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A Fluorescence Resonance Energy Transfer Approach for Monitoring Protein-Mediated Glycolipid Transfer between Vesicle Membranes¹

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Abstract

A lipid transfer protein, purified from bovine brain (23.7 kDa, 208 amino acids) and specific for glycolipids, has been used to develop a fluorescence resonance energy transfer assay (anthrylvinyl labeled lipids; energy donors and perylenoyl labeled lipids; energy acceptors) for monitoring the transfer of lipids between membranes. Small unilamellar vesicles composed of 1 mol% anthrylvinyl-galactosylceramide, 1.5 mol% perylenoyl-triglyceride, and 97.5% 1-palmitoyl-2-oleoyl phosphatidylcholine (POPC) served as donor membranes. Acceptor membranes were 100% POPC vesicles. Addition of glycolipid transfer protein to mixtures of donor and acceptor vesicles resulted in increasing emission intensity of anthrylvinyl-galactosylceramide and decreasing emission intensity of the nontransferable perylenoyl triglyceride as a function of time. The behavior was consistent with anthrylvinyl-galactosylceramide being transferred from donor to acceptor vesicles. The anthrylvinyl and perylenoyl energy transfer pair offers advantages over frequently used energy transfer pairs such as NBD and rhodamine. The anthrylvinyl emission overlaps effectively the perylenoyl excitation spectrum and the fluorescence parameters of the anthrylvinyl fluorophore are nearly independent of the medium polarity. The nonpolar fluorophores are localized in the hydrophobic region of the bilayer thus producing minimal disturbance of the bilayer polar region. Our results indicate that this method is suitable for assay of lipid transfer proteins including mechanistic studies of transfer protein function.

Keywords

Glycosphingolipid; Lipid transfer protein; Phospholipid bilayers; Galactosylceramide; Anthrylvinyl; Perylenoyl

Fluorescence approaches offer a convenient means to monitor a wide variety of biological processes including lipid trafficking and transfer between membranes. Compared to

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alternative methods of monitoring lipid intermembrane transfer, fluorescence approaches generally do not require separation of donor and acceptor membranes and offer the advantage of continuous real-time monitoring of the lipid transfer process. Two variations of the fluorescence approach have been used previously to investigate lipid transfer events. In the first approach, lipid derivatives carrying a single fluorophore type have been used to take advantage of changes in emission responses related to the membrane concentration of the fluorophore. Examples include self-quenching assays based on concentration-dependent changes in the emission intensity as well as excimer assays based on concentration-dependent changes in the emission wavelength spectra. Lipid derivatives commonly used in self-quenching assays include NBD²- (7-nitrobenz-2-oxa-1,3-diazol-4-yl)- and BODIPY-derivatives (e.g., 1-3); whereas pyrene-lipid derivatives are typically used in excimer-based assays (e.g., 4, 5).

A second fluorescence approach typically used to study lipid transfer processes relies on fluorescence resonance energy transfer (FRET) between two different lipid probe molecules. In FRET assays, one of the lipid derivatives is transferable compared to the second relatively immobile lipid derivative. The lipid transfer process produces emission spectral changes that depend upon probe proximity and orientation within the donor and acceptor membranes (6). In earlier studies involving lipid transfer proteins, lipid derivatives carrying NBD as the energy transfer donor and lissamine rhodamine B sulfonate as the energy transfer acceptor were employed using the FRET approach (1, 7, 8). The same donor/acceptor FRET pair has been used to study other aspects of lipid diffusion and distribution in membranes such as transbilayer phospholipid migration (9) and transbilayer lipid distribution (10). In contrast, Silvius and coworkers have used phospholipids labeled with different coumarin moieties as energy transfer donors and with DAB-labeled (dimethylaminophenylazophenyl) lipids as energy transfer acceptors to provide a FRET probe pair with advantages over the NBD/lissamine rhodamine pair (11-13).

Here, we report the utility of the anthrylvinyl and perylenoyl FRET pair for studying intermembrane lipid transfer processes. This FRET energy transfer pair is especially well suited for such studies. The anthrylvinyl emission spectrum effectively overlaps the perylenoyl excitation spectrum and the fluorescence parameters of the anthrylvinyl fluorophore are nearly independent of the medium polarity (14). Both fluorophores reportedly localize to the hydrophobic region of the bilayer and thus produce minimal disturbance of the bilayer interfacial/polar region (14). To illustrate the usefulness of the anthrylvinyl/perylenoyl FRET pair, we have synthesized a galactosylceramide carrying a *trans*-12-[9-anthryl]-11-dodecenoyl acyl chain (AV-GalCer) and a triglyceride (Per-TG) carrying a 9-(3-perylenoyl)nonanoyl acyl chain (Fig. 1), and studied the intervesicular transfer process mediated by glycolipid transfer protein (GLTP).

