BBA 75978

CORRELATIONS BETWEEN FLUORESCENCE, X-RAY DIFFRACTION, AND PHYSIOLOGICAL PROPERTIES IN CYTOPLASMIC MEMBRANE VESICLES ISOLATED FROM *ESCHERICHIA COLI*

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(Received March 3rd, 1972)

SUMMARY

The catalytic properties (*i.e.* transport) of cytoplasmic membrane vesicles isolated from *Escherichia coli* ML strain can be dissociated from their barrier properties. Initial rates of sugar and amino acid transport exhibit sharp activity transitions with respect to temperature at 15 to 20 °C, and optima at 45 to 50 °C. On the other hand, accumulation of maximal intramembranal concentrations of sugar phosphates *via* the phosphoenolpyruvate–phosphotransferase system exhibits temperature optima from 30 to 46 °C depending on the carbon source used for the growth of the parent cells. Moreover, the temperature optimum for accumulation of sugar phosphate in a particular membrane preparation is correlated with an abrupt increase in passive membrane permeability that occurs at that same temperature.

By means of X-ray diffraction and fluorescence techniques applied to membrane vesicles into which dansyl phosphatidylethanolamine has been incorporated, two transitions are detected as functions of temperature. One transition is correlated with the transition in transport activity that occurs at around 20 °C, and coincides with the "melting" of paraffin chains of the membrane phospholipids. The second transition is correlated with the abrupt increases in passive membrane permeability.

INTRODUCTION

Recent studies have described a correlation between the structure of protein-lipid–water phases and the behavior of fluorescent probes incorporated into these systems[1–3]. Specifically, it was demonstrated that fluorescence spectroscopy, under the conditions employed, allowed the discrimination of two major types of protein–lipid interactions, electrostatic and hydrophobic. The studies presented in this paper extend these techniques to a biological membrane system, *i.e.* cytoplasmic membrane vesicles isolated from *Escherichia coli* ML strain.

Abbreviations: α-MG, methyl α-D-glucopyranoside; α-MGP, methyl α-D-glucopyranoside phosphate.

The choice of this biological membrane system has been dictated by a number of criteria: (1) ease of large scale preparation of highly purified membranes which are devoid of both cytoplasmic and cell wall contamination[4-7]; (2) the ability of these preparations to carry out biochemically well-defined membrane functions, *i.e.* the active transport of a wide variety of small molecular weight solutes[4-17]; and (3) the existence of at least two types of temperature-induced transitions in transport-related functions in these preparations[4,5,7,8,10,13].

In the experiments presented here, fluorescence emission spectra, fluorescence depolarization, X-ray diffraction, vectorial phosphorylation *via* the phosphoenolpyruvate–phosphotransferase system[4,8,18], and the passive efflux of sugar phosphate are studied as functions of temperature. The fluorescence properties are those associated with dansyl phosphatidylethanolamine incorporated into the membrane vesicles. Structural changes are observed which coincide with changes in transport parameters, and possible underlying molecular interpretations based on studies of model membrane systems are discussed.

MATERIALS AND METHODS

Membrane preparations

E. coli ML 308-225 ($i^-z^-y^+a^+$) was grown on Medium A[19] containing 0.5% glucose, 0.5% glycerol, or 1% succinate (hexahydrate), or on Medium 63 (ref. 20) containing 1% tryptone (Difco). Vesicles were prepared from these cells by methods described in detail in a previous publication[6].

Uptake studies (α-MGP$_{in}$)

E. coli ML 308-225 membrane samples (100 μl total volume containing approximately 0.5 mg membrane protein) were incubated 15 min after the addition of 0.1 M phosphoenolpyruvate at the desired temperature. α-[^{14}C]Methylglucoside (52.2 mCi/mmole) was then added at a final concentration of $3.64 \cdot 10^{-5}$ M. The experimental conditions are those described previously[8] with the exception that 0.3 M LiCl (rather than 0.25 M potassium phosphate, pH 6.6) was used in the reaction mixtures, and 0.5 M LiCl during dilution, filtration, and washing[6].

Assay for external α-MGP (α-MGP$_{out}$)

α-MG and α-MGP in filtrates from reaction mixtures was collected and assayed as described previously[8] with the exception that silica-gel thin-layer chromatography was performed with a solvent system containing chloroform–methanol–10 M LiCl (60:70:26,v/v/v).

