Properties of the binding sites for the sn-1 and sn-2 acyl chains on the phosphatidylinositol transfer protein from bovine brain
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ABSTRACT: We have studied the properties of the fatty acyl binding sites of the phosphatidylinositol transfer protein (PI-TP) from bovine brain, by measuring the binding and transfer of pyrenylacyl-containing phosphatidylinositol (PyrPI) species and pyrenylacetyl-containing phosphatidylcholine (PyrPC) species as a function of the acyl chain length. The PyrPI species carried a pyrene-labeled acyl chain of variable length in the sn-2 position and either palmitic acid [C(16)], palmitoleic acid [C(16:1)], or stearic acid [C(18)] in the sn-1 position. Binding and transfer of the PI species increased in the order C(18) < C(16) < C(16:1), with a distinct preference for those species that carry a pyrenyloctanoyl [Pyr(8)] or a pyrenyldecanoyl [Pyr(10)] chain. The PyrPC species studied consisted of two sets of positional isomers: one set contained a pyrenylacetyl chain of variable length and a C(16) chain, and the other set contained an unlabeled chain of variable length and a Pyr(10) chain. The binding and transfer experiments showed that PI-TP discriminates between positional isomers with a preference for the species with a pyrenylacyl chain in the sn-1 position. This discrimination is interpreted to indicate that separate binding sites exist for the sn-1 and sn-2 acyl chains. From the binding and transfer profiles it is apparent that the binding sites differ in their preference for a particular acyl chain length. The binding and transfer vs chain length profiles were quite similar for C(16)Pyr(x)PC and C(16)Pyr(x)PI species, suggesting that the sn-2 acyl chains of PI and PC share a common binding site in PI-TP.

Phospholipid transfer proteins belong to a group of soluble cytosolic proteins which presumably take part in the transport of phospholipids between the intracellular membranes (Wirtz, 1982). These proteins are able to discriminate between phospholipid classes and species (Wirtz, 1982; Kader et al., 1983; Zilversmit, 1984). It has been proposed that this property is essential for the maintenance of the phospholipid composition of the intracellular membranes (Kaplan & Simoni, 1985). In view of the important physiological function of phosphoinositides, it is of considerable interest that in all mammalian cells investigated to date a protein has been detected that binds and transfers phosphatidylinositol (PI) between membranes (Helmkamp et al., 1974; DiCorleto et al., 1979; Daum & Paltauf, 1984; George & Helmkamp, 1985). The PI transfer protein from bovine brain (PI-TP) has been studied in considerable detail [for reviews, see Wirtz (1982) and Helmkamp (1985)]. This protein preferentially transfers PI but can also transfer phosphatidylcholine (PC) (DiCorleto et al., 1985).
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Jelsema catalyzing the transport of PI from its site of synthesis, i.e., the endoplasmic reticulum (Benjamins & Agranoff, 1969; Jelsema & Morré, 1978), to the site of metabolism, e.g., the plasma membrane (Abdel-Latif, 1983; Michell, 1975; Hokin, 1985).

In order to carry out phospholipid transport, PI-TP must be able both to interact with a phospholipid membrane surface and to form a complex with a single phospholipid molecule. The effect of membrane composition on transfer activity of PI-TP has been the subject of several studies (Somerharju et al., 1983; Helmkamp et al., 1976; Zborowski, 1979; Helmkamp, 1980a,b). It was clearly established that physical membrane properties, such as charge and fluidity, have a large influence on the activity of PI-TP (Somerharju et al., 1983; Helmkamp, 1980a,b). Much less, however, is known about the properties of the binding site of PI-TP that accommodates the lipid molecule. A recent study using fluorescent parinoyl-phosphatidylcholine and PI-TP has revealed that the sn-2 acyl chain of both lipid classes is completely immobilized inside the transfer protein (Van Paridon et al., 1987a).

