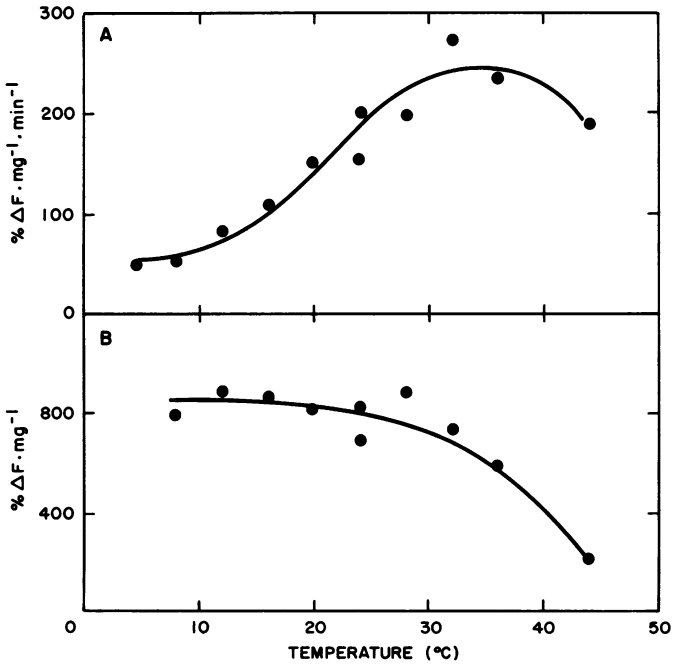

FIG. 1. ATP-dependent proton transport as a function of temperature. Membrane vesicles from the 3,000 to 10,000g mitochondrial pellet were collected on a cushion of 10% Dextran. The cells were grown at 28°C for 5 d. A, Initial rate of quench of acridine orange fluorescence as a function of temperature. B, Maximum quench as a function of temperature. Assay buffer contained 10 mM Tris-HCl (pH 7.3).

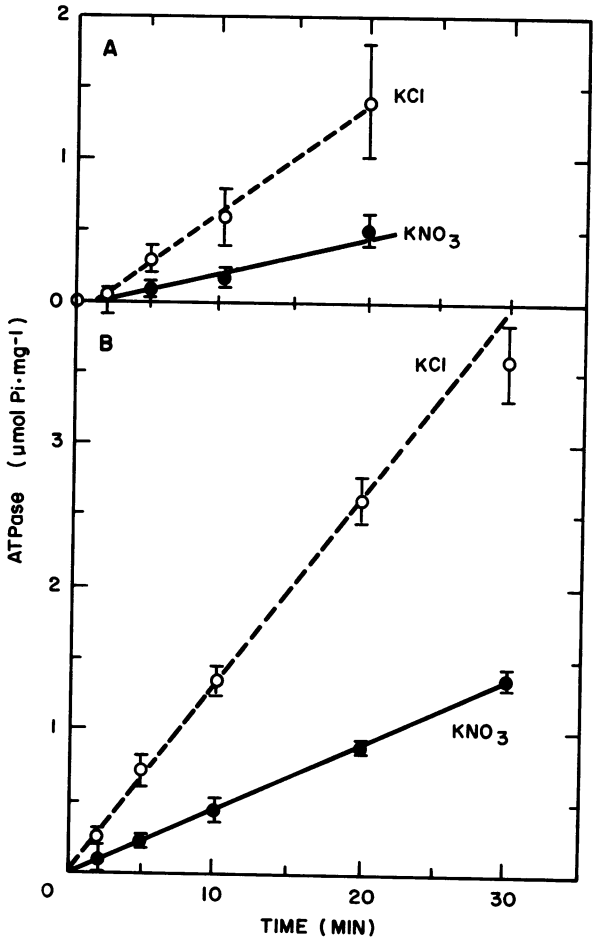

FIG. 2. ATPase activity as function of time. Membrane vesicles from the 10,000 to 100,000g microsomal pellet were collected as in Figure 1 from cells grown at 28°C for 5 d. ATPase activity was assayed at 23°C (A) or 38°C (B) in the presence of 50 mM KCl (O) or 50 mM KNO_3 (●). The assay buffer contained 10 mM Pipes KOH (pH 7.0).

