Reconstituted Human Erythrocyte Sugar Transporter Activity Is Determined by Bilayer Lipid Head Groups†

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ABSTRACT: The effects of bilayer lipid head group on human erythrocyte passive sugar transport protein activity were examined by reconstituting the transporter into bilayers of large unilamellar vesicles (LUVs) formed from lipid classes of identical (or similar) acyl chain composition. Two reconstituted transport parameters were measured as a function of temperature. These were K_m and turnover number [$T_n = V_{max}$ per reconstituted D-glucose-sensitive cytochalasin B binding site (transport molecule)]. T_n for sugar transport was found to be almost entirely a function of the properties of the bulk lipid composition of the reconstituted LUVs. It was found to be independent of both reconstituted transporter density and small amounts (≤3%) of endogenous red cell lipids. With the dimyristoylphospholipids, T_n increases at all temperatures in the order phosphatidylcholine < phosphatidylglycerol < phosphatidic acid < phosphatidylserine (at 50 °C, T_n for transport in dimyristoylphosphatidylcholine is 100-fold lower than T_n for transport in dimyristoylphosphatidylserine). Similar results are found with egg yolk derived lipids. Only dimyristoyl- and dipalmitoylphosphatidylcholine bilayers are incapable of supporting detectable transport activity at temperatures below the bilayer phase transition, and only the phosphatidylcholines show a clear increase in T_n during the bilayer melt. All other bilayer systems studied (phosphatidic acid, phosphatidylglycerol, phosphatidylserine, and sphingomyelin) support a small or negligible increase in T_n during the bilayer melt, the major change in transport being restricted to altered K_m. With the disaturated phosphatidylglycerols (C_{14}–C_{18}), T_n and the activation energy (E_a) for reconstituted transport increase with acyl chain carbon number. Similar results are found with the phosphatidylcholines. Transport in bilayers formed from egg yolk sphingomyelin (a lipid containing a sphingosine rather than a glycerol backbone) is characterized by very high K_m and low T_n parameters. Moreover, protein-mediated transport in sphingomyelin bilayers "spikes" during the bilayer phase transition. These and previous findings [Carruthers, A., & Melchior, D. L. (1984) *Biochemistry 23*, 6901–6911; Connolly, T. J., Carruthers, A., & Melchior, D. L. (1985) *Biochemistry 24*, 2865–2873] indicate that those bilayer factors influencing reconstituted sugar transporter activity are, in order of importance, lipid head group > lipid acyl chain length and saturation/unsaturation > lipid backbone > bilayer "fluidity".

It is known that the activities of many membrane enzymes are governed by their lipid environment [for a review, see Stubbs & Smith (1984)]. This dependency of membrane enzyme activity on lipid environment initially became evident in investigations of the physiological consequences of bilayer phase transitions (Steim et al., 1969; Melchior et al., 1970). In the relatively simple prokaryote, it was observed that as the membrane melted, many membrane activities were altered (Melchior & Steim, 1976; Overath & Thilo, 1978). The melting of a membrane is a drastic process, and considering the concomitant changes in the mobilities of membrane lipids and proteins (Overath et al., 1971; Edidin, 1974), altered membrane enzyme activities should not be unexpected.

Investigations of the modulation of membrane enzyme activity by bilayer lipids were initially facilitated by techniques which allowed the lipids of prokaryotic membranes to be altered by a variety of nutritional and genetic manipulations (Razin & Rottem, 1976). Such studies were later extended to eukaryotic systems (Baldassarre et al., 1979). Interpretive problems exist, however, in studies of native membranes. For example, it is not possible to determine to what extent changes in membrane enzyme activity result solely from changes in bilayer physical state. For instance, during the course of a phase transition in a membrane composed of a mixture of lipid types, membrane enzymes are exposed to continuously varying ratios of different lipid species (Melchior & Steim, 1979). Indeed, it is not really clear whether the effects of a change in the physical state of any mixed lipid bilayer on an enzyme can be practically separated from the accompanying change in lipid composition in the environment of the enzyme. A major unanswered question arising from previous studies is the following: What parameters of a membrane bilayer are most important in determining the activity of a membrane enzyme? Are overall kinetic time-averaged features of a membrane most important, or are specific lipid/protein interactions more important? For example, recent studies with purified enzymes in bilayers of predetermined lipid composition have demonstrated that the parameter "bilayer fluidity" is a very minor factor in determining a membrane enzyme's activity when compared to compositional changes in membrane lipid (East et al., 1984; Carruthers & Melchior, 1984b; Connolly et al., 1985a,b).

In vivo systems with their unavoidable heterogeneous lipid populations, presence of diverse protein species, cytoskeletal protein matrix, bilayer lipid compositional asymmetry, possible existence of lipid domains, etc. are at present too complex to provide an optimal system for the rigorous investigation of the control of membrane enzyme function by membrane lipids. For this reason, recent investigations in this laboratory have

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concentrated on more precise ways of investigating this problem. As a first approach, it was decided to investigate protein-mediated, passive nonelectrolyte transport. To accomplish this, the human erythrocyte passive sugar transport protein (HEPSTP)[1] (Kasahara & Hinkle, 1977; Baldwin et al., 1982) was reconstituted into large unilamellar vesicles of predetermined lipid composition (Carruthers & Melchior, 1984a). The HEPSTP provides an excellent model for studying the regulation of a membrane transport protein by its resident bilayer (Carruthers, 1984; Wheeler & Hinkle, 1985). Using this system, we have previously found (Carruthers & Melchior, 1984b; Connolly et al., 1985a,b) the following for lecithin bilayers of homogeneous lipid composition:

(1) The absolute activity of the reconstituted HEPSTP (V_{max} per reconstituted cytochalasin B binding protein) is strongly governed by the bulk lipid composition of the bilayer. In no instances is there any suggestion that HEPSTP activity is influenced by any physically or compositionally distinct hypothetical "boundary" or "annular" lipid.

(2) The parameter "bilayer fluidity" appears to be a relatively unimportant determinant of HEPSTP-mediated hexose flux.

(3) In all the homogeneous phosphatidylcholine bilayers studied which exist in the crystalline state above 0 °C, only two (DMPC and DPPC) do not support transport in the crystalline state. The others (e.g., DSPC, DAPC, and DEPC) support *significant* protein-mediated transport in the crystalline state. Indeed, at all temperatures studied, crystalline DSPC (T_m = 56 °C) supported substantially better transport (V_{max} per reconstituted transporter) than *fully fluid DOPC* (T_m = −22 °C) or DPOPC (T_m = −36 °C) bilayers.

(4) In a given membrane, the bilayer transition from the crystalline to the fluid state results in increased protein-mediated sugar transport activity. In the one bilayer (DEPC) whose transition temperature allowed measurement of the activation energy for transport (E_a) both above and below the transition, E_a was unaffected by the phase change.

(5) E_a and the Arrhenius constant (A) for transport are dependent on phosphatidylcholine acyl chain length and saturation. For both parameters, the order of increase is DMPC < DPPC = DPOPC < DSPC < DAPC < DEPC = DOPC. Thus, at 59–60 °C, the order of HEPSTP catalytic activity follows the lipid sequence DMPC < DPOPC < DAPC < DOPC < DPPC = DEPC < DSPC.

(6) In more complex bilayers formed from phosphatidylcholines and the major erythrocyte membrane lipid, cholesterol, sugar transport is markedly sensitive to bilayer cholesterol content. Upon introduction of progressively greater amounts of cholesterol into a bilayer, HEPSTP activity does not reflect what would be expected for a bilayer monotonically approaching a state of "intermediate fluidity" (Demel & de Kruyff, 1976). Abrupt, cholesterol-induced bilayer reorganizations occurring at 15–20 and 30 mol % bilayer cholesterol are reflected as dramatic changes in transporter turnover number. For example, at 17 mol % cholesterol, as little as a 2.5% change in bilayer cholesterol can result in as much as a 100-fold change in V_{max} per reconstituted protein.