Leakage studies (α-MGP leakage)

Membrane samples were incubated in the presence of 0.1 M phosphoenolpyruvate and α-[^{14}C]methylglucoside at 46 °C until essentially all of the free α-[^{14}C]-methylglucoside had been phosphorylated and most of it concentrated in the vesicles (see Fig. 5B, ref. 8). The samples were then transferred at various temperatures for 15 min and assayed. Baseline samples were assayed immediately after the 46 °C incubation.

Preparation of liposomes

Aliquots of chloroform–methanol (1:1, v/v) solutions of dansyl phosphatidylethanolamine, phosphatidylcholine (lecithin), and phosphatidylinositol containing 4 mg, 4 mg, and 0.5 mg, respectively, were mixed and evaporated to dryness under vacuum in a 250-ml round-bottom flask. The dried material was redissolved in chloroform–methanol and re-evaporated. This was repeated 3 times. Finally, the dried phospholipid film was suspended in 2.0 ml of 0.1 M KCl at room temperature to give a moderately opalescent solution.

Incorporation of dansyl phosphatidylethanolamine into membrane vesicles

Dansyl phosphatidylethanolamine is incorporated into the vesicles *via* the liposomes described above. 1 ml of the appropriate membrane suspension (approximately 10 mg/ml protein) was added to a 1-ml sample of liposomes. The mixture was then incubated at 40 °C for 5 min or at 30 °C for 15 min, and subsequently centrifuged on a three-layer discontinuous sucrose gradient. The gradient was made up of equal proportions of 1.15 M (bottom), 0.95 M (middle), and 0.25 M (top) sucrose, and centrifugation was carried out at 20000 rev./min in a Beckman 25.1 rotor for 1 h. The membranes sediment to the bottom of the tube, while the liposomes remain on top of 0.25 M sucrose. Prolonged incubation of membranes with liposomes leads to the accumulation of material at the 1.15 M–0.95 M and 0.95 M–0.25 M interfaces. The quantity of dansyl phosphatidylethanolamine incorporated into the vesicles can be approximated by ultraviolet absorption as described below.

Spectroscopic measurements

Spectroscopic measurements were carried out on membrane pellets obtained from density centrifugation as described above. The membrane pellet was transferred to a small fused silica plate and another identical plate was placed on top. When squeezed between fused silica plates the membrane pellet is completely transparent, and spectroscopic measurements can be made with a minimum of light scattering[1,2,21]. A typical absorption curve of membranes without incorporated dansyl phosphatidylethanolamine is shown in Fig. 1 (a). The spectrum displays a typical aromatic absorption maximum at 280 nm. On the other hand, the ultraviolet absorption spectrum of dansyl phosphatidylethanolamine is characterized by two maxima, one at 335 nm (ε_m = 5000) and another at 250 nm (ε_m = 15000), and a pronounced minimum at 285 nm (Fig. 1, b). The ratio of the absorbance at 250 nm to that at 280 nm can be used to approximate the amount of dansyl phosphatidylethanolamine incorporated into the vesicles (Fig. 1, c). This value varies from 0.8 for untreated membranes to 10 for pure dansyl phosphatidylethanolamine. Assuming that most of the absorption at 280 nm is due to tryptophan in the membrane proteins, and that there is approximately 1 tryptophan residue per 50 amino acid residues in the membrane proteins, an $A_{250\,nm}/A_{280\,nm}$ ratio of 1.65 (Fig. 1, c) indicates that there is approximately 1 dansyl phosphatidylethanolamine molecule for every 20 membrane phospholipid molecules, since the ratio of membrane protein to membrane lipid is approximately 1.5. This figure, which is clearly a rough approximation justifies our assumption that the concentration of dansyl phosphatidylethanolamine in the vesicles is low. In any case, in most of the experiments to be presented, an $A_{250\,nm}/A_{280\,nm}$ ratio of 1.1 to 1.2

was obtained (corresponding to approximately 1 mole of dansyl phosphatidylethanolamine per 50 to 100 moles of membrane phospholipid).

