Fusion of Phospholipid Vesicles Induced by Muscle Glyceraldehyde-3-phosphate Dehydrogenase in the Absence of Calcium†

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ABSTRACT: Ca^{2+}-induced fusion of phospholipid vesicles (phosphatidylcholine/phosphatidic acid, 9:1 mol/mol) prepared by ethanolic injection was followed by five different procedures: resonance energy transfer, light scattering, electron microscopy, intermixing of aqueous content, and gel filtration through Sepharose 4-B. The five methods gave concordant results, showing that vesicles containing only 10% phosphatidic acid can be induced to fuse by millimolar concentrations of Ca^{2+}. When the fusing capability of several soluble proteins was assayed, it was found that concanavalin A, bovine serum albumin, ribonuclease, and protease were inactive. On the other hand, lysozyme, L-lactic dehydrogenase, and muscle and yeast glyceraldehyde-3-phosphate dehydrogenase were capable of inducing vesicle fusion. Glyceraldehyde-3-phosphate dehydrogenase from rabbit muscle, the most extensively studied protein, proved to be very effective: 0.1 μM was enough to induce complete intermixing of bilayer phospholipid vesicles. Under conditions used in this work, fusion was accompanied by leakage of internal contents. The fusing capability of glyceraldehyde-3-phosphate dehydrogenase was not affected by 5 mM ethylenediaminetetraacetic acid. The Ca^{2+} concentration in the medium, as determined by atomic absorption spectroscopy, was 5 ppm. Heat-denatured enzyme was incapable of inducing fusion. We conclude that glyceraldehyde-3-phosphate dehydrogenase is a soluble protein inherently endowed with the capability of fusing phospholipid vesicles.

Membrane fusion is an important event in biology. The fusion of membrane occurs in biological processes such as oocyte fertilization (Epel & Vacquier, 1978), muscle embryogenesis (Bischoff, 1978), and exocytotic events (Lawson et al., 1977). Studies on membrane fusion with phospholipid vesicles as relatively simple models are being used extensively. Vesicles are made with different proportions of acidic phospholipids and are induced to fuse by the addition of Ca^{2+}. Such a system, using variable mixtures of phosphatidylcholine (PC)[1] and PA, has been characterized in recent years (Liao & Prestegrad, 1979). Different experimental criteria have been utilized to assess vesicle fusion. Since fusion of phospholipid vesicles involves mixing of vesicle contents and membrane components, assays have been devised accordingly. The use of Tb^{2+} and dipicolinic acid as a fluorescent couple (Wilschut et al., 1980) was employed in a fusion assay involving mixing of vesicle contents. If the emphasis is put upon the intermixture of the membrane components, the use of RET of lipophilic chemicals has proven simple and practical, as reported by several groups (Vanderwerf & Ullman, 1980; Uster & Deamer, 1981; Struck et al., 1981). With simplified artificial membrane models and practical methods to assess the degree of membrane intermixing, the basic mechanism leading to fusion can be a subject of study. The necessity of acidic phospholipids and the presence of Ca^{2+} for fusion to occur (Portis et al., 1979) are generally agreed upon. The role of soluble proteins has, so far, received less attention: Van der Bosch & McConnel (1975) concluded that concanavalin A caused fusion between lecithin vesicles, although a rigorous exclusion of probe diffusion through the aqueous phase was not attempted. Hong et al. (1981) described that synexin, a soluble Ca^{2+} binding protein, reduces the threshold for Ca^{2+}-induced fusion. It is noteworthy that synexin per se is unable to induce fusion in the absence of Ca^{2+}. Very recently, a pH-dependent fusion capability of clathrin (Blumenthal et al., 1983) and albumin (Cestaro et al., 1983) has been communicated. These proteins were unable to induce fusion at physiological pH, and the effect was observed when the pHs were lowered to 6.0 and 3.6, respectively. Fusion capability has also been ascribed to myelin basic protein (Lampe & Nelsestuen, 1982) and melitin (Eytan & Almary, 1983).

In this paper, we report about the capacity of several soluble proteins to induce fusion of artificial phospholipid vesicles made of PA and PC at pH 7.5, in the absence of Ca^{2+}. Among several proteins, GAPDH was the most extensively used. Fusion of phospholipid vesicles induced by GAPDH was studied by five different methods, namely, RET, light scattering, gel filtration, electron microscopy, and intermixing of aqueous contents.