(7) Increasing the cholesterol content of crystalline distearoylphosphatidylcholine bilayers, which physically results in a more fluid bilayer, results in inhibited transporter activity. This finding again emphasizes that fluidity is *not* a major determinant of transport activity. No consistent relationship between bilayer cholesterol content, bilayer apparent partial specific volume, and V_{max} per transporter is observed. Since changes in the volume of a fluid are closely related to changes in the motion of its constituent molecules, bilayer fluidity cannot be a primary determinant of HEPSTP transport activity.

(8) The major influence of bilayer lipid composition on reconstituted HEPSTP activity reported to date is seen primarily as an influence on the V_{max} of the transporter rather than its K_m.

Here we report an extension of our studies to the effects of lipid head groups on reconstituted HEPSTP activity.

Materials and Methods

Solutions. NaCl medium consisted of 25 mM NaCl/5 mM Tris-HCl/0.2 mM EDTA, pH 7.4. Detergent medium consisted of NaCl medium (pH 8) plus 0.5% Triton X-100. In experiments where pH was varied, medium containing 15 mM sodium citrate, 10 mM imidazole, and 15 mM boric acid (CIB medium) was used. This solution provides effective buffering capacity over the pH range 3–10 [temperature coefficients (pH units/°C) at pH 3, 7.4, and 10 are −0.00138, −0.0132, and −0.0142, respectively]. At pH 7.4, reconstituted activity in CIB medium is indistinguishable from that in NaCl medium.

Hexose Transfer Protein Purification. Human erythrocyte sugar transport protein (protein band 4.5, a 55-kilodalton glycoprotein) was purified as described previously (Carruthers & Melchior, 1984a,b). Briefly, integral membrane proteins (bands 3, 4.2, 4.5, 6, and trace amounts of 7) are eluted from red cell ghosts (obtained by lysis of washed red cells from one unit of freshly outdated whole blood) by incubation in detergent medium (5 volumes of detergent medium/1 volume of white ghosts; 1 °C; 30-min agitation-free incubation) and collected as the supernatant fraction of the centrifuged material. Band 4.5 protein is obtained by passing this supernatant (approximately 40 mL) over a DEAE-cellulose column (Whatman DE 52; 8 cm × 3 cm column equilibrated with detergent medium; flow rate 35 mL/h) and collecting 6 bed volumes of eluate. The remaining integral membrane proteins are eluted from the column by using detergent medium containing 1 M NaCl. The band 4.5 eluate is concentrated some 5–6-fold by ultrafiltration (Diaflo YM-10 membranes) and then treated with Bio-Beads (Bio-Rad SM2; prewashed by using methanol followed by distilled water followed by NaCl medium; 1 volume of wet beads to 1 volume of protein solution) for at least 4 h at 4 °C to remove detergent. The resulting suspension consists of band 4.5 protein (4–8 mg) and minor protein contaminants [protein bands 3 (or a dimer of band 4.5; see Lienhard et al., 1984) and 7; see Figure 1] and lipid (8–16 mg) in the form of particles of average diameter 0.1–0.6 μm. These particles are osmotically inactive and, under phase contrast microscopy, are amorphous in appearance.

Functional band 4.5 protein binds the potent competitive sugar transport inhibitor cytochalasin B with a probable stoichiometry of 1 site per transporter molecule (Baldwin et al., 1982; Carruthers & Melchior, 1984a,b). This can be used to determine the number of functional transporter molecules

[1] Abbreviations: HEPSTP, human erythrocyte passive sugar transport protein; PC, phosphatidylcholine; DMPC, dimyristoylphosphatidylcholine; DPPC, dipalmitoylphosphatidylcholine; DSPC, distearoylphosphatidylcholine; DAL, diarachidonoylphosphatidylcholine; DOPC, dioleoylphosphatidylcholine; DEPC, dielaidoylphosphatidylcholine; DPOPC, dipalmitoleoylphosphatidylcholine; PG, phosphatidylglycerol; DMPG, dimyristoylphosphatidylglycerol; DPPG, dipalmitoylphosphatidylglycerol; DSPG, distearoylphosphatidylglycerol; DMPS, dimyristoylphosphatidylserine; PA, phosphatidic acid; DMPA, dimyristoylphosphatidic acid; LUV, large unilamellar vesicle; MLV, multilamellar vesicle; DSC, differential scanning calorimetry; T_m, phase transition onset temperature; T_f, phase transition completion temperature; E_a, activation energy; T_n, turnover number; EDTA, ethylenediaminetetraacetic acid; CCB, cytochalasin B; SM, sphingomyelin.

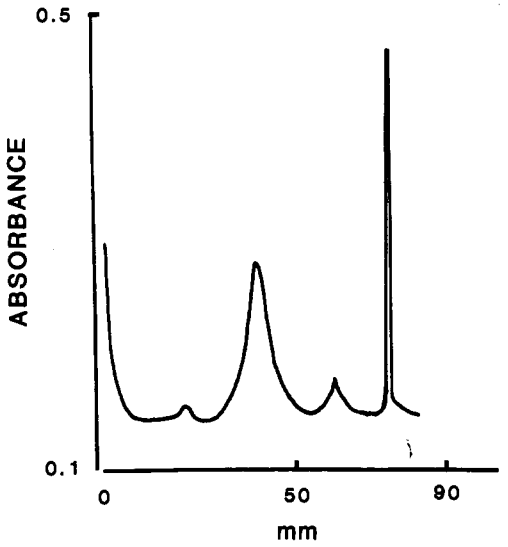

FIGURE 1: Scan of the electrophoretic pattern of purified band 4.5 protein. Five micrograms of band 4.5 was run on a 10% polyacrylamide gel. The Coomassie-stained, dried gel was scanned at 500 nm by using a Beckman DU-8 spectrophotometer in the gel scanning mode. The major peak is band 4.5; the lower molecular weight peak is band 7, and the higher molecular weight peak is a dimer of the band 4.5 protein (Lienhard et al., 1984).

purified. An alternative procedure is to monitor cytochalasin B induced quenching of transport protein intrinsic tryptophan fluorescence (Gorga & Lienhard, 1982; Carruthers, 1986). Under conditions where a significant proportion of ligand is bound to the transporter, the dissociation constant, K, for cytochalasin B binding to transporter is given by

$$K = C_L(1/R - 1) - nC_E(1 - R)$$

where C_L is the total cytochalasin B concentration, R is the fractional saturation of transporter by cytochalasin B, C_E is the total transporter concentration, and n is the number of binding sites per transporter. In practice, R is determined by titrating the transporter with cytochalasin B until no further quenching of intrinsic fluorescence is observed. R is given by

$$R = (E_0 - E_L)/(E_0 - E_M)$$

where E_0 is fluorescence in the absence of cytochalasin B, E_L fluorescence in the presence of cytochalasin B, and E_M fluorescence in the presence of a fully saturating concentration of cytochalasin B. On plotting $1/(1 - R)$ vs. C_L/R, one obtains a straight line of slope $1/K$ and an x intercept of nC_E (Carruthers, 1986). Table I summarizes the results of both [^{3}H]cytochalasin B binding and cytochalasin B induced fluorescence quenching measurements on five band 4.5 preparations. Fluorescence measurements were performed at 23 °C by using a Farrand MK 2 spectrofluorometer with excitation at 295 nm and excitation and emission bandwidths of 5 and 10 nm, respectively. The contents of the cuvette were constantly stirred by using a Spectrocell Inc. cuvette stirrer (Oreland, PA). Both radiolabel and fluorometric analyses are in agreement, indicating that the stoichiometry of cytochalasin B binding to the band 4.5 samples is 15.6 ± 0.7 nmol/mg of protein [molar stoichiometry of (0.86 ± 0.04):1].