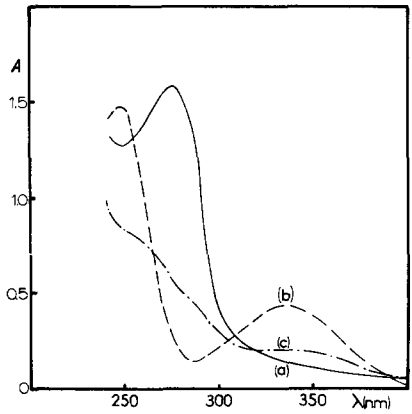


Fig. 1. Ultraviolet absorption spectra of untreated *E. coli* ML 308-225 membrane vesicles (a); dansyl phosphatidylethanolamine in absolute ethanol (b); and ML 308-225 membrane vesicles with incorporated dansyl phosphatidylethanolamine (c). The spectra derived from membrane vesicles (Curves a and c) were recorded using a centrifuged pellet of membranes squeezed between fused silica plates as described in Methods. The quantity of dansyl phosphatidylethanolamine incorporated into the vesicles is determined from the ratio of absorption at 250 nm to the absorption at 280 nm as described in the text.

Fluorescence measurements

The spectrofluorimeter used in these experiments has been described elsewhere[22]. Membrane preparations squeezed between fused silica plates were placed in a temperature controlled sample holder oriented 45° to the incident beam (excitation wavelength = 335 nm), and the fluorescence emission spectrum measured at 90°.

Fluorescence depolarization was performed using the same sample mounting except that a polarizer was placed in the optical path of the excitation beam. Also, filters cutting off light below wavelength 400 nm were placed in the emission beam. The intensity of the fluorescent light was then measured with the analyzer parallel ($P_{\parallel}$) and perpendicular ($P_{\perp}$) to the plane of polarization of the excitation beam. Fluorescence depolarization was determined by the ratio of $P_{\parallel}-P_{\perp}/P_{\parallel}+P_{\perp}$.

X-ray diffraction

X-ray diffraction experiments were performed using techniques and methods described previously[23]. In order to decrease the exposure time, especially at high temperatures, the membrane vesicles were "stacked" by high speed centrifugation on the mica used for X-ray diffraction, followed by drying to 65–70% humidity in a constant humidity chamber. The X-ray diffraction experiments were carried out with the X-ray beam perpendicular to the plane of the "stacked" membranes.

RESULTS

Effect of temperature on α-MG uptake, α-MG phosphorylation and α-MGP leakage

The experiments presented in Fig. 2A demonstrate the effect of temperature on the initial rate of uptake of α-MG and the maximal level of accumulation of α-MGP by

membrane vesicles incubated in the presence of phosphoenolpyruvate. The membranes were derived from *E. coli* ML 308-225 grown on minimal medium with glucose (Panel 1), glycerol (Panel 2), or succinate (Panel 3) as carbon sources. Although the