Experimental Procedures

Materials. Before each experiment, beef heart L-lactic dehydrogenase and yeast and rabbit muscle GAPDH were dissolved in 20 mM Tris-HCl, pH 7.5, and then gel filtered

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[1] Abbreviations: PC, phosphatidylcholine; PA, phosphatidic acid; RET, resonance energy transfer; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; *N*-NBD-PE, *N*-(7-nitro-2,1,3-benzoxadiazol-4-yl)phosphatidylethanolamine; CA9C, cholesteryl anthracene-9-carboxylate; DPA, dipicolinic acid; EDTA, ethylenediaminetetraacetic acid disodium salt; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride.

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through a Sephadex G-25 column (1.3 cm × 28 cm) equilibrated with the same buffer to remove ammonium sulfate and excess NAD. Bovine serum albumin, concanavalin A (type IV), *Bacillus thermoproteolyticus* protease (type X), egg white lysozyme and bovine pancreas ribonuclease (type III-B) were dissolved in 20 mM Tris-HCl, pH 7.5, and used without further modifications. All proteins were purchased from Sigma Chemical Co. (St. Louis, MO). PC was purified from egg yolk by neutral alumina chromatography as described previously (Bloj & Zilversmit, 1976). PA was prepared by the action of cabbage phospholipase D on purified PC (Yang, 1969) and isolated by column chromatography on silicic acid. The purity of the phospholipids was checked by thin-layer chromatography on silica gel H, with chloroform/methanol/NH_4OH (65:35:5) as solvent. They were stored in chloroform at −20 °C in the presence of 0.1 mol % butylated hydroxytoluene. *N*-NBD-PE was obtained from dipalmitoylphosphatidylethanolamine and NBD chloride (Sigma Chemical Co., St. Louis, MO) as described by Monti et al. (1978) and purified by column chromatography on CM-cellulose. CA9C was from Molecular Probes Inc. (Junction City, OR). They were stored in chloroform at −20 °C. Terbium chloride was purchased from Koch-Light Laboratories, and dipicolinic acid was from Sigma. Tris(hydroxymethyl)-aminomethane was recrystallized before use. All other reagents used were analytical grade.

Preparation of Vesicles. Vesicles for RET assay were obtained by mixing 0.9 μmol of PC, 0.1 μmol of PA, and either 0.01 μmol of CA9C or 0.03 μmol of NBD-PE in two different test tubes. Lipids were dried under nitrogen and dissolved in 0.25 mL of ethanol. Vesicles were prepared by microinjection of 25 μL of the ethanolic solution into 2.0 mL of aqueous phase (20 mM Tris-HCl, pH 7.5) according to the method of Batzri & Korn (1973).

To prepare vesicles containing terbium, DPA, or the Tb/DPA complex trapped within, phospholipids (PC/PA, 9:1) were injected into 20 mM Tris-HCl, pH 7.4, containing respectively the following: (i) 15 mM $TbCl_3$ and 150 mM sodium citrate; (ii) 50 mM DPA (sodium salt); or (iii) 15 mM $TbCl_3$ and 50 mM DPA (sodium salt). Nonencapsulated material was eliminated by gel filtration on Sephadex G-75 (Pharmacia) as described in detail by Wilschut et al. (1980).

Resonance Energy Transfer Assay. The RET assay has been described in detail by Hoekstra (1982). The fluorescence donor (CA9C) and acceptor (NBD-PE) were in separate vesicle populations. Equal volumes of these vesicles were mixed, and fusion was initiated by the addition of 5–40-μL aliquots of 200 mM $CaCl_2$, or protein solution. The mixtures were incubated for 5 min at 37 °C. Fusion of phospholipid vesicles results in intermixing of the lipids which brings the donor and acceptor to close proximity. When both fluorescent lipids were in a lipid veesicle at appropriate surface densities (ratio of fluorescent lipid to total lipid), efficient resonance energy transfer was observed.

Steady-state emission spectra of samples containing both CA9C and NBD-PE were obtained by exciting at 360 nm. The efficiency of resonance energy transfer (quenching of the energy donor) is defined by the equation (Fung & Stryer, 1978)

$$\mathrm{RET} = 1 - F/F_0 \qquad (1)$$

where F_0 is the fluorescence emission intensity of CA9C in the absence of fusogenic agents and F the fluorescence in the presence of $CaCl_2$ or the protein to be tested.