In the present study we have investigated the properties of the sites on PI-TP that accommodate the sn-1 and sn-2 acyl chains of PI and PC by determining both binding and transfer of the corresponding pyrenyl phospholipids. These lipids carry a pyrenylacyl chain in either the sn-1 or sn-2 position and an unlabeled acyl chain in the other position. Similar lipids have been used by others to study either passive or protein-mediated phospholipid transfer (Charlton et al., 1978; Doody et al., 1980; Roseman & Thompson, 1980; Massey et al., 1982, 1984, 1985). By varying the position and the length of the pyrenylacyl and unlabeled acyl chain, one can systematically probe the sn-1 and sn-2 acyl binding sites of the protein. The results strongly suggest that PI-TP has specific binding sites for the sn-1 and sn-2 acyl chains of PI and PC and that these sites are possibly shared by PI and PC. Similar studies have recently been carried out with the PC transfer protein from bovine liver (Somerharju et al., 1987).

Materials and Methods

Materials

Didecanoyl-, dilauroyl-, dimyristoyl-, dipalmitoyl-, and distearoyl-PC species and phosphatidase A2 from Crotalus adamanteus were obtained from Sigma. Saturated fatty acids were products of Fluka. Egg yolk PC was purified according to Singleton et al., (1965), and phosphatidic acid (PA) was prepared from this lipid by phospholipase D catalyzed hydrolysis. PI was isolated from bakers’ yeast (Trevelyan, 1966) and purified as described before (Somerharju & Wirtz, 1982). 8-Pyrenyloctanoic acid was a generous gift from Dr. J. Virtanen from KSV Chemicals (Helsinki, Finland). Other pyrenyl fatty acids were synthesized essentially as described by Gallar and Hartman (1981) and were purified by silica gel column chromatography. Diarachidoyl-PC and dipyrenyloctanoyl-PC species were prepared from the glycerophosphocholine–cadium adduct and the corresponding fatty acids (Patel et al., 1979). Lysophosphatidylethanolamines were prepared from the diacyl-PC species by phospholipase A2 treatment and purified by repeated acetone precipitation (Kates, 1972). Mixed-chain PyrPCs (or formulas, see Figure 1) were synthesized from lysophosphatidylethanolamines and fatty acids according to Gupta et al. (1977) and purified by HPLC silica column chromatography. Phosphatidylethanolamines were obtained from egg PC as described previously (Somerharju et al., 1985). TNP-PE was prepared from phosphatidylethanolamine as described by Gordensky and Marinetti (1973) and was purified by silica column chromatography. 1-Acyl-2-pyrenylacyl-PI [C(x)-Pyr(x)PI] species were synthesized from yeast PI and various pyrenyl fatty acids as described by Somerharju et al. (1985) and purified by HPLC using a silica column (Merck LiChrosorb 60). The pyrPI species were eluted with a gradient of methanol in chloroform at approximately 50% (v/v) methanol.

Figure 1: Structure and nomenclature of the PyrPI and PyrPC species. X indicates the total number of carbon units (including the carbonyl one) in the aliphatic chain. R refers to the variable unlabeled acyl chain in the sn-1 position of PyrPI. The corresponding positional isomers of PyrPC carry the pyrenylacyl chain on the sn-1 position and the unlabeled acyl chain on the sn-2 position.

The concentration of the fluorescent phospholipids was estimated by three different methods. The concentration of PyrPC species was determined by measurement of the absorbance at 342 nm in ethanol (ε = 42 000 M⁻¹·cm⁻¹) (Somerharju et al., 1985) or by phosphorous assay (Rouser et al., 1970). The concentration of PyrPI species was estimated from the absorbance at 346.3 nm in DMSO (ε = 37 000 M⁻¹·cm⁻¹) on a Hitachi U-3200 spectrophotometer. When relatively small amounts of PyrPI species were available, the concentration was determined by measuring the fluorescence intensity in DMSO (excitation at 346 nm, emission 378 nm; slits 5 and 1 nm, respectively). The fluorescence signal was calibrated with a PyrPC standard. DMSO was chosen as the solvent for PyrPI because of the poor solubility of PyrPI species in ethanol, which gave rise to distorted absorption spectra.