ATP-dependent proton transport, assayed as quench of acridine orange fluorescence, was measured as a function of temperature. The general pattern, illustrated in Figure 1, was a temperature optimum between 30°C and 40°C for initial rate of quench, and a decline, with temperature, of maximum quench. Although initial rates at 5°C were low, they produced, within 30 min, as

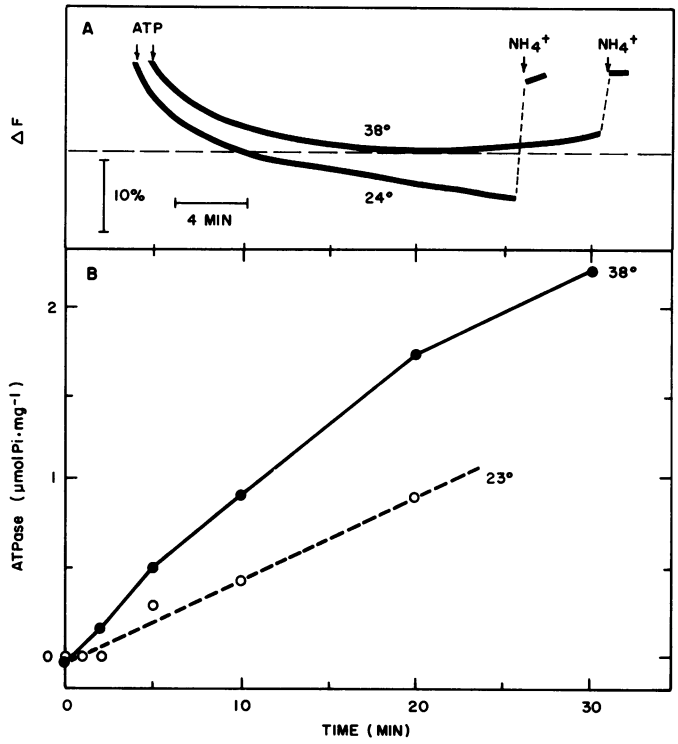

FIG. 3. ATPase activity and fluorescence quench as a function of time. A, ATP-dependent fluorescence quench was assayed at 24°C and at 38°C. Vesicles are the same as in Figure 2. NH_4^+ was added where indicated to achieve a final concentration of 6 mM. B, Nitrate-inhibited ATPase activity was calculated from the data shown in Figure 2.

large a maximum quench as at 30°C. In repeated experiments, using various buffers, Arrhenius plots (not shown) gave a straight line fit from 5 to 20°C, with a Q_{10} of 2 and no obvious break point.

Two methods were used to assess vesicle permeability to protons at different temperatures. One was to compare rates of recovery of quench (21) upon inhibition of the enzyme with KNO_3. The rate of recovery was much lower at 5°C than at 20°C or 30°C when measured at a comparable starting ΔpH.[2] The other was to test recovery of quench after addition of KOH to create an artificial ΔpH (21). Rates of recovery were very slow at 5°C, and rapid at 30°C (data not shown). These results suggest that membrane permeability is lower at lower temperatures.

Figures 2 and 3 compare the time course for ATP hydrolysis with the time course for proton transport at 23 to 24° and 38°C. ATPase activity was assayed in the presence of ammonium molybdate, to inhibit phosphatase, sodium orthovanadate, to inhibit any vanadate-sensitive ATPase that might be present, and NaN_3, to inhibit the mitochondrial ATPase (11). ATPase activity and proton transport were measured as a function of time at 23°C and 38°C in the presence of 50 mM KCl or 50 mM KNO_3 (Figs. 2 and 3A; Table I). The nitrate-inhibitible portion of the ATPase activity is in agreement with the data for quench. The nitrate-inhibitible ATPase activity was linear with time at 23°C over 20 min, but not at 38°C (Fig. 3). At 38°C, quench reached a maximum at 10 min, then began to decline. ATPase activity with time also declined at 38°C. Although the rate of the ATPase was higher at 38°C than at 24°C, initial rates of quench were similar at the two temperatures, and maximum quench was greater at 24°C than at 38°C.