Band 4.5 Reconstitution. Band 4.5 protein (10–80 μg) was reconstituted into bilayers of large unilamellar vesicles (LUVs) by an adaptation of reverse phase evaporation (Carruthers & Melchior, 1984a,b). The appropriate lipid (40–50 mg) was dissolved in chloroform or hexane and shelled in a 50-mL round-bottomed flask under N_2. The flask was then placed under vacuum (100 μmHg) for at least 3 h to remove the remaining solvent. The lipid was brought up in 1.5 mL of

Table I: Cytochalasin B Binding to Band 4.5

method			
[^{3}H]cytochalasin B		fluorescence quenching	
K_d (nM)	nmol of CCB bound/mg of protein	K_d (nM)	nmol of CCB bound/mg of protein
181 ± 27	15.6	140 ± 10	16.4
128 ● 9	14.3	176 ± 9	15.4
107 ± 10	14.7	171 ± 19	15.3
146 ± 8	17.6	97 ± 5	15.5
137 ± 11	17.2	121 ± 7	16.0
140 ± 24[a]	15.9 ± 1.3[a]	141 ± 30[a]	15.7 ± 0.4[a]
	0.87 ± 0.08[b]		0.86 ± 0.02[b]

[a] Mean ± SD. [b] Cytochalasin B:band 4.5 molar ratio.

diethyl ether and sonicated briefly (using a bath-type sonicator), and then 0.3 mL of NaCl medium (±100 mM D-glucose) was added. This mixture was then sonicated for 2–5 min in a bath-type sonicator containing water (and a small amount of detergent) brought up to a temperature above the transition temperature (T_m) of the bilayers formed by the lipid. When a visually homogeneous dispersion was formed, the ether was removed completely by rotary evaporation (under partial vacuum) at a temperature greater than T_m. The protein sample (containing or lacking 100 mM D-glucose) was then added to the resulting lipidic gel and the mixture swirled gently. This results in the formation of LUVs (as judged by phase contrast and electron microscopy and encapsulated volume analyses) whose bilayers contain reconstituted band 4.5 protein. The LUVs were brought up in 2 mL of NaCl medium (±100 mM D-glucose), passed once through a 1.2-μm Millipore filter (an 8-μm filter was used with sphingomyelin LUVs), and collected by centrifugation (30000g for 10 min). The filtration step was found to result in narrower LUV size distributions and to remove particles of lipid that had failed to vesiculate.

Transport Determinations. The V_{max} for infinite-cis D-glucose influx (saturated influx into initially sugar-free LUVs) was obtained by monitoring the time course of equilibration of the glucose-free intravesicular space with 100 mM external D-glucose using turbidimetry [for details, see Carruthers & Melchior (1984a,b) and Connolly et al. (1985a,b)]. The time course data are then analyzed by using the integrated form of the Michaelis–Menten equation for infinite-cis influx (Hankin et al., 1972; Baker & Naftalin, 1979; Carruthers & Melchior, 1983a,1984a) to obtain V_{max} for influx and K_m for efflux into 100 mM D-glucose medium. This analysis requires that the external sites are fully saturated with glucose. In experiments where high K_m values greater than 25 mM were obtained, infinite-cis entry experiments were replaced by zero-trans efflux measurements. Here the LUVs were loaded with 100 mM D-glucose during formation, and the time course of glucose efflux into glucose-free medium was monitored. These data were then analyzed by using the simple, integrated Michaelis–Menten equation (Karlish et al., 1972; Baker & Naftalin, 1979; Carruthers & Melchior, 1983a) to obtain V_{max} and K_m for efflux. The requirements of these types of analysis have been discussed previously (Carruthers & Melchior, 1984; Connolly et al., 1985b). The major technical requirements are that the LUVs behave as perfect osmometers (both below and above the melting point of their bilayers) and that non-protein-mediated transbilayer sugar flux (leakage) is small. These requirements were investigated in the following manner. The rate constant for hydraulic water conductivity, K_P, was a linear function of transbilayer osmotic gradients established by using glucose [see also Carruthers & Melchior (1983b)]

Table II: Physical Characteristics of Reconstituted Systems

	DMPG	DPPG	DSPG	DMPA	DMPS	egg PC	egg PG	egg PA	egg SM
LUV diameter (μM)	0.17 ± 0.06	0.35 ± 0.07	0.24 ± 0.06	0.48 ± 0.12	1.09 ± 0.26	0.69 ± 0.15	0.48 ± 0.1	0.54 ± 0.1	5.62 ± 0.73
onset of bilayer melt (°C)	21.5	38.6	52.9	47.3	38.4	−20	−43.3, −53.1	8.6	35.1
K_d for CCB binding (μM)	0.39 ± 0.07	0.17 ± 0.07	0.33 ± 0.03	0.25 ± 0.12	0.18 ± 0.05	0.03 ± 0.01	0.22 ± 0.04	0.08 ± 0.02	0.42 ± 0.05
lipids per CCB binding site	2.24×10^5	1.08×10^6	7.8×10^5	4.4×10^4	1.28×10^5	1.5×10^5	1.42×10^5	1.3×10^5	1.72×10^5
sites per vesicle	1	1	1	33	66	17	10	8	1152
sites per μm^2	18	4	5	90	31	27	28	31	23
fatty acid carry-through (mol %)	1.2	1.5	1.6	1.3		1.1	1.2	0.8	0.9
Triton X-100 contamination (mol %)	0.08	0.09	0.09	0.06	0.07	0.07	0.05	0.03	0.05
osmotic water permeability at 50 °C ($\times 10^3$ cm/s)	9.2 ± 0.7	8.7 ± 0.3	9.5 ± 0.4	11.6 ± 1.3		11.9 ± 1.2	10.3 ± 1.0	5.2 ± 0.3	3.7 ± 0.3
K_1 at 50 °C ($\times 10^9$ cm/s)[b]	3.6 ± 0.4	3.9 ± 0.6	2.1 ± 0.2	2.7 ± 0.4	104 ± 23[a]	1.3 ± 0.2	1.5 ± 0.2	0.8 ± 0.1	0.9 ± 0.2
K_{sat} for D-glucose at 50 °C ($\times 10^6$ cm/s)[c]	0.34 ± 0.04	0.7 ± 0.05	1.2 ± 0.06	2.6 ± 0.3	2.75 ± 0.4	0.79 ± 0.05	2.14 ± 0.3	3.1 ± 0.2	0.98 ± 0.06

[a] Measured by using D-[^{3}H]glucose. [b] K_1, bilayer D-glucose permeability in the presence of 50 μM cytochalasin B and 100 μM phloretin. [c] K_{sat}, bilayer D-glucose permeability in the absence of cytochalasin B and phloretin.

and gave no indication of rectification (K_P for influx = K_P for efflux). The rate constant for D-glucose leakage, K_1, was obtained by measuring transbilayer hexose fluxes in the presence of 50 μM cytochalasin B and 100 μM phloretin. These two agents are potent competitive inhibitors of human red cell D-glucose efflux and influx, respectively (Carruthers, 1984). As with our previous studies, the criterion we applied required that leakage (K_1, [D-glucose]) was at most 5% of the total transbilayer D-glucose flux. Occasionally, some LUV preparations gave higher rates of glucose leakage. The reasons for this were not systematically investigated, but may be related to the amount of detergent coreconstituted with the protein in that specific preparation. In any event, these leaky preparations were not employed in this study.

With DMPS LUVs, barely any light scattering could be detected in spite of the obvious presence of LUVs detected by phase contrast microscopy and encapsulated volumes. Here, D-glucose transport (efflux) was monitored at 24 and 50 °C by use of D-[^{3}H]glucose as described previously (Carruthers & Melchior, 1984a).

Calculation of Sugar Fluxes. The V_{max} for sugar transport obtained by the above procedures has units of moles per liter of intravesicular water per unit time. These units were converted to moles per square centimeter of LUV surface area per unit time by calculating the surface area of that number of LUVs whose encapsulated volume is 1 L. This was obtained from LUV size analyses. Knowing the density of reconstituted transporters (sites per unit LUV surface area), V_{max} (moles per unit of LUV surface area per second) was converted to T_n (turnover number, V_{max} per site) with units of moles of transporter per second.