Preparation of Phospholipid Vesicles. Single bilayer donor vesicles containing fluorescent phospholipids were prepared in the cuvette by injecting the appropriate lipids dissolved in ethanol (5–10 µL) into 2 mL of 20 mM Tris-HCl/5 mM EDTA/100 mM NaCl (pH 7.4) (Batzi & Korn, 1973). When PI was present in the donor lipid mixture, the lipids were first dissolved in a small volume of DMSO (Merck, Uvasol), before the ethanol was added. The DMSO concentration was always lower than 25%. After the injection of the donor lipid
solution, the donor vesicles were equilibrated for 2 min prior to the start of the binding or transfer experiments. The single bilayer character of the vesicles was established (see Results). To prepare the acceptor vesicles for the transfer assay, the lipids were dried down from a chloroform solution, dispersed in the Tris/EDTA/NaCl buffer and sonicated for 10 min on ice with a Branson probe sonicator (60-W output). Titanium particles and any undispersed lipid were removed by centrifugation (8000 rpm for 20 min).

**Fluorescence Measurements.** The fluorescence measurements were carried out on a SLM-Aminco SPF-500C spectrophotometer equipped with a thermostated cuvette holder and a magnetic stirring device. Excitation was at 346 nm (slit 2–4 nm). The pyrene monomer fluorescence intensity was monitored at 378 nm (slit 10 nm). All experiments were carried out at 37 °C.

**Phospholipid Binding Assay.** Binding of PyrPI and PyrPC by PI-TP was determined by titrating quenched vesicles consisting of PyrPC, TNP-PE, PA, and egg PC (10:10:30:50 mol %; 2 nmol of total lipid) with PyrPI, TNP-PE, yeast PI, and egg PC (10:10:30:50 mol %; 2 nmol of total lipid) with PyrPC and PI-TP (aliquots of 1 μg, see arrows). Curve C represents a titration in the absence of vesicles. The increments of fluorescence yield as a function of the amount of PI-TP added are presented in the insert.

To prepare the acceptor vesicles for the transfer assay, the solution, the donor vesicles were equilibrated for 2 min prior to the start of the binding or transfer experiments. The single bilayer character of the vesicles was established (see Results). The increments of fluorescence yield as a function of the amount of PI-TP added are presented in the insert.

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**RESULTS**

**Fluorescent Lipid Distribution in Donor Vesicles.** In order to be able to compare binding and transfer of fluorescent PC and PI species, it is necessary that the vesicle bilayer distribution is known. To establish this distribution, vesicles were prepared from pure C(16)Pyr(10)PC by ethanol injection. These vesicles only exhibit pyrene excimer fluorescence (λmax = 480 nm, see Figure 3). Incubation of these donor vesicles with a 100-fold excess of acceptor vesicles in the presence of PI-TP resulted in transfer of PyrPC molecules to the acceptor vesicles. This transfer is reflected in a time-dependent increase of the pyrene monomer fluorescence (λmax = 378 nm) and a concomitant decrease of the excimer fluorescence in the donor vesicles. As one sees from Figure 3, an equilibrium is reached at which the excimer fluorescence remains constant at 37% of the original value. This value represents the amount of PyrPC in the inner vesicle monolayer, in agreement with the earlier observations that ethanol injection yields small single bilayer vesicles (Batzri & Korn, 1973; Kremer et al., 1977). Disruption and total mixing of the donor and acceptor vesicle lipids by addition of Triton X-100 (final concentration of 0.025%) completely abolished the excimer fluorescence and
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Triton X-100 micelles as compared to that in vesicles (Levade et al., 1987). The donor vesicles used in the present study consisted of a mixture of pyrene-labeled and unlabeled phospholipids and the internal quencher TNP-PE (see Methods). In this case neither monomer nor excimer fluorescence is observed. Transfer experiments similar to the one described above were carried out with donor vesicles consisting of the C(16)Pyr(x)PC and C(16:1)Pyr(x)PI species. From the fluorescence yield at equilibrium relative to the yield obtained after addition of Triton X-100, it was calculated that the amount of PyrPC and PyrPI, available for transfer, was approximately 60% (see Figure 4). This indicates that the ethanol injection method yields single bilayer vesicles in which the pyrene-labeled phospholipids, independent of acyl chain length and polar head group, display a similar distribution over the bilayer.