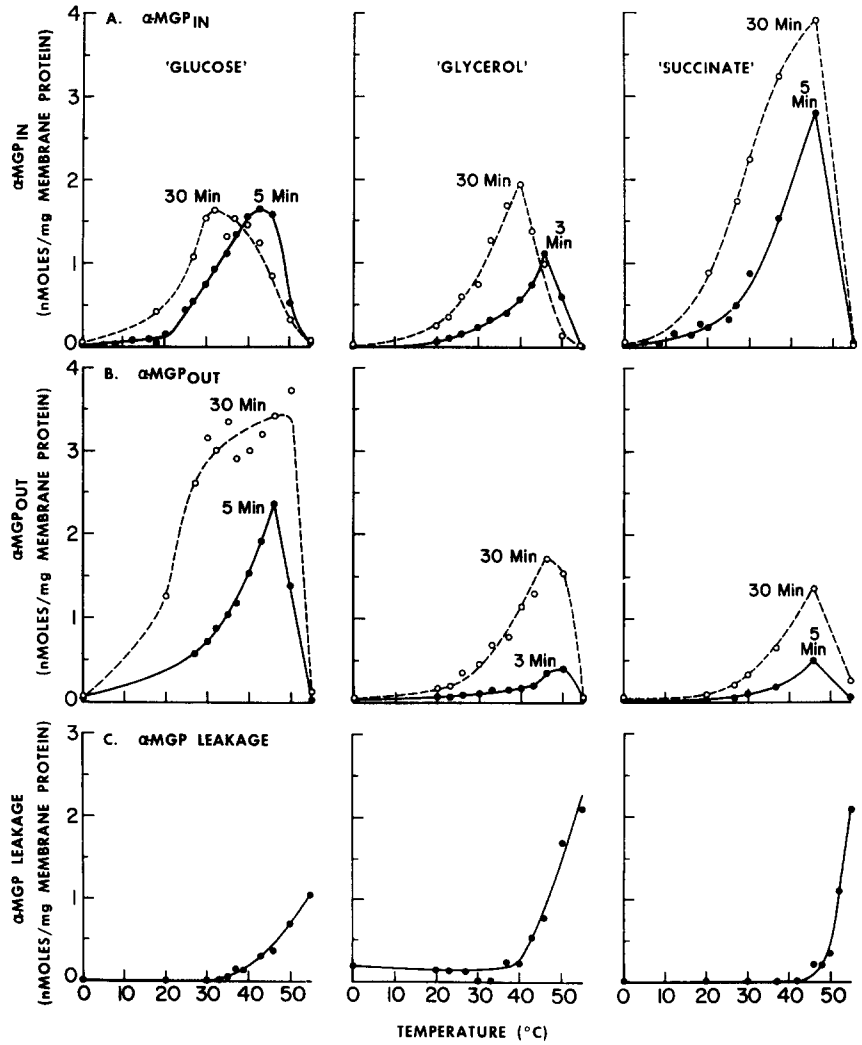


Fig. 2. A. Effect of temperature on the initial rate of α-methylglucoside uptake and the level of α-methylglucoside phosphate accumulation. α-MGP_{in} is expressed in nmoles α-methylglucoside taken up per mg of membrane protein in 3 min (●—●), 5 min (●—●), or 30 min (○---○) incubations. The "glucose", "glycerol" and "succinate" membranes contain 0.48, 0.61, and 0.41 mg membrane protein, respectively. B. Effect of temperature on the appearance of α-methylglucoside phosphate in the filtrate (α-MGP_{out}) from the samples assayed in A. C. Effect of temperature on the initial rate of leakage of α-methylglucoside phosphate (see Methods). Incubation times at 46 °C were 7, 10, and 25 min for "glucose", "glycerol", and "succinate" membranes, respectively. The baseline samples assayed immediately after the 46 °C incubation contained 1.25, 1.9, and 4.5 nmoles α-methylglucoside phosphate per mg membrane protein for "glucose", "glycerol", and "succinate" membranes, respectively. Although not shown, membrane vesicles prepared from cells grown on minimal medium with tryptone as carbon source (see Methods) behave qualitatively in a manner identical to that shown for "succinate" membranes.

data are not shown, membranes prepared from *E. coli* ML 308-225 grown on minimal medium with tryptone as a carbon source behave similarly to "succinate" membranes. With increasing temperature, the maximal level of α-MGP accumulated (uptake in 30 min) increases until it reaches an optimum at approximately 30 °C, 40 °C, or 46 °C with "glucose", "glycerol", or "succinate" membranes, respectively. At temperatures exceeding these optima, the intramembranal level of α-MGP declines markedly. On the other hand, the initial rates of uptake (uptake in 3 or 5 min) in all three preparations display sharp optima at 46 °C.

It is also noteworthy that the increase in the initial rates of transport as a function of temperature in all three membrane preparations is very slow from 0 to approximately 20 °C. Above 20 °C, the initial rates of uptake increase much more rapidly up to 46 °C. A discontinuity at 15 to 20 °C has also been observed with D-lactate dehydrogenase activity and with the D-lactate dehydrogenase-coupled transport of lactose[14]. Moreover, similar discontinuities have been observed more recently with the D-lactate dehydrogenase-coupled transport systems for amino acids, galactose, arabinose, glucuronic acid, gluconic acid*, and glucose 6-phosphate**.

The data presented in Fig. 2B represent α-MGP recovered from the filtrates of the reaction mixtures shown in Fig. 2A. The appearance of α-MGP in the medium bears an inverse relationship to the ability of the membranes to retain transported α-MGP, *i.e.* "glucose" membranes compared to "succinate" membranes accumulate less α-MGP, but more α-MGP is found in the external medium. "Glycerol" membranes represent an intermediate situation. Also, it should be clear from the data that the initial rates of phosphorylation, regardless of transport, are optimal at approximately 46 °C in all three membrane preparations.

α-MGP leakage (Fig. 2C) was studied by allowing each membrane preparation to accumulate α-MGP at 46 °C until all of the α-MG in the reaction mixtures was converted to α-MGP and most of it was concentrated within the vesicles[5,7,8]. Since all of the free sugar in the reaction mixtures was phosphorylated and the vesicles can neither hydrolyze nor transport sugar phosphate under these conditions, retention of radioactivity is a strict function of the passive permeability of the vesicle membrane to α-MGP[5,7-10]. By this method, the ability of membrane vesicles to retain high concentrations of α-MGP can be assessed as a function of temperature. As shown, there is no significant loss of α-MGP from 0 to just below 30, 40, and 46 °C with "glucose", "glycerol", and "succinate" membranes, respectively. Above each of these temperatures, the rate of leakage in each preparation increases markedly and becomes maximal at about 55 °C. Although not shown, the effects of incubation at temperatures up to and including 46 °C on accumulation and phosphorylation are completely reversible. Above 48 °C, there is irreversible inactivation of the phosphotransferase activity of the system.