Analytical Procedures. The concentration dependence of equilibrium [^{3}H]cytochalasin B binding to reconstituted LUVs was determined as described previously (Carruthers & Melchior, 1984a,b). Triton X-100 assays were as previously described (Carruthers & Melchior, 1984a). Protein assays were according to Lowry et al. (1951) with modification as described previously (Carruthers & Melchior, 1984a,b). Fatty acid analysis was as reported previously (Carruthers & Melchior, 1984a,b). Differential scanning calorimetry (DSC) was carried out with the Du Pont Instruments 1090 thermal analyzer. DSC plans were loaded with LUVs (0.3–1.5 mg of lipid) and scanned at 3 °C/min. Slower scans produced identical thermograms. Lipids whose phase transition occurred below 0 °C were run in the DSC in 1:1 buffer/ethylene glycol to lower the water melt below that of the lipids. Integration of thermograms, determination of onset temperatures, and decomposition of curves were performed by computer using the Du Pont general analysis utility program (version 1.0). LUVs were sized by using photon correlation spectroscopy [Coulter N4 submicron particle analyzer with size distribution processor analysis and multiple scattering angle detection options; Coulter Electronics, Hialiah, FL; see Connolly et al. (1985b)]. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis was carried out on 8–10% gels as described by Laemmli (1970). Kinetic constants for saturable cytochalasin B binding to LUVs were obtained by curve fitting to the Michaelis–Menten formalism. This was achieved by weighted nonlinear regression (Carruthers & Melchior, 1984b).

Results

Physical Properties of the Reconstituted System. Our previous studies with lecithin bilayers have examined how lipid acyl chain carbon number, saturation/unsaturation, and bilayer cholesterol content affect the properties of the reconstituted human red cell sugar transport protein (Carruthers & Melchior, 1984b; Connolly et al., 1985a,b). The approach used was to reconstitute the transporter into LUVs formed from phosphatidylcholines of selected acyl chain composition and varying cholesterol content. The major aim of the present study was to determine how (if at all) the lipid head group affects the catalytic properties of the human red cell hexose transport protein and to extend our findings on the effect of hydrocarbon chain length to other lipid classes. Our approach here (where technically feasible) was to reconstitute the transport protein into bilayers formed from lipids of fixed acyl chain composition but varying head-group composition. Several technical considerations somewhat limited the scope of these studies. We found that it was not possible to reconstitute the transporter into pure dipalmitoylphosphatidic acid bilayers. This may be due to the high melting temperature of these bilayers (67 °C at neutral pH). We also found that dimyristoylphosphatidylethanolamine did not form osmotically active LUVs. Thus, the use of these particular lipids was

precluded in these studies on single lipid-reconstituted systems.

Table II summarizes the major characteristics of the various reconstituted systems employed in this study. Data are presented from at least three separate reconstitutions with each lipid system. The lipid vesicles formed by reverse-phase evaporation were unilamellar structures as judged by two critera (Carruthers & Melchior, 1984b): (1) Electron microscopy and phase contrast microscopy demonstrated the absence of multilamellar structures; (2) the mean encapsulated volumes of the vesicles were consistent with those calculated for unilamellar vesicles of average diameters obtained by photon correlation spectroscopy [inclusion of one or more intravesicular lamellae (diameter 10–90% of the external lamella) in these calculations results in significant deviations from the experimental encapsulation volume].

Reconstitution of band 4.5 protein into LUV bilayers results in the incorporation of red cell lipids copurified with the protein and residual Triton X-100. The extent of lipid contamination was estimated by fatty acid analysis of the reconstituted LUVs by gas chromatography and varied from 8 red cell lipids per 1000 exogenous lipid molecules to 16:1000. Triton X-100 contamination varied from 3 detergent molecules per 10000 lipid molecules (6 detergent molecules per LUV) to 9:10000 (20 detergent molecules per LUV). The number of reconstituted cytochalasin B binding proteins per LUV varied from 8 to 1152. Transporter characteristics (turnover number) were invariant (within error) for all experiments on each lipid type despite variable exogenous lipid to cytochalasin B binding ratios. After correction for LUV size, the number of reconstituted transport proteins per square micrometer of LUV surface area varied from 4 to 90. The magnitude of protein-mediated sugar flux in the reconstituted vesicles is directly proportional to the number of cytochalasin B binding proteins reconstituted. In DMPG, DPPG, and DSPG LUVs, the V_{max} for D-glucose efflux increases (1.8 ± 0.2)-, (2.6 ± 0.3)-, and (2.3 ± 0.2)-fold for a (1.7 ± 0.3)-, (2.7 ± 0.2)-, and (2.4 ± 0.4)-fold increase in reconstituted cytochalasin B binding sites, respectively. Table II displays estimates of LUV osmotic water permeability (K_P), leakage-mediated (non-protein-mediated flux) D-glucose permeability (K_1), and transport-mediated D-glucose permeability (K_{sat}). K_1 was obtained by monitoring transbilayer D-glucose fluxes in the presence of the D-glucose transport inhibitors cytochalasin B and phloretin. As in our previous studies (Carruthers & Melchior, 1983b, 1984a,b), K_1 was on the order of 2×10^{-9} cm/s at 50 °C. This means that leakage-mediated hexose flux at 100 mM D-glucose is some 1–4% of the V_{max} for protein-mediated transport.

Effects of Lipid Head Group on Reconstituted Transport. As seen in Figure 2, thermograms of PG LUVs are very similar to those of PC LUVs with analogous hydrocarbon chains, e.g., egg PG/egg PC (mixed chains) and DMPG/DMPC (saturated C_{14} chains). A comparison of the thermograms of the series DMPG, DPPG, and DSPG (saturated C_{14}, C_{16}, and C_{18} chains) is likewise similar to the series DMPC, DPPC, and DSPC (Melchior & Carruthers, 1984). In contrast to their similar thermograms, the transport profiles of PG and PC differ significantly (Figure 3). Unlike DMPC which does not support transport until the onset of its transition, DMPG bilayers support transport below the transition with V_{max} remaining constant below and throughout the transition. At the completion of the transition, V_{max} increases exponentially until 60 °C. DMPC begins to support transport at the onset of the transition; transport increases rapidly throughout the DMPG transition and then increases at a lesser but constant exponential rate until protein denaturation. K_m values for the

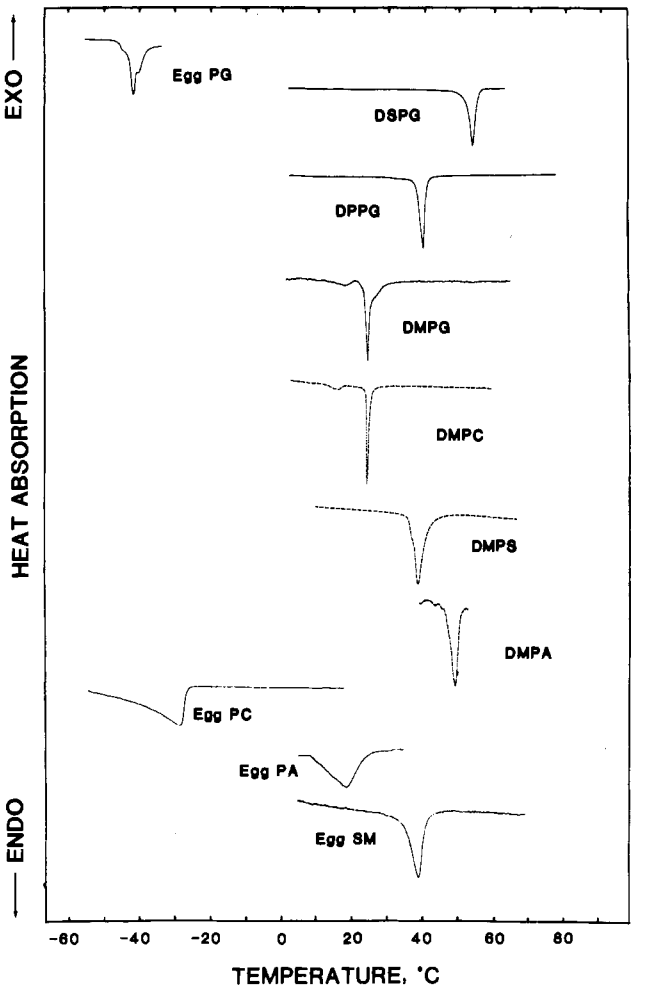

FIGURE 2: DSC thermograms of the various reconstituted systems. Ordinate, heat flow; abscissa, temperature in degrees centigrade. The lipid composition of the LUVs is shown beside each record. For the LUVs whose endotherms occur below 0 °C, samples were run in 1:1 buffer/ethylene glycol. Samples were scanned at a rate of 3 °C/min and contained from 0.3 to 1.5 mg of lipid. The LUVs contained 1.2 μg of band 4.5 protein/mg of lipid.