It is concluded from the data of Figures 1 to 3 and from the permeability measurements that at temperatures above 30°C the abrupt decline in maximum quench is largely a function of increased passive efflux of protons from the membrane vesicles but also of denaturation of the ATPase.

Several experiments indicated that membranes prepared from cells maintained at 9°C had a lower temperature optimum for quench than membranes from cells maintained at 28°C. In the following experiments, this adaptation is studied in detail and the effect of both time and temperature are considered. A similar response to temperature ws obtained using Tris buffer (pH 7.3) (Fig. 1) or Pipes-KOH (pH 7.0) (Fig. 4), though initial rates of quench were lower using Pipes than using Tris. We preferred to use Pipes-KOH for the fluorescence assay buffer because its pK changes only slightly with temperature.

Although total membrane protein per g fresh weight declined between day 5 and day 10 for cells grown at 28°C and the yield of ATP-dependent proton transport was low for the day 10 stationary phase cells (Table II), the specific activity and temperature profile did not change between day 5 and day 10 (Fig. 4). The cultures maintained at 9°C slowly increased in fresh weight, doubling in fresh weight in 2 weeks, then had no further increase in weight whether maintained in the same medium or transferred to new medium. After 2 weeks at 9°C, the cultures were gray, but when homogenized the gray color was found largely in the cell wall remnants on the Miracloth filter and in the 3,000*g* pellet. Yields of total protein were similar for day 5 cells maintained at 28°C and for cells that passed 1, 8, or 15 d at 9°C, while the yield of proton transport activity measured at 24°C increased after exposure to cold (Table II).

The temperature profile for proton transport changed with exposure to chilling temperatures. The temperature optimum for initial rate of quench broadened and shifted to a lower temperature range when cells were maintained at 9°C for from 1 to 2 weeks (Fig. 5). Figure 6 shows a detailed comparison of the effect of temperatures between 4°C and 20°C on initial rates of quench. The initial rate of quench for cells maintained at 9°C was much greater than initial rates of quench for cells maintained at 28°C, though at higher temperatures (Fig. 5) rates were similar.

The results suggest that the tonoplast of the tomato cells can adapt to low temperatures and maintain higher rates of proton transport than those observed in nonadapted cells. The increase in initial rate of proton transport by the tonoplast vesicles adapted to low temperatures may reflect alterations in the enzyme itself, in the lipid bilayer, in the interaction of the enzyme with the membrane and/or an increased permeability at low temperatures to the accompanying anion. An increased rate of ion uptake after

[2] Abbreviation: ΔpH, pH gradient.

Table I. *Effect of Nitrate on Proton Transport and ATPase Activity*

Vesicles are the same as shown in Figures 2 and 3. ATP-dependent proton transport was measured as initial rate of quench of acridine orange fluorescence at 24°C.

Temperature	Proton Transport Rate			ATPase		
	+KCl	+KNO_3	Inhibition	+KCl	+KNO_3	Inhibition
°C	$\%\Delta F \cdot mg^{-1} \cdot min^{-1}$		%	$\mu mol\ Pi \cdot mg^{-1} \cdot h^{-1}$		%
23	124 ± 19	15 ± 5	88	4.2 ± 1.3	1.5 ± 0.4	64
38	109 ± 18	29 ± 11	74	7.9 ± 0.5	2.7 ± 0.1	66

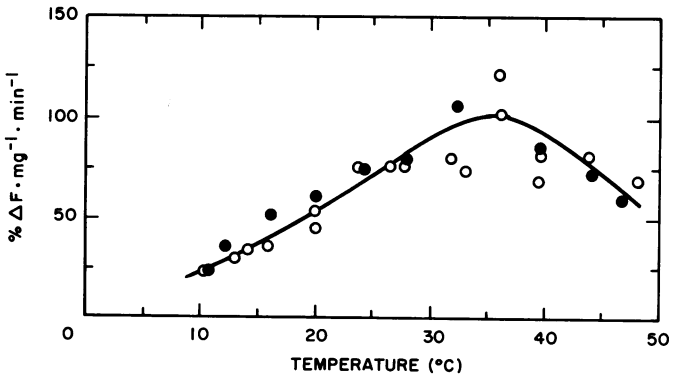

FIG. 4. Initial rate of quench as a function of temperature. Effect of time in passage. Vesicles were prepared as in Figure 2 from cells grown at 28°C for 5 d (●) or 10 d (○). See also Table II. The assay buffer contained 10 mM Pipes KOH (pH 7.0).