Thus, the phosphotransferase activity of the membranes increases dramatically from approximately 20 to 46 °C, while the membranes become "leaky" above approximately 30 °C ("glucose"), 40 °C ("glycerol"), or 46 °C ("succinate" and "tryptone") resulting in 30-min temperature optima at these respective temperatures. Despite leakage at 46 °C, the rate of influx of α-MGP is considerably faster than its passive efflux, resulting in accumulation of α-MGP only until phosphorylation (and

* G. K. Kerwar and H. R. Kaback, unpublished information.

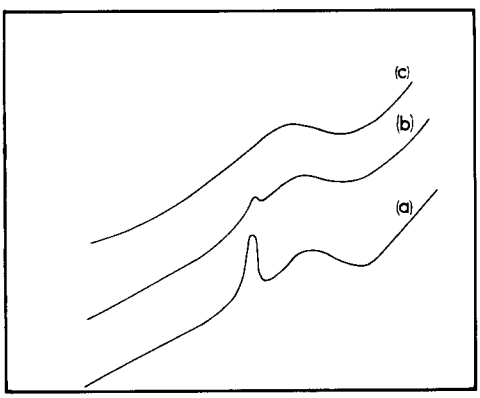
** P. Bhattacharyya, F. J. Lombardi and H. R. Kaback, manuscript in preparation.

transport) stop because free α-MG, the transport substrate, becomes rate-limiting. If additional α-MG is added at this time, uptake resumes immediately[5,7].

The results shown in Figs 2A, 2B, and 2C are identical when glucose, fructose, or mannose, rather than α-MG, are used as transport substrates (data not shown). Moreover, although the data are not presented, membrane vesicles containing dansyl phosphatidylethanolamine manifest no significant differences in the transport activities studied. The phosphoenolpyruvate-dependent vectorial phosphorylation of α-MG, as well as the active transport of lactose and proline are essentially the same as control preparations.

X-ray diffraction studies

X-ray diffraction measurements carried out with "stacked" membrane vesicles at low temperatures reveal a sharp reflexion at 4.2 Å superimposed on a broad band at 4.5 Å. A typical densitometer tracing from such an X-ray diffraction pattern is shown is Fig. 3, Trace a. The 4.2-Å reflexion is characteristic of rigid lipid paraffin chains, while the 4.5-Å band is characteristic of "liquid-like" lipid paraffin chains[23–27]. From 0 to approximately 15 to 20 °C, the intensity of the 4.2-Å reflexion remains unchanged. Above 15 to 20 °C, the intensity of the 4.2-Å reflexion decreases (Fig. 3, Trace b), until 30 °C, when it can no longer be detected (Fig. 3, Trace c). The ratio of the integrated intensity of the 4.2-Å reflexion to the total intensity of the 4.2-Å and 4.5-Å reflexions as a function of temperature is shown in Fig. 4. Clearly, a transition



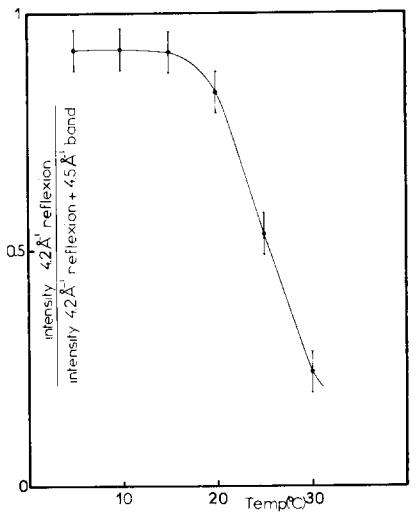


Fig. 3. X-ray diffraction of membrane vesicles. The curves presented represent densitometer tracings from X-ray films obtained as described in Methods. The studies were carried out with centrifugally "stacked" membrane vesicles at 5 °C (Tracing a), 25 °C (Tracing b), and 30 °C (Tracing c). The sharp reflexion shown primarily in Tracing a is at 4.2 $Å^{-1}$; the diffuse band present in all three tracings is at 4.5 $Å^{-1}$. Identical results were obtained with "glucose", "glycerol", "succinate", and "tryptone" membrane vesicles.