HEPSTP drop during the course of the transition while showing similar constant values both below and above the transition. With DMPC (and DPPC), the HEPSTP K_m cannot be measured in the crystalline bilayer due to the absence of transport. During the transition with the onset of transport activity, K_m drops in value and increases from the completion of the transition onward. Denaturation of the HEPSTP occurs at 60–62 °C. This is similar to the value of 65 °C for transport in red cell ghosts found by Jung et al. (1971). Further differences between the PG's and PC's are seen with the dipalmitoyl- and distearoylphospholipids (Figure 4). Unlike the PC's, transport can always be supported by crystalline PG bilayers. Furthermore, unlike the PC's, there is no obvious increase in V_{max} with the melting of PG bilayers. Turnover numbers for transport in DPPG and DSPG LUVs are indistinguishable between 7 and 59 °C whereas T_n for transport in DPPC bilayers is considerably lower than that for transport in DSPC membranes [see Carruthers & Melchior (1984b)]. The temperature dependence of K_m for reconstituted HEPST activity in PG bilayers again contrasts with that found for the transporter in PC bilayers. While there was no change in K_m during the melt of DPPC and DSPC bilayers (Carruthers & Melchior, 1984b), the K_m for transport in PG bilayers is modified by and closely follows the lipid bilayer transition (Figure 4B).

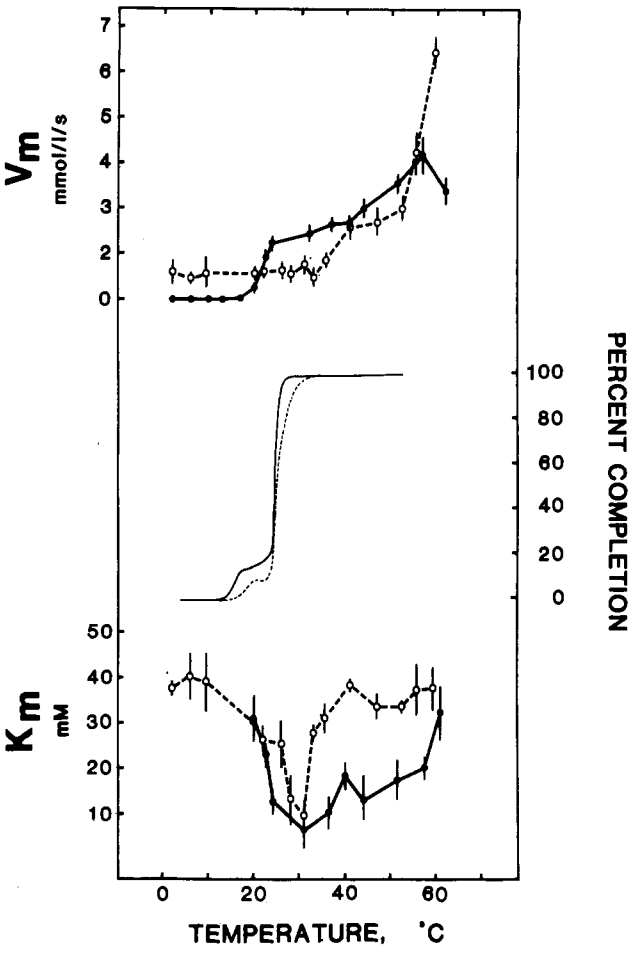

FIGURE 3: Values of V_m and K_m as a function of temperature for the human erythrocyte passive sugar transport protein reconstituted into LUVs of dimyristoylphosphatidylcholine (—) and dimyristoylphosphatidylglycerol (---). Also shown (center) is the integrated heat flow of the bilayer transition of each LUV system. Percent completion of the transition is approximately equal to the percentage of the bilayer in the fluid state. Number of LUV preparations per zero-trans efflux transport determination per point, 3 or more. Points shown as mean ± 1 SE.

Figure 5 summarizes K_m and turnover numbers (V_{max} per reconstituted cytochalasin B binding site) for a variety of reconstituted systems at two selected temperatures, 24 and 50 °C. With the glycerophospholipids (DMPC, DMPG, DMPA, egg PC, egg PG, and egg PA), K_m increases over the temperature range 24–50 °C. The one non-glycerophospholipid studied (sphingomyelin) not only produced K_m parameters larger than those found in the other bilayers but also caused K_m to fall with increasing temperature from 24 to 50 °C. V_{max} per site is primarily dependent upon lipid head group and secondarily dependent upon bilayer physical state. For any given lipid system, a liquid-crystalline to fluid phase transition can result in anywhere from zero to infinite activation of transport. With DMPC, no activity is observed in crystalline bilayers, yet transport occurs in fluid bilayers. With DMPG and DMPA bilayers, increasing the temperature over the range of the transition onset temperature (T_m) to completion temperature (T_f) is without significant effect on V_{max} per site. This means that the activation of transport activity seen in PC bilayers (Carruthers & Melchior, 1984b), upon melting the bilayer, is not found in PG and PA bilayers of identical acyl chain composition; i.e., with respect to HEPSTP activity, lipid head groups can override the effects of greatly increased disorder in the bilayer. With the C_{14} disaturates (dimyristoyl lipids), the order of V_{max} per site at all temperatures is DMPS

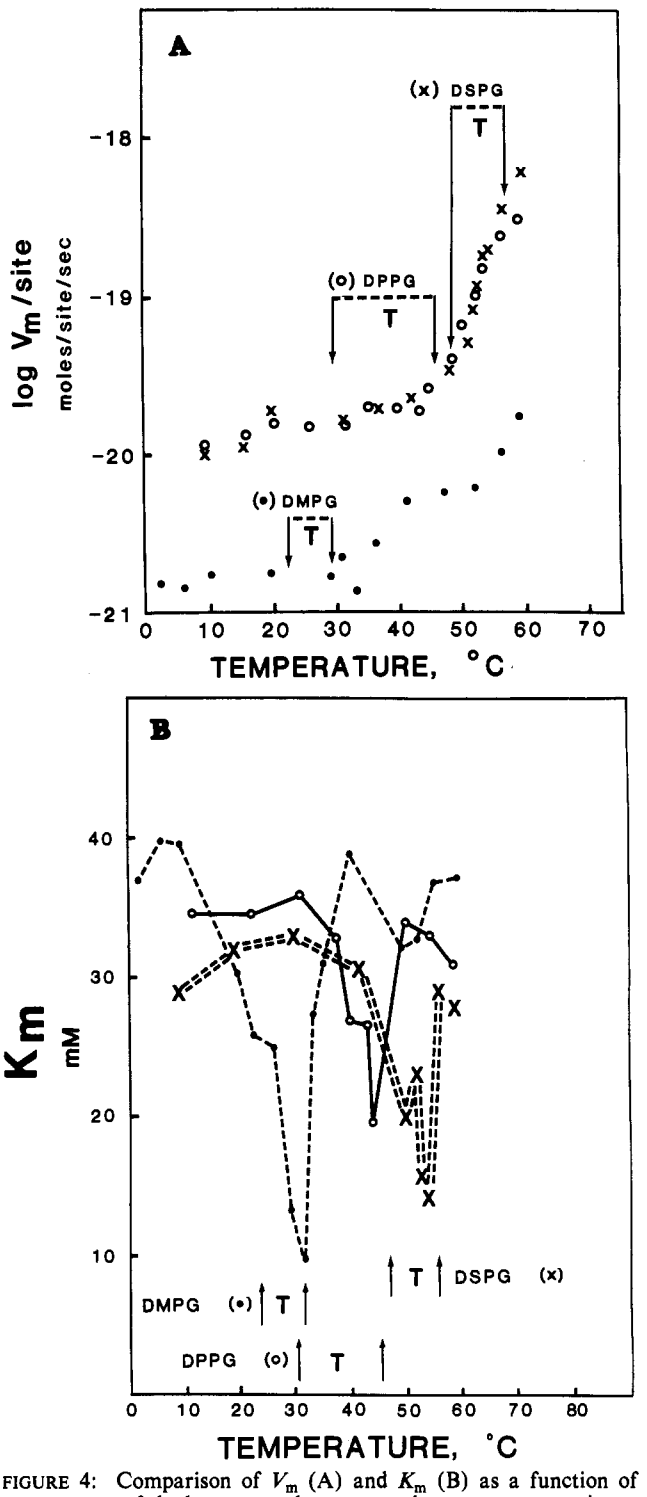

FIGURE 4: Comparison of V_m (A) and K_m (B) as a function of temperature of the human erythrocyte passive transport protein reconstituted into lipid bilayers formed from a series of disaturated phosphatidylglycerols with increasing hydrocarbon chain lengths: (●) dimyristoylphosphatidylglycerol (C_{14}); (○) dipalmitoylphosphatidylglycerol (C_{16}); (×) distearoylphosphatidylglycerol (C_{18}). The arrows indicate the temperature range over which each type of bilayer goes from a crystalline to a fluid state. Number of LUV preparations per efflux determination per point, 3 or more.