²Abbreviations used: FRET, fluorescence resonance energy transfer; GLTP, glycolipid transfer protein; NBD, 7-nitrobenz-2-oxa-1,3-diazol-4-yl; AV, anthrylvinyl; AV-GalCer, N-[(11*E*)-12-(9-anthryl)-11-dodecenoyl]-1-O- β -galactosylsphingosine; AV-PC, 1-acyl-2-[(11*E*)-12-(9-anthryl)-11-dodecenoyl]-*sn*-glycero-3-phosphocholine; AV-SPM, N-[(11*E*)-12-(9-anthryl)-11-dodecenoyl]-sphingosine-1-phosphocholine; Per, perylenoyl; Per-TG, *rac*-1,2-dioleoyl-3-[9-(3-perylenoyl)nonanoyl]glycerol; GalCer, galactosylceramide; POPC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; TG, triglyceride.

MATERIALS AND METHODS

Materials

1-Palmitoyl-2-oleoylphosphatidylcholine (POPC) was purchased from Avanti Polar Lipids (Alabaster, AL) and gave a single spot when analyzed by thin layer chromatography. The fluorescent probes (Fig. 1), N-[(11*E*)-12-(9-anthryl)-11-dodecenoyl]-1-O- β -galactosylsphingosine [AV-GalGer] (15), 1-acyl-2-[(11*E*)-12-(9-anthryl)-11-dodecenoyl]-*sn*-glycero-3-phosphocholine [AV-PC] (16), N-[(11*E*)-12-(9-anthryl)-11-dodecenoyl]sphingosine-1-phosphocholine [AV-SPM] (17) and *rac*-1,2-dioleoyl-3-[9-(3-*perylene*oyl)-nonanoyl]glycerol (18) were prepared as described earlier. The glycolipid transfer protein was purified to homogeneity from bovine brain as described earlier (19).

Preparation of Vesicles

Small unilamellar vesicles, prepared by rapid ethanol injection (20), served as donor vesicles in the assay. POPC was mixed with 1% AV-GalCer (0.4 nmol) and 1.5% Per-TG (0.6 nmol) from stock solutions (hexane:ethanol; 95:5) and dried under nitrogen and re-dissolved immediately before use in absolute ethanol. The mixture (40 nmol) was rapidly injected with a 25 μ l Hamilton syringe into a 10 mM sodium phosphate buffer (containing 1 mM dithiothreitol and 0.02 % sodium azide) under rapid stirring at 37°C. Final concentration of donor vesicles in the assay was 13 μ M, and the final ethanol concentration was less than 0.2 %. The acceptor vesicles were prepared as described earlier (21). Briefly, POPC was dried under vacuum and suspended in the sodium phosphate buffer (pH 7.4) at a concentration of 50 mM by vortexing. The suspension was sonicated under nitrogen and centrifuged for 90 minutes at 100,000 g to remove probe particles and multilamellar vesicles. The final donor vesicle concentration used in the assay was 133 μ M. The size of the vesicle populations reportedly averages about 25 nm in diameter (20).

Fluorescence Measurements

Fluorescence experiments were performed with a SPEX Fluoromax instrument (Instruments S.A., Inc. Edina, NJ). The excitation and emission bandpass were 5 nm and the sample cuvette holder was temperature controlled to 37°C $\pm$ 0.1 °C (Neslab, RTE-111). Donor vesicles were formed first by ethanol injection into a sodium phosphate buffer under rapid stirring; next sonicated acceptor vesicles were added at an acceptor/donor ratio of 10. The transfer of AV-GalCer (400 pmol per assay) was started by addition of catalytic amounts of glycolipid transfer protein to the vesicle mixture and the stirring rate was reduced to about 100 rpm. Emission spectra were obtained by scanning (1 nm/sec) from wavelengths 390 nm – 560 nm at different time intervals, while exciting the AV-GalCer at 370 nm.