Tb/DPA Fusion Assay and Tb Release. Intermixing of aqueous contents during vesicle fusion was carried out with a 1:1 mixture of Tb- and DPA-containing vesicles in 20 mM Tris-HCl, pH 7.4, and 0.2 mM EDTA at a final lipid concentration of 50 μM, as determined by phosphate analysis (Ames, 1966). The fluorescence of the Tb/DPA complex was measured by exciting at 280 nm using a Corning 3-71 cut-off filter between the sample and the emission monochromator.

Tb release was determined with Tb/DPA vesicles (50 μM final lipid concentration) in 20 mM Tris-HCl, pH 7.4, and 1 mM EDTA. The maximum release was determined after lysing the vesicles with 0.01% (v/v) Triton X-100.

Fluorescence and Light-Scattering Measurements. Steady-state emission spectra were obtained by using a 4048 C SLM polarization spectrofluorometer with crossed polarizers to reduce light scattering. Light-scattering changes were recorded with an Aminco Bowman spectrophotofluorometer with the excitation and emission monochromators set at 370 nm.

Electron Microscopy. Samples of phospholipid vesicles (PC/PA, 9:1) obtained by injecting an ethanolic solution of lipids in 20 mM Tris-HCl, pH 7.5, and 100 mM NaCl were fused with calcium or muscle GAPDH, concentrated by ultrafiltration, and prepared for thin sectioning as follows: vesicles at a lipid concentration of 0.25 μmol/mL were mixed with an equal volume of 5% agarose and rapidly cooled. The resulting gels were fixed, sliced, and stained according to Gad et al. (1982).

Analysis of Vesicle Fusion by Gel Filtration. A vesicle mixture containing donor (CA9C) and acceptor (*N*-NBD-PE) probes was subjected to calcium or muscle GAPDH-induced fusion and applied to a Sepharose 4-B column (1.3 cm × 26 cm) equilibrated and eluted with 20 mM Tris-HCl, pH 7.5. Fractions of 2.0 mL were collected, and the fluorescence emission of each probe was measured.

Results

Fusion of Phospholipid Vesicles Induced by Ca^{2+}. Previous studies (Uster & Deamer, 1981) have shown that there is a significant overlap between the emission band of CA9C (donor) and the excitation band of *N*-NBD-PE (acceptor). Consequently, energy transfer between this donor/acceptor pair is expected to be relatively efficient. To test the feasibility of using RET to detect fusion events, we employed the calcium-induced fusion of a mixture of PC and PA vesicles as a model system. When a preparation of vesicles containing CA9C and *N*-NBD-PE was excited at 360 nm, emission maxima at 452 and 530 nm were observed (Figure 1A). The first peak arises from the emission fluorescence of the donor (CA9C). Fusion was initiated by the addition of calcium. The spectral changes associated with fusion were a marked reduction in the emission peak at 452 nm with a concomitant increase in the fluorescence yield from *N*-NBD-PE (530 nm). It should be noted that the spectral change shown in Figure 1A was absolutely dependent upon the addition of calcium. Lipid vesicles incubated at room temperature for up to 60 h in the absence of Ca^{2+} did not show any fluorescence emission changes. The dependence of RET efficiency on calcium concentration is shown in Figure 1B. With increasing calcium concentrations, the transfer efficiency was increased, reaching a plateau in the presence of 5 mM calcium. A simultaneous increase in the light scattering which is consistent with the results of RET was found (Figure 1B).

The fusion of phospholipid vesicles (PC/PA, 9:1) was also followed conveniently by using the fluorescent assay of Wilschut et al. (1980). This assay monitors the mixing of vesicle aqueous contents between two populations of vesicles containing $TbCl_3$ and DPA. The formation of a Tb/DPA complex produces a 10^4-fold enhancement in Tb fluorescence.