Binding and Transfer of PyrPI Species. Three sets of PyrPI species carrying a pyrenylacyl chain of variable length in the sn-2 position and a C(16), C(16:1), or C(18) acyl chain in the sn-1 position (see Figure 1) were used to study the effects of acyl chain modification on binding and transfer of PI by PI-TP. In all binding and transfer experiments reported here, the fraction of the pyrenyl lipid in the donor vesicles was small (10 mol %) to ensure a minimal perturbation of the vesicle membrane by the presence of the pyrene moieties. The binding of PI was strongly dependent on the length of the pyrenylacyl chain, with the highest affinity for the species carrying Pyr(8) or Pyr(10) chains (Figure 5A). Variation of the unlabeled chain in the sn-1 position also had a marked effect on binding. Elongation of the C(16) chain by two methylene units diminished the affinity by a factor of 2 or more. On the other hand, the introduction of a double bond into the C(16) chain enhanced binding significantly.

The rates of transfer of the PyrPI species were also found to be dependent on the length of both the labeled chain in the sn-2 position and the unlabeled chain in the sn-1 position (Figure 5B). For all three sets of species, the shapes of the transfer rate vs chain length profiles were quite similar to the binding profiles, although the optima appear to have shifted slightly toward longer pyrenyl chains. It is of note that, in contrast to the binding experiments, the C(16)Pyr(x)PI and C(16:1)Pyr(x)PI species behaved quite similarly in the transfer experiments.

Binding of PyrPC Species. In a previous study (Van Paridon et al., 1987a) it was demonstrated that similar to PI the sn-2 acyl chain of PC was strongly bound to PI-TP, despite the fact that this protein has a relatively low affinity for PC (Demel et al., 1977). To further explore the characteristics of the acyl binding sites of PI-TP, the binding of various PyrPC species was determined. To study the effect of increasing the length of the pyrenylacyl chain on the binding affinity, two sets of PyrPC species were used. The lipids of the fist set [C(16)Pyr(x)PC] contained a C(16) acyl chain in the sn-1 position and a pyrenylacyl chain in the sn-2 position. The second set [Pyr(x)C(16)PC] consisted of the positional isomers of the first set (see Figure 1). The results from these binding experiments are shown in Figure 6A. Clearly, the binding of the fluorescent lipids is strongly dependent on the length of the pyrenylacyl chain in both the sn-1 and sn-2 positions. Furthermore, markedly different affinity vs chain length

FIGURE 4: Amount of PyrPC and PyrPI in the outer monolayer of single bilayer donor vesicles as a function of pyrenylacyl chain length. Lipid transfer was measured between donor and acceptor vesicles in the presence of PI-TP until an equilibrium was reached. For details see Methods. (●) C(16)Pyr(x)PC; (▲) C(16:1)Pyr(x)PI; (■) C(18)Pyr(x)PI.

enhanced the total monomer fluorescence 2.8 times instead of the anticipated 1.6 times. This apparent discrepancy is due to the higher monomer fluorescence yield of PyrPC in mixed Triton X-100 micelles as compared to that in vesicles (Levade et al., 1987). The donor vesicles used in the present study consisted of a mixture of pyrene-labeled and unlabeled phospholipids and the internal quencher TNP-PE (see Materials and Methods). This indicates that the ethanol injection method yields single bilayer vesicles in which the pyrene-labeled phospholipids, independent of acyl chain length and polar head group, display a similar distribution over the bilayer.

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FIGURE 5: Effect of acyl chain composition on binding and transfer of PyrPI species by PI-TP. (A) Binding of C(16)Pyr(x)PI (●) and C(16:1)Pyr(x)PI (▲); x varied from 6 to 14 carbon atoms. Binding of PyrPI species was measured by titration of quenched PyrPI/TNP-PE/yeast PI/egg PC (0.2/0.2/0.4/1.2 nmol) donor vesicles with PI-TP as described under Materials and Methods. (B) Transfer of PyrPI species by PI-TP. Transfer was measured from quenched donor vesicles (composition and concentration identical with those of the binding experiments) to an excess of egg-PC/PA (85/15 nmol) acceptor vesicles as described under Materials and Methods.

transfer rate vs chain length profiles were quite similar to the binding profiles, although the optima appear to have shifted slightly toward longer pyrenyl chains. It is of note that, in contrast to the binding experiments, the C(16)Pyr(x)PI and C(16:1)Pyr(x)PI species behaved quite similarly in the transfer experiments.