Fig. 4. X-ray diffraction of membrane vesicles. The data shown were obtained from densitometer tracings obtained as described in Fig. 3 and Methods. The ordinate represents the ratio of the intensity of the 4.2-Å reflexion to the total intensity of the 4.2-Å reflexion and the 4.5-Å band. Identical results were obtained with "glucose", "glycerol", "succinate", and "tryptone" membranes. The bars indicate the range of variations in the measurements with the four membrane preparations.

occurs between 20 and 30 °C such that the ratio remains essentially constant up to 15 to 20 °C, decreases sharply between 20 and 30 °C, and reaches practically zero at 30 °C. Identical results have been obtained with membrane vesicles prepared from *E. coli* ML 308-225 grown on glucose, glycerol, succinate, and tryptone (leakage transitions at 30, 40, 46, and 46 °C, respectively). Identical results have also been obtained with membrane preparations into which dansyl phosphatidylethanolamine has been incorporated (data not shown).

Fluorescence depolarization of dansyl phosphatidylethanolamine incorporated into membrane vesicles and model systems

Since fluorescence depolarization reflects primarily the mobility of the fluorescent chromophore, variations in this parameter as a function of temperature using dansyl phosphatidylethanolamine as the fluorescent probe were studied.

Fluorescence depolarization measurements of dansyl phosphatidylethanolamine incorporated into "glucose" and "succinate" membrane vesicles as a function of temperature are shown in Fig. 5. This parameter yields similar results with both membrane preparations—a linear, relatively mild decrease in depolarization of fluorescence from 0 to approximately 20 °C; a plateau region from 20 to 25 °C (this region although small, is highly reproducible); and a linear, relatively marked decrease above 25 °C. Thus, the "melting" of lipid paraffin chains of the membrane phospholipids as demonstrated by X-ray diffraction is reflected by the mobility of dansyl phosphatidylethanolamine incorporated into the membrane as detected by fluorescence depolarization. Similar variations in fluorescence depolarization are observed when dansyl phosphatidylethanolamine is incorporated into membrane model systems where an order–disorder transition of the paraffin chains is known to take place.

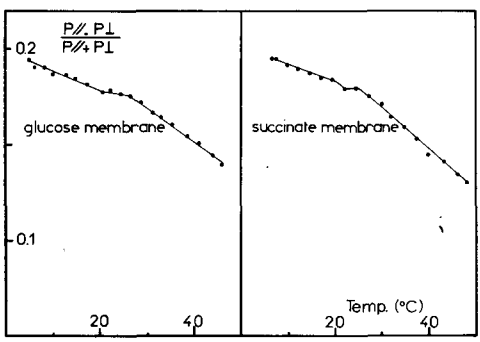


Fig. 5. Fluorescence depolarization of dansyl phosphatidylethanolamine incorporated into membrane vesicles. Dansyl phosphatidylethanolamine was incorporated into "glucose" and "succinate" membranes as described in Methods. Fluorescence depolarization was measured as a function of temperature as described in Methods.

Fluorescence emission maximum (λ^{E}_{max}) of dansyl phosphatidylethanolamine incorporated into membrane vesicles

Fluorescence emission maxima of dansyl phosphatidylethanolamine incorporated into "glucose", "succinate", and "tryptone" membrane vesicles as functions of temperature are shown in Figs 6A, 6B, and 6C, respectively. The emission maxima display the same general behavior for all three membrane preparations—two linear functions with different slopes and a discontinuity. Moreover, the temperatures at

which the discontinuities occur vary from one membrane preparation to another (*i.e.* 30 °C for "glucose" membranes, 38 °C for "succinate" membranes, and 40 °C for "tryptone" membranes), and coincide reasonably well with the temperatures at which the membrane vesicles exhibit leakage transitions (*cf.* Fig. 2).