Table II. *Effect of Different Time and Temperature Regimes upon Initial Rate of Proton Transport and Membrane Protein*

Membrane vesicles from the 10,000 to 100,000*g* microsomal pellet were collected on a cushion of 10% Dextran. ATP-dependent proton transport was measured as initial rate of quench of acridine orange fluorescence at 24°C.

Growth Temp	Time	Total Protein	Proton Transport Rate	
			Specific activity	Total activity
°C	*d*	*mg·g fresh wt*$^{-1}$	*%F·mg*$^{-1}$*·min*$^{-1}$	*%F·min*$^{-1}$
28[a]	5	0.22	70.0	15.40
28	10	0.03	70.0	2.10
9[b]	1	0.19	50.0	9.50
9	8	0.18	94.0	16.92
9	15	0.21	120.0	25.20

[a] The cultures were grown at 28°C.

[b] The cultures were grown at 28°C for 4 d, then transferred to 9°C for the indicated time.

exposure to low temperatures has been reported for roots of rye and barley (6, 28) and for the fresh water alga *Chara corallina* (26). It was suggested that the increases were a function both of alterations in lipid composition and increase in number of transport proteins (6, 26). Also, Hellergren *et al.* (13) reported an increase in the specific activity of a Mg^{2+}-ATPase in a plasma membrane fraction prepared from cold-hardened pine needles.

Many plant species adjust the temperature dependence of photosynthesis to the growth temperature (3) and there is evidence that one aspect of acclimation of photosynthetic tissues to temperature includes changes in membrane fluidity (22 and references therein). Our data show a change in the behavior of an intrinsic membrane protein. The nature of this change as well as other changes which occur in membranes of higher plants in response to temperature are not known. The tonoplast vesicles may be a useful experimental tool to explore the effect of minor changes in membrane composition on membrane function.

The original goal of the experiments reported here was to search for evidence of membrane dysfunction at low temperature that might be related to chilling injury of tomato plants. A careful reading of the literature revealed that, although an increase in rate of electrolyte leakage from plant tissues is commonly cited as a symptom of chilling injury (3, 15), chilling does not usually result in an immediate increase in electrolyte leakage (29). Chilling of tomato plants at 0°C did not result in increased electrolyte leakage from leaf discs until the plants had experienced at least

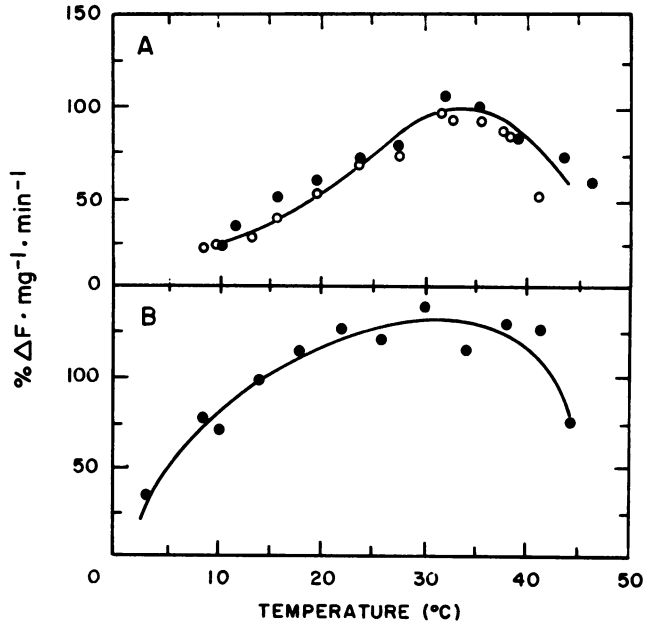

FIG. 5. Initial rate of quench as a function of temperature. Effect of growth temperature. Vesicles were prepared as in Figure 2 from cells grown at 28°C for 5 d (A) or from cells that were transferred from 28°C to 9°C at day 4 and grown at 9°C for 15 d (B). Each point represents a single assay. Assayed as in Figure 4.