Light Scattering Measurements

Light scattering was measured at 90° relative to incident light using a FluoroMax spectrofluorimeter. Temperature in the cell was maintained at 37°C using a circulating water bath (Neslab RTE-111). The intensity at 320 nm was measured as a function of time to assess changes in vesicle aggregation state or fusion using the basic approach described by Nelsestuen and co-workers (22, 23).

RESULTS

FRET Assay Design

For efficient monitoring of lipid transfer between membranes, our goal was to maximize the total change in fluorescence signal response over the experimental time course while minimizing the required quantities of the anthrylvinyl-labeled glycolipid and perylenoyl-labeled triglyceride (Per-TG). To accomplish this goal, a large excess of acceptor vesicles (e.g., 10-fold) was deemed desirable and both FRET lipid probes initially were incorporated in the donor vesicles during their preparation (see Methods). By placing both probes in the minority vesicle population, i.e., donors, interbilayer energy transfer could be avoided should vesicle aggregation occur. To test the effectiveness of the assay design, the following experiments were performed. Donor vesicles containing both AV-GalCer and Per-TG were prepared (see Methods). Because the emission wavelengths of anthrylvinyl overlap with the excitation wavelengths of perylenoyl (Fig. 2), exciting the AV-GalCer at 370 nm (arrow) resulted in the expected resonance energy transfer to the perylenoyl residue of triglyceride. Due to their close proximity within the donor vesicles, the anthrylvinyl emission signal was quenched but the perylenoyl emission signal was observed (Fig. 3; trace 2). This finding agreed well with earlier results showing resonance energy transfer between other anthrylvinyl- and perylenoyl-lipid derivatives in model bilayer vesicles (24).

Upon addition of POPC acceptor vesicles in 10-fold excess relative to donor vesicles, only a small change in the emission spectra was observed. However, subsequent addition of catalytic amounts of glycolipid transfer protein (GL TP) (2 μ g, 84.4 pmol) produced an immediate increase in the anthrylvinyl signal and a concomitant decrease in the perylenoyl signal was observed as a function of time when exciting at 370 nm (Fig. 3). The observed signal response was consistent with the expected resonance energy transfer changes that would occur during transfer of AV-GalCer, but not Per-TG, from the donor to the acceptor vesicles. Because a similar signal response might occur in response to fusion between donor and acceptor vesicles, control experiments were performed to eliminate this possibility (see Discussion).

Emission scans obtained after one hour (Fig. 3; 60, 90 or 120 min.) show almost no further increase in the relative AV-GalCer intensity suggesting that the intervesicular transfer process is close to equilibrium. To test this interpretation, Triton X-100 was added to the vesicle mixture after the end-point equilibrium was reached. Because Triton X-100 reportedly quenches NBD fluorescence by about 50% in micelles (25), separate control experiments were performed to determine the effects on AV-GalCer fluorescence. These controls revealed that Triton X-100 did not quench anthrylvinyl fluorescence but did produce a nearly constant elevation (~30%) of the “intensity” across the AV-GalCer emission wavelength range (390 to 500 nm) in buffer alone. Presumably, this intensity elevation is due to changes in light scattering produced by Triton X-100 micelles (data not shown). Figure 3 (dotted line) shows the emission scan obtained by donor and acceptor vesicles along with GLTP after treatment with Triton X-100. This emission scan has been corrected for Triton X-100 induced changes due to light scattering effects. Detergent treatment disrupted the vesicles and effectively diluted the FRET probe pair so as to

minimize energy transfer. The total AV-GalGer signal intensity of the emission scan produced after Triton X-100 treatment (Fig. 3; dotted line) is about 35% higher than the signal intensity achieved at transfer equilibrium between donor and acceptor bilayer vesicles. This result appears to be consistent with the expected accessibility and distribution of GalGer within small-diameter unilamellar donor vesicles such as those produced by rapid ethanol injection (20). In such donor bilayer vesicles, the AV-GalGer on the inner bilayer leaflets would not be accessible to the glycolipid transfer protein because GalGer transbilayer diffusion (flip-flop) is extremely slow and cannot be detected after 5 h (26). If the AV-GalGer probe is mass distributed in the outer and inner leaflets of the small and highly curved donor vesicles similar to the POPC matrix lipid (27), then approximately 33.3% of the AV-GalGer originally present would be expected to remain in the inner membrane leaflet, be quenched by the perylenoyl-TG, and be inaccessible to GLTP. At equilibrium and with a donor-to-acceptor vesicle ratio of 1:10, the GLTP would transfer 10/11ths (90.9%) of the AV-GalGer on the outer bilayer leaflet to the acceptor vesicles. Considering the preceding, approximately 60-65% recovery of the AV-GalCer emission signal would be expected when intervesicular transfer equilibrium is achieved. This is what we observed.