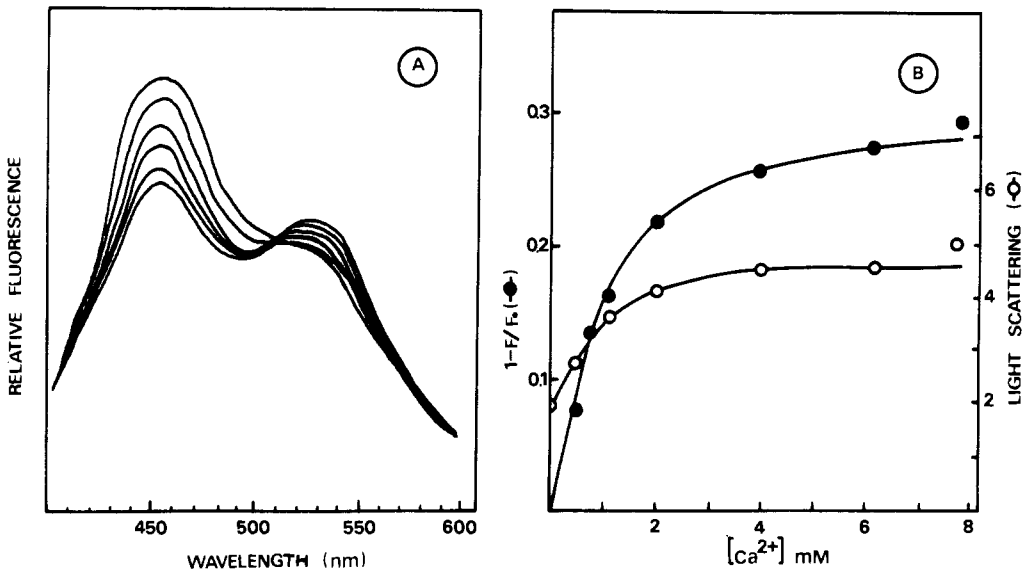

FIGURE 1: Effect of $CaCl_2$ on the emission spectra of mixed CA9C- and *N*-NBD-PE-labeled vesicles. Vesicles were prepared as described under Experimental Procedures. The fusion mixture (2.0 mL final volume; 20 mM Tris-HCl, pH 7.5) contained 200 nmol of phospholipids (PC/PA, 9:1), 1 nmol of CA9C, and 3 nmol of *N*-NBD-PE. Fusion carried out at 37 °C was initiated by the addition of $CaCl_2$. (A) In descending order, the emission spectra (excitation at 360 nm) of fusion mixtures containing 0, 0.5, 1.0, 2.0, 4.0, and 8.0 mM $CaCl_2$, respectively. (B) Energy transfer efficiency (●) and light scattering (○) measured according to Experimental Procedures as a function of $CaCl_2$ concentration.

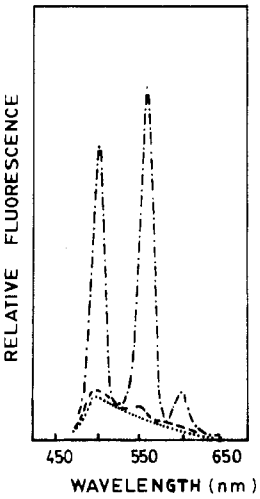

FIGURE 2: Ca^{2+}- and GAPDH-induced fusion of phospholipid vesicles (PC/PA, 9:1) measured by the Tb^{3+}/DPA assay. A mixture of Tb^{3+} and DPA vesicles (1:1) at a total lipid concentration of 50 μM was incubated for 3 min at 37 °C in the absence of fusogenic agents (···) or in the presence of 5 mM $CaCl_2$ (–·–) or 0.1 μM GAPDH (- - -). Fluorescence emission spectra of the $Tb(DPA)_3^{3-}$ complex were recorded by exciting at 280 nm.

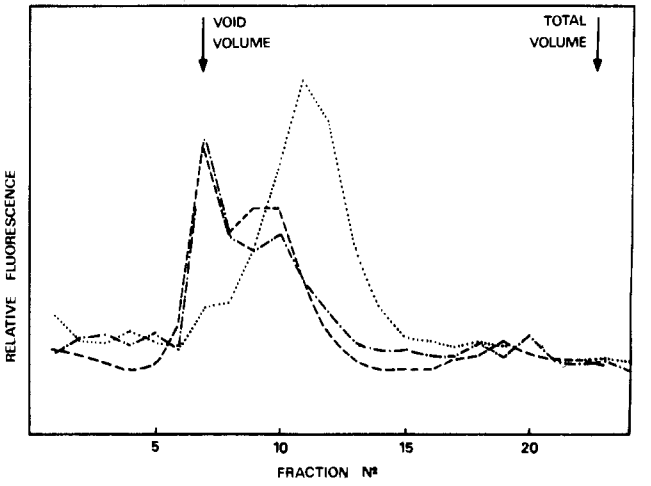

FIGURE 3: Analysis of phospholipid vesicle mixtures by gel filtration before and after calcium- or GAPDH-induced fusion. Phospholipid vesicles prepared as described in Figure 1 were incubated at 37 °C for 10 min either alone (···) or in the presence of $CaCl_2$ (4 mM) (–·–) or GAPDH (0.2 mg/mL) (- - -). The samples were applied to a Sepharose 4B column (1.3 × 26 cm) equilibrated and eluted with 20 mM Tris-HCl, pH 7.5. Fractions of 2.0 mL were collected, and the fluorescence of CA9C was recorded.