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FIGURE 6: Effect of acyl chain length on the binding of PyrPC species by PI-TP. (A) Binding of Pyr(x)C(16)PC (●) and C(16)Pyr(x)PC (▲); x varied from 6 to 14 carbon atoms. (B) Binding of C(x)Pyr(10)PC (▲) and Pyr(10)(x)PC (■); x varied from 10 to 20 carbon atoms. Binding of PyrPC species was measured by titration of quenched PyrPC/egg PC/PA-/TNP-PE (0.4/2.1/2.0/2.0 nmol) donor vesicles with PI-TP as described under Materials and Methods.
In this study we have investigated the structural properties of the acyl binding sites of PI-TP. Earlier studies have shown a strong influence of the fatty acyl composition of PC on the transfer activity of PI-TP (Zborowski, 1979; Helmkamp, 1980a). However, in these studies it remains unclear to what extent these effects can be attributed to alterations in the physical properties of the membranes. It has been clearly demonstrated that, for instance, membrane fluidity strongly influences PI-TP transfer activity (Helmkamp, 1980b). Here we have used pyrenyl phospholipids to investigate the acyl chain specificity of the lipid binding site of PI-TP. The use of these fluorescent phospholipids provides simple and very sensitive methods to measure both lipid transfer (Charlton et al., 1978; Doody et al., 1980; Roseman & Thompson, 1980) and binding (Thompson, 1982). Thus far, most studies on the specificity of PI-TP have relied on the measurement of rates of transfer (Demel et al., 1977; DiCorleto et al., 1979; Kasper et al., 1980). However, in these studies it remains unclear to what extent these effects can be attributed to alterations in the physical properties of the membranes. It has been clearly demonstrated that, for instance, membrane fluidity strongly influences PI-TP transfer activity (Helmkamp, 1980b). Here we have also measured lipid binding to assess the affinity of PI-TP for a certain lipid species. Since the determination of binding is an equilibrium measurement, it is much less sensitive toward the physical properties of the membranes involved.

The binding and transfer curves for PyrPC positional isomers (Figures 6 and 7) strongly suggest that PI-TP has specific binding sites for both acyl chains. This decrease could be explained by assuming that long acyl chains are poorly accommodated in the hydrophobic acyl binding site. On the other hand, long acyl chains may have also measured lipid binding to assess the affinity of PI-TP for PC. The use of these fluorescent phospholipids provides simple and very sensitive methods to measure both lipid transfer and binding (Thompson, 1982). Thus far, most studies on the specificity of PI-TP have relied on the measurement of rates of transfer (Demel et al., 1977; DiCorleto et al., 1979; Kasper & Helmkamp, 1981; George & Helmkamp, 1985). Here we have also measured lipid binding to assess the affinity of PI-TP for a certain lipid species. Since the determination of binding is an equilibrium measurement, it is much less sensitive toward the physical properties of the membranes involved.

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energetically favor the presence of this PC molecule in the bilayer. Spontaneous monomer transfer between phospholipid membranes gradually decreases with increasing chain lengths, possibly due to this lower energy level (Massey et al., 1982, 1984). Closely similar binding profiles were observed for C(x)Pyr(10)PC and Pyr(x)C(16)PC species and also for Pyr(10)C(x)PC and C(16)Pyr(x)PC (Figures 6A and 8). This is particularly evident for the sn-1 position. Apparently, the pyrenylacyl chain and the unlabeled acyl chain behave quite similarly toward the two binding sites on PI-TP. Specifically, the contribution of the pyrene moiety approximately equals that of four methylene units. This correlation could also be derived from studies on the passive transport of PyrPC species (Massey et al., 1984, 1985).