Dependence of Glycolipid Transfer on GLTP

To show more clearly the extent of AV-GalGer transfer that occurred at the discrete time points when emission spectra were collected, the anthrylvinyl portion of the FRET emission spectrum was used and glycolipid transfer was expressed as the emission intensity at 425 nm relative to that achieved after addition of Triton X-100. As is shown in Figure 4, rapid transfer of AV-GalGer occurred during the initial five minutes following addition of GLTP regardless of whether 1, 2, or 4 μ g was added. However, when no GLTP was added to the transfer assay, the rate of GalGer intervesicular transfer was very slow. The slow spontaneous transfer rate of AV-GalCer agrees well with the previously published spontaneous transfer rates for different uncharged glycosphingolipids (28,29). The half-time transfer rate between vesicles for galactosylceramide reportedly is greater than 20 hours and increases dramatically when the amide-linked acyl chain is lengthened (30).

In other control experiments in which catalytic amounts of GLTP (84.4 pmol) were added to donor vesicles directly, without any acceptor vesicles present, no increase in the anthrylvinyl emission intensity was detected. Yet, subsequent addition of acceptor vesicles to the cuvette resulted in immediate and rapid transfer of AV-GalCer (data not shown). Also, if other proteins with similar size and isoelectric point (e.g., ribonuclease) or with a general affinity for lipids (e.g., bovine serum albumin) were substituted for GLTP, then no change in AV-GalCer signal response was observed regardless of whether or not acceptor vesicles were present.

GLTP Specificity for Different AV-Lipid Derivatives

To determine whether the presence of the anthrylvinyl fluorescent probe altered GLTP specificity for glycolipids, experiments were performed in which AV-labeled phospholipid, either AV-PC or AV-SPM was incorporated into the donor vesicles instead of AV-GalCer. As is evident in Figure 4, little or no protein-mediated transfer of phospholipid was

observed. The selectivity of GLTP for glycolipids agrees with earlier studies (29, 31, 32) performed in the absence of fluorescent probes.

Real-Time, Continuous Monitoring of Lipid Transfer

To provide an effective means to obtain the initial rates of GLTP-mediated AV-GalCer transfer, we monitored anthrylvinyl emission continuously at a fixed wavelength (425 nm) as a function of time (Fig. 5). At time point A, 40 nmol donor vesicles containing 1 mol% AV-GalCer (0.4 nmol) and 1.5 mol% Per-TG (0.6 nmol) were injected to the cuvette containing a sodium phosphate buffer at 37°C. Resonance energy transfer between AV-GalCer and Per-TG resulted in significant fluorescence quenching of AV-GalCer. Upon addition of acceptor vesicles (POPC, 400 nmol) at time point B, AV-GalCer began to spontaneously transfer very slowly to the acceptor vesicles while the nontransferable Per-TG remained in the donor vesicle. As AV-GalCer molecules transferred to the acceptor vesicles, they were no longer within the minimum distance [20-50 Å, (6)] required for efficient quenching via resonance energy transfer to Per-TG. Upon addition of GLTP (Fig. 5; point C), the rate of GalCer departure from the donor vesicles greatly increased. The half-time ($t_{1/2}$) for the equilibrium of the transfer process could be determined as illustrated in the inset panel in Figure 5. Following the initial burst in the GLTP-mediated intervesicular transfer of AV-GalCer, a dramatic slowing of glycolipid transfer was observed (Fig. 5; points C to D). To determine whether this response signified the 91% depletion of AV-GalCer from the donor vesicle outer surface which is predicted near equilibrium at donor-to-acceptor vesicle ratios of 1:10, additional experimental manipulations were performed. First, more acceptor vesicles were added (Fig. 5; point D). Only a very small increase in emission intensity resulted probably due mostly to light scattering as well as a small amount of AV-GalCer transfer. To rule out GLTP denaturation as the cause for the lack of AV-GalCer transfer, more protein was added (Fig. 5; point E) and still no change in emission response was evident. However, subsequent addition of more donor vesicles at point F (Fig. 5) sparked another rapid burst in transfer activity. In separate control experiments, reversing the order of addition of more acceptor vesicles (point D) and more GLTP did not change the outcome of the experiment. Taken together, the results indicate that the diminishing transfer rates that follow the bursts (points C to D and following point F) are due to a depletion of AV-GalCer from the outer surfaces of the donor vesicles and not from denaturation of GLTP or from acceptor vesicle limitations.