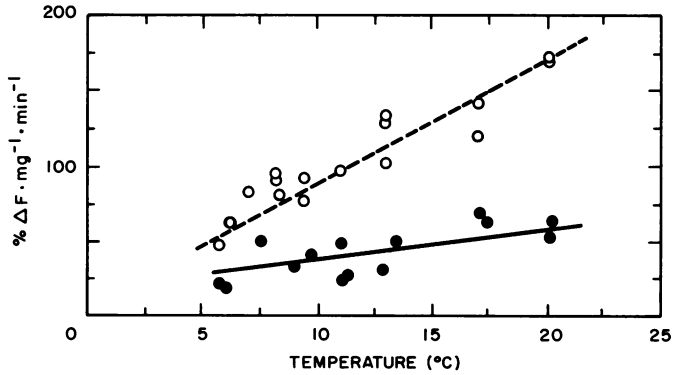

FIG. 6. Initial rate of quench as a function of temperature. Effect of growth temperature. Cells were grown at 28°C for 5 d (●) or transferred to 9°C for 15 d (○). The same vesicle preparations were used for Figure 5. Assayed as in Figure 4.

72 h exposure to the low temperature (14). Papers by Nobel (18) and by Murata *et al.* (17) are sometimes cited as demonstrating an increase in permeability of tomato chloroplasts at chilling temperatures. The actual data, however, may be correlated with a decrease in the permeability of tomato chloroplasts at chilling temperatures. Our data also show decreased permeability at low temperatures. It is unlikely, therefore, that chilling injury of tomatoes can be explained by invoking a direct physical effect on the membrane that causes an immediate increase in permeability. There is an increase in ion (but not water, or proton) permeability when a bulk phase change occurs in the lipid bilayer of lipid vesicles or biological membranes (10, 20). However, no such bulk phase change occurs in polar lipids from tomato leaves at temperature above 0°C; at most, a change involving 4% of the total polar lipids can be detected with differential scanning calorimetry (4). The initial characterization of the effect of temperature on the tonoplast-like vesicles of tomato cells agreed with the literature on effects of temperature on permeability of mem-

branes (10, 16, 20). Permeability increased as temperature increased, and permeability at chilling temperatures was low.