Table I: Effect of Proteins on the Light Scattering and Fluorescence Energy Transfer of Phospholipid Vesicles[a]

proteins	concn (mg/mL)	light scattering (arbitrary units)	RET (%)
ribonuclease	250	1.87	0
concanavalin A	500	2.00	0
bovine serum albumin	500	2.20	0
protease	0.40	1.90	0
glucagon	250	3.20	0
lysozyme	0.04	3.30	17.0
L-lactic dehydrogenase	0.04	4.00	8.5
GAPDH (muscle)	0.02	4.15	29.6
GAPDH (yeast)	0.04	3.66	14.9
		1.90	0

[a] The experimental details are given under Experimental Procedures.

Formation of the fluorescent Tb/DPA complex in the external medium is prevented by the presence of EDTA which rapidly dissociates the complex. Figure 2 shows that fusion of phospholipid vesicles by 5 mM $CaCl_2$ occurred with an increase of Tb/DPA complex fluorescence which indicates a large intermixing of aqueous contents within 3 min after the initiation of the reaction.

To provide further evidence that the observed changes in the efficiency of energy transfer and the intermixing of aqueous contents were due to vesicle/vesicle fusion, the vesicle mixture was analyzed by gel filtration before and after the addition of calcium. When a mixture of fluorescent-labeled vesicles was chromatographed on a Sepharose 4-B column, most of the vesicle population eluted at a position corresponding to small unilamellar vesicles. If this mixture was exposed to calcium (5 mM) prior to chromatography, a significant portion of the vesicles eluted at the column void volume (Figure 3).

Fusion of Phospholipid Vesicles Induced by Proteins. In the following experiments, RET and light scattering were used

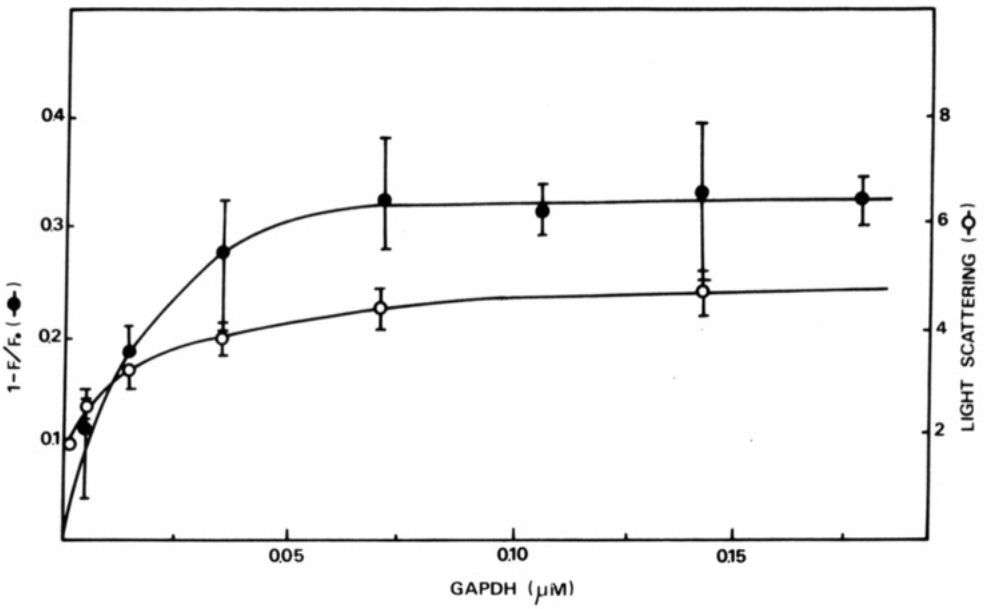

FIGURE 4: Dependence of the extent of fusion on GAPDH concentration. Phospholipid vesicles were prepared as described in Figure 1. Fusion was initiated by the addition of a desired concentration of enzyme. Fluorescence energy transfer efficiency was calculated as described under Experimental Procedures.

to test the capability of several soluble proteins to induce vesicle/vesicle fusion at pH 7.5 and 37 °C. According to the results summarized in Table I, the proteins tested can be classified in three groups.