> DMPA > DMPG > DMPC. Such hierarchical effects are less pronounced in bilayers formed from mixed chain phospholipids (egg PC, egg PG, egg PA). The one non-glycerophospholipid studied, sphingomyelin, supported the lowest activities. Unlike the other phospholipids studied, the V_m for cytochalasin B sensitive transport in sphingomyelin bilayers spiked in value through the transition (n = 3). The value

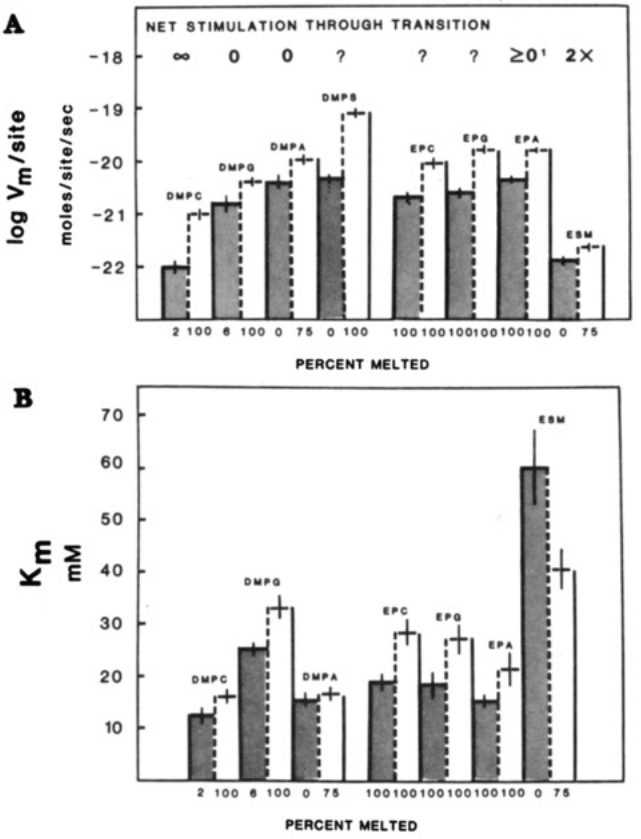

FIGURE 5: (A) V_m and (B) K_m values for the human erythrocyte passive sugar transport protein reconstituted into a variety of lipid bilayers. Values for these parameters are compared at two temperatures: 24 °C (shaded bars); 50 °C (open bars). The numbers at the base of the histograms indicate to what extent the bilayers are melted at either of the two temperatures. In (A), values for the net stimulation through the transition are unknown for EPC and EPG since these bilayers melt below 0 °C and also for DMPS since of all the lipids studied transport measurements were taken at only two temperatures. The superscript 1 by EPA indicates that a clear association of an increase in V_m with the melting of the bilayer could not be made. Number of LUV preparations per condition, 3 or more. Data shown as mean ± 1 SE.

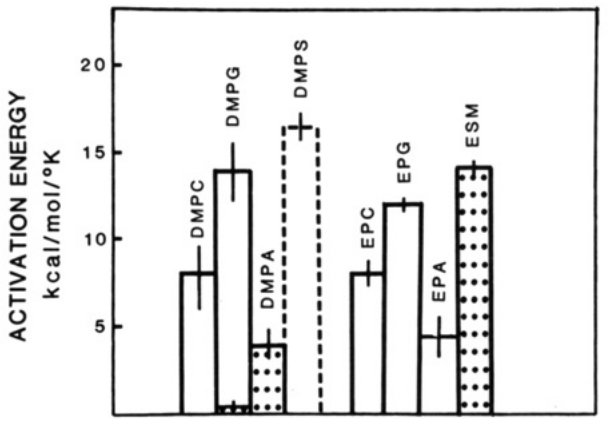

FIGURE 6: Comparison of the activation energy of the human erythrocyte passive sugar transport protein in bilayers of varying head groups with similar hydrocarbon chains. The dimyristoyl lipids all have C_{14} hydrocarbon chains while the egg lipids all contain a similar mixture of saturated and unsaturated hydrocarbon chains. E_a is shown for transport in fluid (open bars) and crystalline (dotted bars) membranes. DMPS is shown in a dashed outline since here flux values were obtained at only two temperatures and the calculation for E_a assumed a simple exponential dependence of activity on temperature. Data shown as mean ± 1 SE of at least three separate LUV preparations.

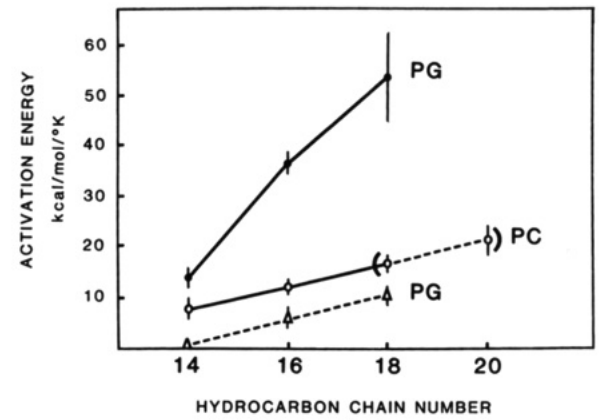

FIGURE 7: Dependency of the activation energy of the reconstituted human erythrocyte passive sugar transporter on the hydrocarbon chain length and physical state of disaturated phosphatidylglycerol and phosphatidylcholine bilayers. Dashed lines represent crystalline bilayers while solid lines represent fluid bilayers. In phosphatidylglycerol bilayers (PG), transport occurs in both the crystalline (---) and the fluid (—) state, a different dependency on chain lengths existing in either of the two states. Bilayers formed from either dimyristoylphosphatidylcholine (C_{14}) or dipalmitoylphosphatidylcholine (C_{16}) do not support detectable sugar transport activity. Due to the denaturation temperature of the transport protein (60 °C), activation energies cannot be obtained for the C_{18} or C_{20} phosphatidylcholine bilayers in the fluid state. Unlike PG, PC bilayers appear to show the same dependency on hydrocarbon chain length in both its crystalline (—) and fluid (---) state. Number of LUV preparations per point, 3 or more.

reported in Figure 5 for net stimulation through the transition is that of V_m at the onset as compared to that at the completion of the melt. While in Figure 5 data are presented as measurements at 24 and 50 °C, full temperature dependencies (e.g., see Figure 3) were obtained for each lipid studied.