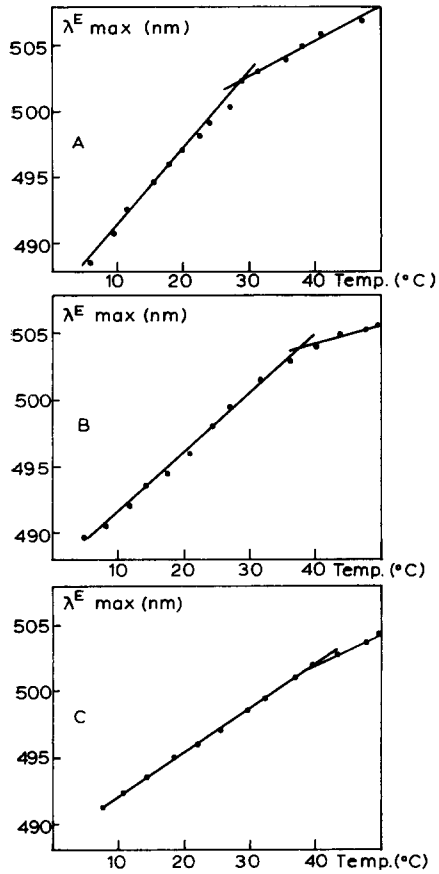


Fig. 6. Fluorescence emission maximum (λ^{E}_{max}) of dansyl phosphatidylethanolamine incorporated into membrane vesicles. Dansyl phosphatidylethanolamine was incorporated into "glucose", "succinate", and "tryptone" membrane vesicles as described in Methods. Fluorescence emission maxima were then measured at each of the temperatures shown as described in Methods. A. "Glucose" membrane vesicles. B. "Succinate" membrane vesicles. C. "Tryptone" membrane vesicles.

The observations that these discontinuities occur at different temperatures in each vesicle preparation indicates that it is not an intrinsic property of the probe itself. Further evidence for this conclusion is provided by studies of liposomes made up-of phosphatidylcholine containing 1 to 3 % dansyl phosphatidylethanolamine (*i.e.* an amount similar to that contained in the bacterial membrane vesicles). No transition is observed over a temperature range from 0 to 50 °C with these liposomes (data not shown). Moreover, at room temperature, the fluorescence emission maximum of the liposomes is displaced to the red by approximately 15 nm compared to that of the membrane vesicles (*i.e.* λ^{E}_{max} = 510 nm for lipsomes *versus* 495 nm for vesicles). These results, especially when considered in conjunction with the X-ray diffraction

and fluorescence depolarization data presented above, indicate that the behavior of dansyl phosphatidylethanolamine reflects the properties of the membrane into which it has been incorporated and is not the result of a trivial attachment of the liposomes to the membrane vesicles.

DISCUSSION

Within recent years, important advances have been made in our understanding of the energetics of bacterial active transport mechanisms. Specifically, the phosphoenolpyruvate–phosphotransferase system[4,18] has been implicated in the vectorial phosphorylation[4] of certain sugars; and more recently, dehydrogenase-coupled transport systems have been demonstrated to be involved in the constitutive transport of amino acids and the inducible transport of many carbohydrates in *E. coli* and other organisms[7,11–17].

Since the efflux of sugar phosphates accumulated by isolated membrane vesicles *via* the phosphotransferase system is limited by passive diffusion, this system has been useful for the study of the barrier properties of membrane vesicles as an isolated functional entity[4,5,7,8,10]. The results presented here demonstrate that initial rates of transport and phosphorylation of α-MG, glucose, fructose, and mannose exhibit maxima at 46 °C, whereas the ability of vesicles to accumulate maximal intramembranal concentrations of sugar phosphate exhibits optima from 30 to 46 °C, depending on the carbon source used for the growth of the parent cells. Moreover, the temperature optimum for accumulation of sugar phosphate by a particular membrane preparation is correlated with a leakage transition that occurs at the same temperature.

In addition to passive leakage transitions, sharp discontinuities have also been demonstrated in the activities of the phosphotransferase-mediated transport of α-MG, as well as D-lactate dehydrogenase and the D-lactate dehydrogenase-coupled transport of a wide variety of amino acids and carbohydrates[14,*]. In each case, the transport activity of membrane vesicles with respect to temperature increases very slowly from 0 to approximately 20 °C, followed by a much more rapid increase in activity from 20 to 45–50 °C.

Utilizing membrane vesicles into which dansyl phosphatidylethanolamine has been incorporated, it has been demonstrated that two structural transitions can be detected as functions of temperature. The first transition can be detected by both X-ray diffraction and fluorescence depolarization techniques, occurs above 20 °C, and is correlated with abrupt changes in phosphotransferase- and D-lactate dehydrogenase-mediated transport activity.