DISCUSSION

Fluorescence resonance energy transfer is a non-destructive method that can provide real time insights into different processes in biological systems (for reviews see (33) and papers cited therein). Here, we have presented a new FRET assay for determining glycolipid transfer activity by utilizing lipid derivatives carrying anthrylvinyl and perylenoyl fluorescence probes.

GLTP appears to function by selectively enhancing the transfer of AV-GalCer, but not the perylenoyl-labeled triglyceride, from the donor vesicles. Admittedly, the changes in FRET observed under our assay conditions could be due to the simultaneous transfer of both FRET

probes via a GLTP-mediated vesicle fusion of donor and acceptor vesicles. To rule out this possibility, we monitored the aggregation/fusion state of the donor and acceptor vesicles before and after GLTP addition by light scattering approaches (see Methods). Figure 6 shows that the 90° light scattering signal obtained after addition of donors, followed by acceptors (10×), and then GLTP produced no changes consistent with vesicle aggregation or fusion. Measurements were carried out at 320 nm to avoid fluorescence contributions from the anthrylvinyl and perylenoyl probes (Fig. 2). While the scattering intensity increased very abruptly with each vesicle addition due to the increased number of scattering objects (e.g., large jump with 10× acceptors; Fig 6, point C), the signal response stabilized rapidly and remained unchanged for several minutes. Addition of GLTP (Fig. 6; point D) produced almost no change in scattering because of its much smaller size (23.7 kD) relative to the donor and acceptor vesicles (~ 2,000 kD). The unchanging scattering response observed over time intervals that coincide with large changes in FRET during the assay (Fig. 5; points C to D and following point F) rules out vesicle aggregation/fusion as the reason for the FRET response.

The anthrylvinyl/perylenoyl FRET pair provides distinct advantages over the NBD/lissamine rhodamine FRET pair often used to monitor protein-mediated lipid transfer and related processes (1, 7-9, 34). The NBD emission wavelength maxima and quantum yield depends significantly upon environmental factors and the NBD probe is relatively sensitive to photobleaching reactions. When used as a substrate derivative for lipid transfer proteins, the NBD label is usually attached to the acyl chain of a phosphoglyceride in order to avoid altering the lipid polar headgroup region which provides the structural specificity for different lipid transfer proteins (e.g., 35). However, due to their inherent polarity, the NBD/rhodamine FRET donor and acceptor fluorophores most often reside in the polar region of the lipid bilayer (36). This can disturb substantially the membrane interface region and impact on lipid transfer processes. Moreover, phospholipids having the relatively polar NBD group at the end of one of their acyl chains may not orient themselves like natural phospholipids. In fact, Chattopadhyay and London have shown that phosphatidylcholines having the NBD at the end of a 6- or 12-carbon *sn*-2 acyl chain were oriented such that the NBD was located at the polar interface of a model membrane. This suggested that the NBD-containing acyl chain was 'looping out' to the polar membrane interface (37, 38). This effect could have significant impact on the interfacial properties of the membrane such as charge, which can be critical for a lipid transfer protein finding its substrate [(39), Mattjus & Brown, unpublished observations].

This is not to say that the anthrylvinyl and 3-perylenoyl energy transfer pair are without a few minor drawbacks. The fact that significant emission response from the perylenoyl TG persists after the addition of Triton X-100 (Fig. 3; dotted line) suggests the excitation wavelength used (370 nm) with a 5 nm bandpass may produce excitation of the perylenoyl probe. This may explain why the signal response of the perylenoyl-TG did not appear as sensitive as that of AV-GalCer as the transfer process proceeded. Additional experiments will be required to completely resolve such matters.

Nonetheless, the apolar nature of both anthrylvinyl and perylenoyl probes give them a great advantage over polar probes such as NBD and rhodamine (14). Apolar probes linked to a

lipid acyl chains mimic the behavior of natural lipids since the head group resembles and retains the same structure and molecular shape as of the natural lipid. In the case of anthrylvinyl and perylenoyl, both fluorophores localize to the hydrophobic region of the bilayer and thus produce minimal disturbance of the bilayer interfacial/polar region (14). The emission wavelength maximum and quantum yield of the anthrylvinyl fluorophore are nearly independent of the surrounding media polarity. Although this is not the case for the perylenoyl probe (14), attaching this probe to triglyceride, which is not a substrate for the GLTP and is functionally nontransferable, keeps the fluorophore in a relatively fixed environmental position immersed in the hydrophobic region of the bilayer.