Ribonuclease, concanavalin A, bovine serum albumin, and protease did not show an effect either on the light scattering or on the RET even at concentrations of 250–500 mg/mL. Glucagon exerted an increase in the light scattering but not on the RET. The dehydrogenases and lysozyme induced an increase in light scattering and RET, suggesting that these proteins exhibit fusogenic activity under our experimental conditions.

Fusogenic Capacity of GAPDH. The fusogenic capacity of muscle GAPDH was studied in more detail. When the fusion process was assayed by RET, half of the maximum effect was obtained around 35 nM enzyme concentration which is equivalent to 0.05 μg/mL. A parallel increase in the light scattering was observed (Figure 4). Several control experiments were run to rule out the presence of divalent cations in the preparation. The Ca^{2+} concentration in the medium was determined by atomic absorption spectroscopy and found to be 5 ppm. The same results as those shown in Figure 4 were obtained in the presence of 5 mM EDTA. Gel filtration of the vesicles after treatment with GAPDH showed a pattern very similar to that exhibited by the vesicles after Ca^{2+}-induced fusion (Figure 3). When the fusion induced by GAPDH was monitored with the Tb/DPA assay to register the coalescence of the internal vesicle volume, no appreciable degree of mixture of contents occurred (Figure 2). It is possible that within large aggregates of vesicles the aqueous contents leak rapidly into intervesicular spaces and the Tb/DPA complex is dissociated by competitive chelation of EDTA. To investigate this possibility, we prepared Tb^{3+}/DPA vesicles and after the addition of GAPDH followed the release of the Tb/DPA complex to the external medium in the presence of EDTA. Under our experimental conditions, a large (80% or more) leakage of vesicle contents was observed after about 2 min (Figure 5). Identical results were obtained with carboxyfluorescein release (not shown).

Electron microscopy confirms that fusion of phospholipid vesicles (PC/PA, 9:1) occurs upon exposure to GAPDH.

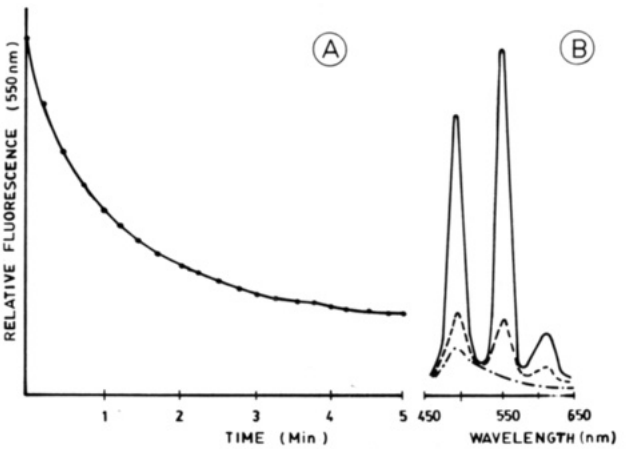

FIGURE 5: Release of contents of PC/PA (9:1) vesicles during fusion by GAPDH. Tb^{3+}/DPA vesicles (total lipid concentration 50 μM) were incubated at 37 °C with 0.1 μM GAPDH in 20 mM Tris-HCl, pH 7.4, and 0.1 mM EDTA. (A) Kinetic decay of the Tb^{3+}/DPA complex fluorescence initiated by addition of GAPDH. (B) Fluorescence emission spectra of the Tb^{3+}/DPA complex before GAPDH addition (—), after 5 min of incubation with GAPDH (---), and in the presence of 0.01% (v/v) Triton X-100 (-·-). Excitation wavelength, 280 nm.

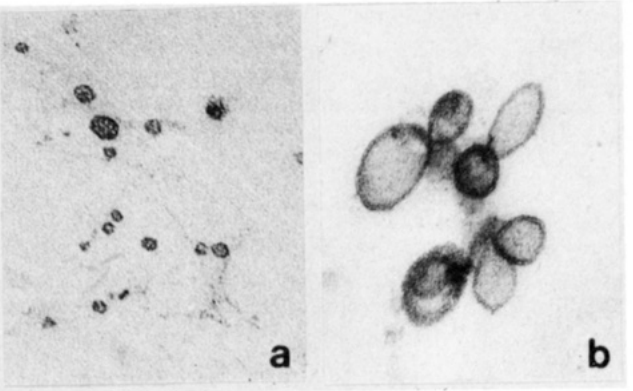

FIGURE 6: Calcium- and GAPDH-induced fusion studied by electron microscopy. Phospholipid vesicles (PC/PA, 9:1) were prepared, treated, and processed as described under Experimental Procedures. (a) Vesicles without treatment; (b) treated with 0.1 μM GAPDH. Magnification 105000×.