Figures 6 and 7 summarize these temperature dependencies as activation energies (E_a) for transport. DMPS flux data were obtained at only two temperatures (24 °C where the bilayers are crystalline and 50 °C where the bilayers are almost fully fluid), and the calculation of E_a assumed a simple exponential dependence of activity on temperature. This assumption may not be valid, and E_a given for DMPS could be either overestimated or underestimated. E_a data are presented where possible as E_a for transport in crystalline or fluid bilayers. Further indication of the differences of the transport process of the HEPSTP in PC and PG bilayers can be seen in Figure 7. Here it is evident that PC and PG bilayers support transport with different but characteristic activation energies (E_a). Moreover, while studies with DEPC indicated no difference between E_a for transport in crystalline and fluid PC bilayers (Carruthers & Melchior, 1984b), E_a for transport in crystalline and fluid PG bilayers differs markedly. Again E_a depends largely upon the lipid head group (see Figure 6) and for a given head group (PC and PG) is directly related to lipid acyl chain length (Figure 7) and bilayer physical state (see also Figure 6). The hierarchical order of E_a values for C_{14} disaturates is DMPS > DMPG > DMPC > DMPA (Figure 6). For the mixed chain lipids, this order is egg SM > egg PG > egg PC > egg PA. Of the C_{14} disaturates studied, DMPA gave the highest E_a for transport in crystalline bilayers. E_a for transport in red cell membranes is 18 kcal mol^{-1} K^{-1} (Carruthers & Melchior, 1984b).

The order of catalytic activity in the various disaturated phospholipid bilayers is DMPS > DMPA > DMPG > DMPC. It is possible that this order reflects the surface potential of the bilayers where the order of negativity is DMPS > DMPA > DMPG > DMPC (Hauser & Phillips, 1979).

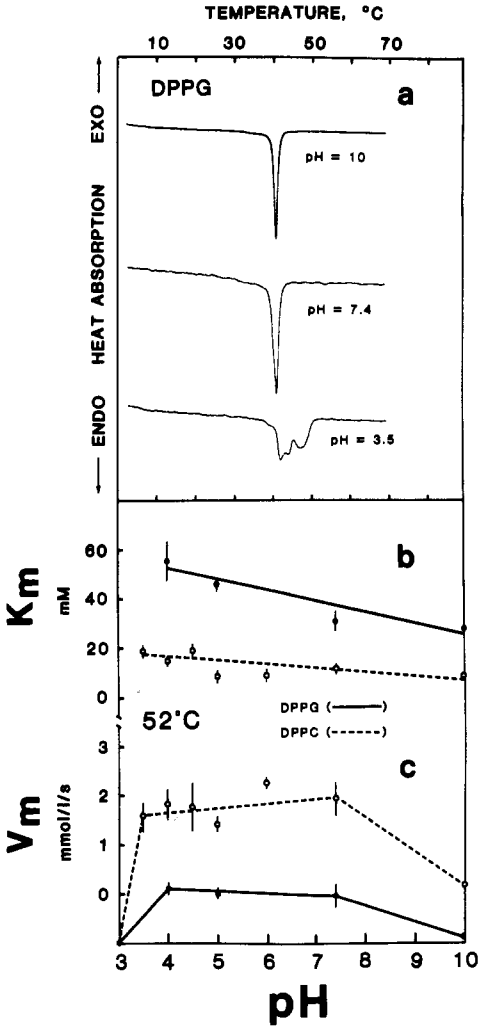

FIGURE 8: Dependence of the K_m and V_m of the human erythrocyte passive sugar transport protein on pH at 52 °C in bilayers formed from dipalmitoylphosphatidylglycerol (solid line) and dipalmitoylphosphatidylcholine (dashed line). Both lipids were in the fully fluid state at 52 °C. Number of LUV preparations per point, 3 or more. The thermograms shown are those of dipalmitoylphosphatidylglycerol. Those for dipalmitoylphosphatidylcholine were not affected by altered pH.

One means of testing this hypothesis is to titrate the reconstituted systems with protons and to determine the effect on activity. Figure 8a illustrates the pH dependence of the thermograms of DPPG. Unlike DPPC whose thermograms (not shown) are invariant with respect to pH, between pH 7.4 and 10 the DPPG endotherm shows a progressive broadening and increase in onset temperature indicating that protonation has altered interactions in the bilayer lattice. This effect of reduced pH is independent of the presence of reconstituted band 4.5 protein and also occurs in PG MLVs. The pH dependence of transport in DPPG bilayers is, however, indistinguishable from that in DPPC membranes. K_m for transport falls slightly with increasing pH, and transport activity is lost below pH 3.5 and somewhere between pH 7.4 and 10 (Figure 8b). This loss of activity is irreversible and reflects protein denaturation (Jung et al., 1971). Preincubation at the indicated pHs for 30 min followed by transport determinations at pH 7.4 results in identical pH profiles for transport (Figure 8). NaCl (100 mM) was without significant effect on transport in PC and PG bilayers.

Denaturation Temperatures. In all systems studied, irreversible loss of transport activity occurred between 60 and 65 °C. The rate of protein denaturation over this temperature range was not systematically investigated.

Discussion

Thermograms of LUVs containing reconstituted HEPSTP were found to be similar to those obtained with MLVs formed from the corresponding pure lipid classes alone. The differences—somewhat broadened endotherms with depressed onset temperatures—followed the same pattern as previously reported for pure phosphatidylcholine LUVs (Carruthers & Melchior, 1983; Düzgunes et al., 1984). The similarity of the thermal behavior of reconstituted LUVs to that of corresponding pure lipid systems is largely due to the low levels of band 4.5 incorporated into the bilayers (1 transporter molecule per 70 000–1 000 000 lipid molecules). The native molecular ratio of sugar transport protein to red cell lipid is on the order of 1:2500. Dilute protein to lipid ratios (28–400-fold dilutions of the native transporter:lipid ratio) were used to minimize the effects of any carry-through lipid or detergent. In addition, low protein to lipid ratios in reconstitution studies avoid significant perturbations in membrane phase behavior and lipid environment. Increasing protein:lipid ratios to supraphysiological values can perturb bilayer lipid packing to a significant extent (Papahadjopoulos, 1977). Our aim was to examine the effects of the transporter's lipid environment on its catalytic properties, not the effects of high concentrations of proteins upon bilayer structure. Low LUV transporter density also discourages significant protein/protein interactions.

The turnover number of the reconstituted transporter at any given temperature and for any given bilayer system was independent of the amount of transporter reconstituted. This indicates that over the range of transporter:lipid ratios employed, reconstituted activity is directly proportional to reconstituted transporter density and independent of random encounters between transporter molecules. Moreover, since (as we shall discuss below) the turnover number is governed by the various physical properties of the reconstituted bilayer, we can conclude that over the range of protein concentrations employed, protein (and coreconstituted red cell lipid) concentration is without significant effect on overall bilayer properties. Reconstituted activity is governed both by the lipid class and by the lipid species of the reconstituted bilayer. Each lipid bilayer system is seen to confer specific catalytic characteristics to the reconstituted transporter. This means either that any lipids of red cell origin initially associated with the transport molecule must be dispersed upon reconstitution or (if they play a role in governing protein activity) that their properties are in turn governed by the bulk properties of the bilayer.

One of the approaches we have used in this study is to examine how thermotropic phase transitions affect the transport characteristics of reconstituted band 4.5 protein. In such experiments, the temperature is raised (or decreased) to cause the bilayer to undergo a characteristic phase transition (crystalline to fluid state or vice versa), and transport activity is monitored. In principle, such analyses indicate how the catalytic properties of the bilayer protein change as its lipid environment is transformed from, for example, a crystalline to a fluid state. This, as should be noted, is as extreme a manipulation of membrane fluidity as can be made. Over a narrow range of temperature (1–4 °C), the lateral mobility of bilayer lipid molecules increases from what is essentially an immobile state in crystalline bilayers to values of 10^{-8}–10^{-7} cm^2/s in fluid bilayers (Edidin, 1974). The results of such analyses are often analyzed in the form of Arrhenius and van't Hoff plots. If catalytic activity is affected by the bilayer phase transition, a break in the plot may be detected over the phase

transition temperature range. This form of analysis is valid provided that parallel measurements of bilayer physical state are made and the catalytic parameters plotted in Arrhenius and van't Hoff form are V_{max} and Michaelis constants, respectively.