The bovine brain GLTP used in our study is specific for various glycolipids including neutral glycosphingolipids and gangliosides, but does not stimulate phospholipid or neutral lipid intermembrane transfer (29, 40). The protein has a molecular weight of 23.7 kDa and an isoelectric point near pH 9.0. Several characteristics of bovine and porcine brain GLTP suggest that this protein is different from other lipid transfer proteins [for reviews, see (41, 42)]. Yet the mechanism of action and the membrane parameters that regulate the kinetics of the transfer mediated by GLTP are not well understood. The FRET assay is currently being used to investigate these issues (Mattjus and Brown, unpublished observations).

Despite the focus on an anthrylvinyl-labeled glycolipid, the FRET intervesicular transfer assay reported here can be adapted easily to other types of lipids as well. The results indicate that this method is suitable for monitoring intermembrane lipid transfer activities during isolation of lipid transfer proteins or for studies of the functions and mechanisms of the transfer proteins themselves. The anthrylvinyl group also offers another advantage for investigations of lipid-protein interactions because its excitation maximum overlaps with the emission maximum of tryptophan.

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FIG 1.

Schematic representation of the (A) anthrylviny-galactosylceramide and the (B) perylenoyl-triglyceride fluorescent probes.

FIG. 2.

Normalized spectra of the two FRET fluorophores in POPC vesicles in sodium phosphate buffer at 37°C (pH 7.4): (a) Excitation of AV-GalCer (λ_{em} 425 nm). (b) Emission of AV-GalCer, at λ_{ex} 370 nm (arrow). (c) Excitation of Per-TG (λ_{em} 520). (d) Emission of Per-TG at λ_{ex} 425 nm.

FIG. 3.

Emission spectra scanned at different time intervals at 37°C: (1) The emission scan of sodium phosphate buffer alone showing a small Raman scatter peak. (This scan was subtracted from each subsequent scan). (2) Emission spectrum of donor vesicles (λ_{ex} 370 nm). (3) Addition of acceptor vesicles. Subsequent scans were recorded at 10, 30, 45, 60, 90, and 120 minutes after GLTP addition (2 μg). The dotted line represents the scan obtained after Triton X-100 addition to the reaction mixture (3.5% final Triton-X-100 conc). This scan has been corrected for the change in light scattering that occurs upon addition of Triton X-100 by subtracting a buffer plus Triton X-100 blank.

FIG. 4.

Glycolipid transfer protein mediated AV-GalCer transfer as a function of time. Anthrylvinyl emission intensity at 425 nm from the different scans was plotted relative to the intensity obtained after Triton X-100 disruption of the vesicles. The Triton X-100 scan (Fig. 3, dotted line) was used as 100% transfer. Intensities have been corrected for the small Raman scatter intensity produced in buffer alone and shown in Figure 3, curve 1.

FIG. 5.

Use of fluorescence resonance energy transfer to measure the kinetics of GLTP mediated AV-GalCer transfer between vesicles. Recording of increasing fluorescence at 425 nm (λ_{ex} 370 nm) as AV-GalCer transfers from donor to acceptor vesicles at 37°C. (A) Addition of 40 nmol donor vesicles (POPC; 0.4 nmol AV-GalCer) to sodium phosphate buffer. (B) Addition of 400 nmol acceptor vesicles (POPC). (C) Addition of 2.0 μ g bovine brain GLTP (84.4 pmol). (D) Addition of 400 nmol acceptor vesicles. (E) Addition of 2.0 μ g bovine brain GLTP. (F) Addition of 40 nmol donor vesicles. The phospholipid ethanolic solution was injected into 0.5 ml sodium phosphate buffer, which was then added to the assay. The insert describes schematically how the transfer half-time, $t_{1/2}$ can be obtained for the reaction.

FIG. 6.

Light scattering intensity measured as a function of time. Measurements were performed at 320 nm and at 37°C as described in the Methods. (A) Phosphate buffer; (B) Addition of 40 nmol of donor vesicles (ethanol injection); (C) Addition of 400 nmol of acceptor vesicles (sonicated); (D) addition of GL TP (2 µg; 84.4 pmol).