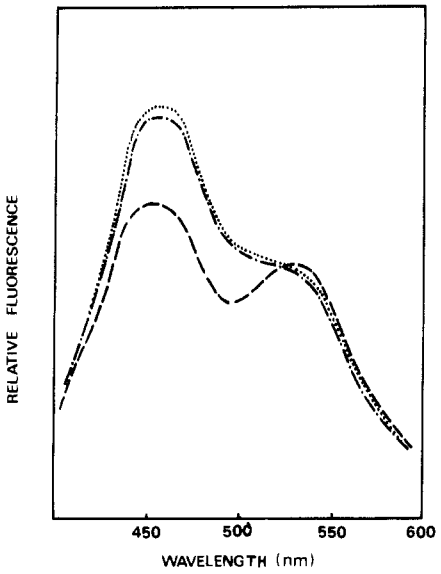

FIGURE 7: Effect of denatured GAPDH on the emission spectra of mixed CA9C- and *N*-NBD-PE-labeled vesicles. Fusion mixture preparation and composition were as described in Figure 1. Fusion was initiated by adding 20 μg of native or denatured (by heating 2 min at 100 °C) enzyme. The emission spectra of the fusion mixture containing no protein (···), native enzyme (- - -), and denatured enzyme (-·-) were scanned, exciting at 370 nm.

Figure 6a shows a micrograph of lipid vesicles without treatment. A well-dispersed population of vesicles was seen with a mean diameter of approximately 200 Å, indicating that no aggregation of fusion had occurred. However, the addition of 0.1 μM GAPDH for 5 min at 37 °C produced aggregates of vesicles which were larger in size (1000 Å) and more irregular in shape (Figure 6b). It should be noted that a minority (10%) of the small vesicles remains unalterable in the fused preparations. Similar results were obtained when fusion was carried out by Ca^{2+}. Finally, as shown in Figure 7, when GAPDH was heated for 5 min at 100 °C, its fusogenic capacity was lost.

Discussion

Previous studies have established that RET between a suitable pair of fluorophores can be used to monitor vesicle/vesicle fusion (Uster & Deamer, 1981; Struck et al., 1981; Vanderwerf & Ullman, 1980). To ascribe the changes in the fluorescence spectra to fusion, one must discard spontaneous intervesicular transfer of the fluorophores. The pair that we have chosen, CA9C and dipalmitoyl-NBD-PE, does not tend to redistribute between vesicles since we could not detect spontaneous movement even after 60 h of incubation at room temperature. The efficiency of this pair is somewhat low, however, on the order of 30%. Higher efficiencies (around 80%) could be achieved recently in our laboratory, using the egg phosphatidylethanolamine/NBD derivatives instead of dipalmitoylphosphatidylethanolamine/NBD derivatives employed in this work. Using RET, we demonstrate that 10% phosphatidic acid in the vesicles is enough to promote vesicle/vesicle fusion in the presense of Ca^{2+} or some soluble proteins. The fusogenic capability of GAPDH, which was apparently independent of the source (Table I), is indeed surprising. Incubation of phospholipid vesicles with GADPH resulted in complete fusion within 120 s at pH 7.5 (not shown).

It can be calculated that at the concentration needed to reach the maximal degree of vesicle fusion (about 0.1 μM), there are about three protein molecules per phospholipid vesicle. Previous works (Wooster & Wrigglesworth, 1975) have demonstrated the affinity of GAPDH for mixed monolayers of phosphatidylcholine and phosphatidic acid, but this is the first time that its fusogenic activity has been demonstrated. Although the kinetic characteristics of the final product obtained when fusion was induced by GAPDH were similar to those obtained by calcium, the mechanism involved could be different and in order to elucidate this question more studies are needed. Unfortunately, the mechanism by which calcium promotes vesicle fusion still remains controversial (Paphadjopoulos et al., 1979; Hoekstra, 1982; Ohki, 1982). Because of the release of vesicle contents during fusion of phospholipid vesicles by GAPDH, the relevance of the experiments shown here could be questioned since a number of fusion events in vivo are supposedly nonleaky. However, it should be noted that the structure and composition of the artificial bilayer used in this work are far from those of the biological membranes. In addition, previous studies have shown that leakage could be reduced by changing the pH, the phospholipid composition (Sundler & Papahadjopoulos, 1981), or the vesicle size (Wilschut et al., 1980).