This current study emphasizes the potential problems associated with this type of analysis. From a kinetic standpoint, an Arrhenius plot consisting of catalytic activities derived from measurements made at only a single substrate concentration is of negligible value (Segel, 1975). Not only is it impossible to obtain measurements of the enthalpy, entropy, and free energy changes of the reaction by using these data but also changes in the slope with temperature could result from changes in either K_m, V_{max}, or both parameters. This is illustrated if we compare the temperature dependence of transport activity in DMPC and DMPG bilayers (Figure 3). With phosphatidylcholine, V_{max} increases and K_m falls during the bilayer melt whereas only K_m changes during the melt of the phosphatidylglycerol bilayer. Moreover, it is not possible to obtain information regarding alterations in bilayer physical state from Arrhenius plots alone. The V_{max} for transport in phosphatidylcholine bilayers increases markedly during the bilayer phase transition [see here and Carruthers & Melchior (1984b)] but is unaffected by the phase transition in phosphatidylglycerol bilayers. These considerations indicate that the turnover number of a membrane enzyme is not always significantly affected by extreme changes in the physical state (fluid/crystalline) of its lipid environment and serve to illustrate the unsuitability of even kinetically precise Arrhenius plots as a means of indicating phase changes in the bulk lipid environment of a membrane enzyme. This underscores our previous findings (Carruthers & Melchior, 1985) showing that membrane fluidity is not necessarily an important factor governing membrane enzyme activity.

Our previous studies on the influence of lipid environment upon the activity of the reconstituted sugar transport protein examined the effects of bilayer cholesterol content and lipid acyl chain length and saturation/unsaturation using phosphatidylcholine as the model phospholipid (Carruthers & Melchior, 1984b; Connolly et al., 1985a,b). We concluded that activity (turnover number) was directly related to lecithin acyl chain carbon number and was depressed in diunsaturated PC bilayers and, although activity increased during the bilayer liquid-crystalline to fluid phase transition, that bilayer "fluidity" was of secondary importance in determining the catalytic activity of the transport protein. One major characteristic of the phosphatidylcholines is that crystalline dimyristoyl- and dipalmitoyl-PC (C_{14} and C_{16} disaturates) bilayers do not support detectable catalytic activity. The activation energy (E_a, a measure of temperature sensitivity) for reconstituted activity is also directly related to bilayer lipid acyl chain carbon number and is somewhat greater with diunsaturates than with disaturates. We have now extended these analyses to phosphatidylglycerol bilayers. These results together with those described in this paper for lipids with other head groups underscore the problems involved in generalizing the effects of lipids on membrane enzyme activity using just one lipid class as a model. Unlike their PC counterparts (the "classical" model bilayer lipid), DMPG and DPPG bilayers support significant sugar transport activity in the crystalline state. Moreover, activity does not increase markedly (if at all) during the liquid-crystalline to fluid phase transition for DMPG, DPPG, and DSPG. This is particularly interesting, for the physical properties of PG bilayers are sufficiently close to those of PC bilayers to result in similar melting temperatures. Similar melting temperatures are indicative of an equivalent balance of attractive and repulsive forces between the lipid molecules constituting the bilayer lattice. Turnover number is related to acyl chain carbon number (T_n in DMPG bilayers < DPPG = DSPG), and E_a for transport in both crystalline and fluid bilayers increases with acyl chain carbon number. However, E_a values calculated for transport in crystalline and fluid PG bilayers are somewhat arbitrary. As no distinct increase in activity was detected during the bilayer melt, we have simply calculated E_a for transport over those temperatures where the bilayers are in either a crystalline or a fluid state. These calculations result in greater E_a values for transport in fluid DSPG bilayers than in fluid DPPG bilayers. However, close examination of Figure 4A indicates that the temperature sensitivities of reconstituted activity in these bilayers are identical. It would be equally valid to conclude from these data that E_a for transport is independent of acyl chain carbon number.

Of the PC bilayers, only DMPC shows large changes in K_m during the bilayer melt (see Figure 3). Even this observation is limited, for activity was not detectable in crystalline DMPC bilayers. However, DSPC and DEPC bilayers which support activity in the crystalline state show little change in the K_m for transport during the transition (Carruthers & Melchior, 1984b). All significant changes in catalytic activity in phosphatidylcholine bilayers undergoing the liquid-crystalline to fluid transition are limited to the V_{max} for transport. The converse is true for PG bilayers. Only K_m is sensitive to the bilayer liquid-crystalline to fluid phase transition. Nevertheless, although a reversible decrease in K_m occurs during the PG melt, K_m's for transport in crystalline and fluid PG bilayers are indistinguishable. These considerations (K_m changes, identical T_n parameters and temperature sensitivities in DPPG and DSPG bilayers, lower T_n and E_a values in DMPG bilayers) suggest that the reconstituted transporter is less sensitive to factors such as bilayer "fluidity" or order than to changes in lipid acyl chain number (C_{14} to C_{16}).

HEPSTP behavior in PG bilayers is thus seen to be quite different from its behavior in PC bilayers. To what extent do other lipid head groups affect the transporter's properties? We examined this by reconstituting the transporter into DMPC, DMPG, DMPA, and DMPS bilayers. Of the bilayers studied, only DMPC supports a marked increased in V_{max} during the bilayer phase transition. Turnover number is sensitive to lipid head group and increases markedly in the order DMPC < DMPG < DMPA < DMPS (T_n in DMPS is 100-fold greater than in DMPC). An obvious conclusion that might be made from these observations is that T_n is, in some way, sensitive to bilayer surface potential or charge which even at physiological pH increases in the order PC < PG < PA < PS [see Hauser & Phillips (1976)].

Titrating the surface potential with protons has quantitatively similar effects on K_m and V_{max} for transport in DPPC and DPPG bilayers. This pH sensitivity is almost identical with that observed in the native membrane (Jung et al., 1971) and most likely reflects the direct actions of altered pH on the transporter molecule rather than secondary effects arising from titration of bilayer lipid surface potential. Activity is irreversibly lost or reduced between pH 3.5 and 3 and at pH 10. Unfortunately, loss of protein activity at these pHs could mask any effect resulting from full protonation of the bilayer itself. Thus, it is not possible using the available data to determine to what extent transport activity is affected by bilayer surface potential or charge. Although the mixed hydrocarbon chains comprising the egg phospholipids apparently render turnover

differences less pronounced than our findings with the disaturated dimyristoyl lipids, turnover number increases in the order egg PC < egg PG < egg PA. These less pronounced differences may be due to the heterogeneous hydrocarbon chains of those lipids resulting in a looser lipid packing. These findings also underscore the fact that the determination of transport activity by a lipid is a consequence of a balance of various factors, i.e., head group and hydrocarbon chain. No obvious correlations between head group and K_m are observed.

Thus far, we have discussed the effects of lipid head group and hydrocarbon chains on transporter activity. It is interesting that the one lipid studied, egg sphingomyelin, that has the same head group as the phosphatidylcholines but a sphingosine rather than glycerol backbone behaves differently than all the glycerophospholipids. It has an extremely high K_m and is the only lipid that is observed to cause transport activity to spike during the course of its phase transition. In our study of the relationship between bilayer water permeability and bilayer lipid composition and physical state, sphingomyelin was also found to have different properties from the other glycerol phospholipids studied (Carruthers & Melchior, 1983b).

Conclusions

Our studies to date (Carruthers & Melchior, 1984b; Connolly et al., 1985a,b; see also this study) indicate that the catalytic activity of the human erythrocyte hexose transfer protein is influenced (in decreasing order of importance) by bilayer lipid class (lipid head group), bilayer lipid acyl chain carbon number, saturation/unsaturation (possibly manifested as bilayer thickness), lipid backbone, and, to the least extent, bilayer order ("fluidity"). In addition, certain marked effects of cholesterol on the activity of the transporter are not causally related to the sterol's ability to modify "membrane fluidity". The molecular basis of these effects must await a detailed understanding of the relationship between transporter tertiary structure and catalytic activity. The observed sensitivity of sugar transport protein activity to bilayer lipid properties discussed in this and earlier papers, however, clearly indicates that in addition to providing a simple structural matrix for membrane enzyme insertion, bilayer lipids assume a critical role in determining a membrane protein's activity.

Acknowledgments

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Registry No. DMPG, 61361-72-6; DPPG, 4537-77-3; DSPG, 4537-78-4; DMPA, 30170-00-4; DMPS, 64023-32-1; DMPC, 13699-48-4; D-glucose, 50-99-7; cytochalasin B, 14930-96-2.

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