LITERATURE CITED

1. BENNETT AB, SB O'NEILL, RM SPANSWICK 1984 H^+-ATPase activity from storage tissue of *Beta vulgaris*. I. Identification and characterization of an anion sensitive H^+-ATPase. Plant Physiol 74: 538–544
2. BENNETT AB, RM SPANSWICK 1983 Optical measurement of Δ-pH and Δ-psi in corn root membrane vesicles: kinetic analysis of chloride effects on a proton-translocating ATPase. J Membr Biol 71: 95–107
3. BERRY J, O BJORKMAN 1980 Photosynthetic response and adaptation to temperature in higher plants. Annu Rev Plant Physiol 31: 491–543
4. BISHOP DG, JR KENRICK, JH BAYSTON, AS MACPHERSON, SA JOHNS, RI WILLING 1979 The influence of fatty acid unsaturation on fluidity and molecular packing of chloroplast membrane lipids. *In* JM Lyons, D Graham, JK Raison, eds, Low Temperature Stress in Crop Plants. Academic Press, NY, pp 375–389
5. BREIDENBACH RW, AJ WARING 1977 Response to chilling of tomato seedlings and cells in suspension cultures. Plant Physiol 60: 190–192
6. CLARKSON DT 1976 The influence of temperature on the exudation of xylem sap from detached root systems of rye (*Secale cereale*) and barley (*Hordeum vulgare*). Planta 132: 297–304
7. DUPONT FM, DL GIORGI, RM SPANSWICK 1982 Characterization of a proton-translocating ATPase in microsomal vesicles from corn roots. Plant Physiol 70: 1694–1699
8. DUPONT FM, LC STARACI, B CHOU, B THOMAS, BG WILLIAMS, JB MUDD 1984 Effect of chilling temperatures upon cell cultures of tomato. Plant Physiol 77: 64–68
9. DUPONT FM, G ZABALA 1984 Preparation of membrane vesicles enriched in ATP-dependent proton transport from suspension cultures of tomato cells. Plant Physiol 77: 69–73
10. ELAMRANI K, A BLUME 1983 Effect of the lipid phase transition on the kinetics of H^+/OH^- diffusion across phosphatidic acid bilayers. Biochim Biophys Acta 727: 22–30
11. GALLAGHER SR, RT LEONARD 1982 Effect of vanadate, molybdate and azide on membrane-associated ATPase and soluble phosphatase activities of corn roots. Plant Physiol 70: 1335–1340
12. HANDA S, RA BRESSAN, AK HANDA, NC CARPITA, PM HASAGAWA 1983 Solutes contributing to osmotic adjustment in cultured plant cells adapted to water stress. Plant Physiol 73: 834–843
13. HELLERGREN J, S WIDELL, T LUNDBORG, A KYLIN 1983 Frost hardiness development in *Pinus sylvestris*: The involvement of a K^+-stimulated Mg^{2+}-dependent ATPase from purified plasma membranes of pine. Physiol Plant 58: 7–12
14. KING AI, MS REID, BD PATTERSON 1982 Diurnal changes in the chilling sensitivity of seedlings. Plant Physiol 70: 211–214
15. LEVITT J 1980 Responses of Plants to Environmental Stresses. Academic Press, New York
16. LYONS JM, JK RAISON, PL STEPONKUS 1979 The plant membrane in response to low temperature: an overview. *In* JM Lyons, D Graham, JK Raison, eds, Low Temperature Stress in Crop Plants. Academic Press, New York, pp 375–389
17. MURATA N, DC FORK 1975 Temperature dependence of the light-induced spectral shift of carotenoids in *Cyanidium caldarum* and higher plant leaves. Biochim Biophys Acta 461: 365–378
18. NOBEL PS 1974 Temperature dependence of the permeability of chloroplasts from chilling-sensitive and chilling-resistant plants. Planta 115: 369–372
19. ONO TA, N MURATA 1982 Chilling susceptibility of the blue-green alga *Anacystis nidulans*. III. Lipid phase of cytoplasmic membrane. Plant Physiol 69: 125–129
20. PAPAHADJOPOULOS D, K JACOBSON, S NIR, T ISAC 1973 Phase transitions in phospholipid vesicles. Fluorescence polarization and permeability measurements concerning the effect of temperature and cholesterol. Biochim Biophys Acta 311: 330–348
21. PERLIN DS, RM SPANSWICK 1982 Isolation and assay of plasma membrane vesicles from corn root with reduced proton permeability. Biochim Biophys Acta 690: 178–186
22. PIKE CS 1980 Membrane lipid physical properties in annuals grown under contrasting thermal regimes. Plant Physiol 70: 1764–1766
23. POOLE RJ, DP BRISKIN, Z KRATKY, RM JOHNSTONE 1984 Density gradient localization of plasma membrane and tonoplast from storage tissue of red beet. Plant Physiol 74: 549–556
24. RAISON JK 1980 Membrane lipids: Structure and function. *In* PK Stumpf, The Biochemistry of Plants, Vol 4. Academic Press, New York, pp 57–81
25. REENSTRA WW, DG WARNOCK, VJ YEE, JG FORTE 1981 Proton gradients in renal cortex brush border membrane vesicles. J Biol Chem 256: 11663–11666
26. SANDERS D 1981 Physiological control of chloride transport in *Chara corallina*. Plant Physiol 67: 1113–1118
27. SANUI H 1974 Measurement of inorganic orthophosphate in biological materials: extraction properties of butyl acetate. Anal Biochem 60: 489–504
28. SIDDIQI MY, AR MEMON, ADM GLASS 1984 Regulation of K^+ influx in barley. Effects of low temperature. Plant Physiol 74: 730–734
29. WILSON JM 1979 Drought resistance as related to low temperature stress. *In* JM Lyons, D Graham, JD Raison, eds, Low Temperature Stress in Crop Plants: The Role of the Membrane. Academic Press, New York, pp 47–65
30. WOLOSIN JM, JG FORTE 1983 Kinetic propeties of the KCl transport at the secreting apical membrane of the oxyntic cell. J Membr Biol 71: 195–207