Results not shown indicate that release of intravesicle contents represents the rupture of the proper vesicles, since there was no evidence for an increased rate of diffusion from the fused vesicles.

The fact that GAPDH, a more widely distributed protein, also induces fusion raises some interesting questions. It would be of particular interest to see if the enzyme is capable of inducing fusion of natural membrane vesicles and what, if any, is the physiological relevance of the phenomenon. Although the experiments were carried out with vesicles containing 10% phosphatidic acid, it remains to be determined if the acidic phospholipid is an essential requirement for the fusogenic activity of the enzyme. Finally, the fate of the protein after vesicle/vesicle fusion will be investigated. This point will be of great interest in any attempt to elucidate the mechanism by which GAPDH exerts its fusogenic activity.

Acknowledgments

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Registry No. GAPDH, 9001-50-7; Ca, 7440-70-2; lysozyme, 9001-63-2; L-lactate dehydrogenase, 9001-60-9.

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Effect of Bovine Basic Protein Charge Microheterogeneity on Protein-Induced Aggregation of Unilamellar Vesicles Containing a Mixture of Acidic and Neutral Phospholipids†

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ABSTRACT: Two of the charge isomers (components 1 and 2) normally found as microheteromers of myelin basic protein were isolated, and their abilities to aggregate vesicles consisting of mixed phospholipids were studied. Component 1 (the most cationic of the microheteromers) aggregated phosphatidylcholine (PC) vesicles containing 7.8 mol % phosphatidylserine (PS) more rapidly and at lower protein concentrations than component 2, which differs from component 1 by 1 net positive charge. Modification of components 1 and 2 in vitro by phosphorylation with rabbit muscle protein kinase decreased the ability of both components to aggregate vesicles. The greater the extent of phosphorylation, the less effective were the isomers at inducing aggregation. Decreasing the charge of either component 1 or component 2 by removal of the two C-terminal arginyl residues also decreased the ability of the isomers to induce aggregation. Therefore, charge microheterogeneity, whether arising in vivo or generated in vitro, markedly affected the ability of these microheteromers to aggregate PC vesicles containing 7.8 mol % PS. Because a small difference in the charge of the protein had a marked effect on vesicle aggregation, we propose that charge microheterogeneity may play an important and dynamic role in the structure and function of normal myelin.

The myelin sheath is the membranous structure that surrounds the axons of the nervous system. Although derived from the plasma membrane of the oligodendroglia cell, its multilayer structure and its low protein/lipid ratio (about 1/4) make it a unique biological membrane structure. The transmission of impulses along the axon is dependent on the integrity of this multilamellar structure. The role of protein–lipid interactions in generating and maintaining this structure is not fully defined as yet.

One of the major proteins in the myelin membrane is an extrinsic protein called basic protein (BP),[1] which accounts for approximately 30% of the myelin protein (Boggs & Moscarello, 1978). The ability of this positively charged protein to organize phosphatidylglycerol bilayers into multilamellar structures characteristic of myelin (Brady et al., 1981), and to aggregate vesicles containing acidic phospholipids (Lampe & Nelestuen, 1982; Young et al., 1982; Lampe et al., 1983) or those prepared from myelin (Young et al., 1982), suggested that BP may play a structural role in either the development or maintenance of the multilamellar profile of the myelin sheath.

Purified BP with a molecular weight of 18 400 migrates as a single component on high ionic strength sodium dodecyl sulfate (SDS) gels with M_r ~21 000. However, at alkaline pH (10.6), the protein resolves into several components characteristic of microheterogeneity based on net charge (Martenson et al., 1969).

The BP charge microheterogeneity, which results from posttranslational modifications such as phosphorylation (Chou

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[1] Abbreviations: PC, phsophatidylcholine; PS, phosphatidylserine; PA, phosphatidic acid; PMSF, phenylmethanesulfonyl fluoride; TCA, trichloroacetic acid; MES, 2-(*N*-morpholino)ethanesulfonic acid; BP, myelin basic protein; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; EDTA, ethylenediaminetetraacetic acid.

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