The relationship between transport and state of the paraffin chains of the membrane phospholipids has been widely discussed lately[25,28–32]. A recent study carried out with *E. coli* fatty acid auxotrophs raised some questions as to the existence of a correlation between transport and "melting" of the paraffin chains of the lipids as detected by X-ray diffraction[25]. Our observations, however, suggest that in *E. coli* membrane vesicles derived from the wild-type ML strain, the "melting" of the paraffin chains of the phospholipids is the "starting point" for the active transport catalyzed by both the phosphotransferase and the D-lactate dehydrogenase mechanisms.

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* G. K. Kerwar and H. R. Kaback, unpublished information.

The second transition is reflected by variations in fluorescence emission maximum with temperature, and coincides reasonably well with the passive leakage transitions observed in the three vesicle preparations studied. It is known that the position of λ^{E}_{max} of dansyl phosphatidylethanolamine is strongly dependent of the polarity of its environment (*i.e.* the higher the polarity, the more λ^{E}_{max} is displaced to the red[2,33,34]). It has been found moreover that λ^{E}_{max} is essentially independent of temperature from 0 to 50 °C in a given organic solvent. Thus the behaviour of dansyl phosphatidylethanolamine in the vesicles cannot be due to an intrinsic property of the probe itself. Rather, the discontinuities observed as a function of temperature must be the result of qualitative changes in the polarity of the environment surrounding the probe.

Although the underlying molecular change in the membrane that causes the abrupt increase in passive permeability at a particular temperature is not obvious, one possible explanation is suggested by studies conducted with artificial model membrane systems. When dansyl phosphatidylethanolamine is incorporated into a lysozyme–cardiolipin–water system which exhibits "hydrophobic" interactions only (as determined by X-ray diffraction), λ^{E}_{max} varies linearly with temperature up to about 50 °C (*cf.* Fig. 7, Curve a). On the other hand, when dansyl phosphatidylethanolamine is incorporated into a lysozyme–phosphatidylinositol–water system, a transition is observed at approximately 35 °C (*cf.* Fig. 7, Curve b). It has been established by X-ray diffraction studies with the latter system that this discontinuity coincides with an increase in hydrophobic interactions between the paraffin chains of phosphatidylinositol and a hydrophobic site in the lysozyme molecule. This change in the nature of the lipid–protein interactions leads to a considerable decrease in the thickness of the phospholipid bilayer. Although observations with highly simplified model systems can hardly be extrapolated directly to biological membranes, it is interesting that the lysozyme–phosphatidylinositol–water system exhibits the same type of variation of

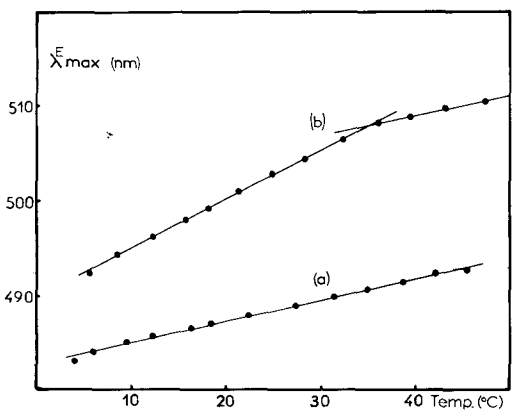

Fig. 7. Fluorescence emission maximum (λ^{E}_{max}) of dansyl phosphatidylethanolamine incorporated into model membrane systems. The fluorescence measurements were carried out at the temperatures indicated as described in Methods. The lower curve (a) was obtained using a lysozyme–cardiolipin (diphosphatidylglycerol)–water phase which displays hydrophobic lipid–protein interactions[21]. The upper curve (b) was obtained using a lysozyme–phosphatidylinositol–water phase prepared at pH 8.0 which displays electrostatic lipid–protein interactions below 35–40 °C and hydrophobic lipid–protein interactions above 40 °C[21]. Each phase contained approx. 2% dansyl phosphatidylethanolamine (relative to total phospholipid).

λ^{E}_{max} as that observed in the biological system. Moreover, if observations made with model systems have any validity in terms of biological membranes, it is a reasonable hypothesis that the changes in passive permeability observed in bacterial membrane vesicles may result from thinning of the bilayer secondary to an abrupt increase in hydrophobic lipid–protein contacts within the membrane.

ACKNOWLEDGEMENTS

The authors would like to thank Dr V. Luzzati for his continual interest in this work and for his many suggestions, and Dr M. Iwatsubo for discussions related to the fluorescence measurements.

This work was supported in part by grants from the Délégation Générale à la Recherche Scientifique et Technique, Comité des Membranes Biologiques.

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