A correlation between the binding and transfer profiles was apparent although distinct differences were observed (see Figures 6 and 7). We assume that these differences are due to the fact that, in contrast to binding, which is an equilibrium measurement, transfer is not just determined by the specificity of the acyl binding sites but also by kinetic parameters (i.e., activation energy). In general, the transfer optima appear to be shifted toward longer acyl chains as compared to the binding optima. For example, the fact that Pyr(12)C(16)PC is optimally transferred (Figure 7A), while the binding affinity of this lipid is relatively low (Figure 6A), suggests that somehow the activation energy barrier involved in the exchange process of this PC species is relatively small. This is in contrast with the effect of chain length on the passive PC transfer rates (Massey et al., 1984, 1985). It is possible that the activation energy is strongly dependent on the interaction of the acyl chains with the lipid binding site.

Comparison of the binding and transfer profiles for the C(16)Pyr(x)PC and C(16)Pyr(x)PI species (Figure 8) demonstrates a strong resemblance with respect to the sn-2 acyl chain specificity for both phospholipid classes. This suggests that the sn-2 acyl chains of PC and PI may be accommodated in the same binding site. A similar conclusion was drawn from time-resolved fluorescence anisotropy measurements on 2-parinaroyl-PC and -PI in the lipid-transfer protein complex (Van Paridon et al., 1987a). Elongation of the C(16) acyl chain in the sn-1 position to C(18) reduces the binding affinity for both PC and PI (Figures 5A and 6B). This suggests that possibly also the sn-2 acyl chains of both lipid classes share a common acyl binding site. The difference between optimally bound and poorly bound (transferred) species is larger for PC than for PI. This may be due to the fact that the binding affinity is also dependent on the phospholipid head group–protein interactions, which are approximately 15-fold stronger for PI than for PC (Van Paridon et al., 1987b). In other words, the fractional contribution of the acyl chains to the binding affinity could be much larger in the case of PC. This would then imply that an identical change in acyl chain affinity has a larger effect on the binding affinity for PC than for PI.

The affinity constants determined for C(16)Pyr(10)PC and C(16)Pyr(10)PI compare very well to those of egg PC and yeast PI, respectively (Van Paridon et al., 1987b). Hence, we think that extrapolation of the results from this study to unlabeled natural PC and PI species is allowed. The most abundant species of PI in mammalian tissues is C(18)C(20:4)PI (Holub & Kuksis, 1978). Here we have clearly shown that the C(18) acyl chain in the sn-1 position and a C(20) acyl chain in the sn-2 position is not favored by PI-TP. However, because of the occurrence of four double bonds in the arachidionate chain, it remains to be determined whether or not this PI species is efficiently transferred by PI-TP. We feel that the major differences observed in the affinity of PI-TP for various PI species warrants further investigation, particularly in view of the proposed role of PI-TP in the intracellular traffic of PI (Helmkamp, 1985).

Binding and transfer of PyrPC species by PC-TP have also been investigated (Sommerharju et al., 1987). Whereas PC-TP preferentially binds and transfers PyrPC species with the pyrenylacyl chain in the sn-2 position, PI-TP has a reversed isomer preference. This difference in behavior clearly illustrates the influence of the acyl binding site on the protein-mediated lipid transfer. Studies with the nonspecific lipid transfer protein have shown that this protein has an acyl chain dependence which is very similar to the passive transport profile (Van Amerongen et al., unpublished data). These observations support the hypothesis that this protein acts by facilitating the transfer of lipid molecules between membrane interfaces without actually binding the lipids (Thompson, 1982; Nicholls & Pagano, 1982). Variation of the acyl chain length has different effects on PI-TP and PC-TP. Binding of PyrPC species by PC-TP appears to be more sensitive toward steric factors, such as the position of the bulky pyrene moiety on the acyl chain, while binding by PI-TP seems to be more strongly influenced by the hydrophobicity of the individual acyl chains.

References
Spectroscopic and Functional Characterization of an Environmentally Sensitive Fluorescent Actin Conjugate

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ABSTRACT: Rabbit skeletal muscle F-actin has been selectively labeled at a cysteine residue with the environmentally sensitive fluorophore 6-acryloyl-2-(dimethylamino)naphthalene. The fluorescent actin conjugate behaves similarly to native actin with respect to the polymerization kinetics, critical monomer concentration, and ability to form F-actin paracrystals. Upon polymerization to F-actin, the absorption of the actin conjugate is red-shifted, whereas the fluorescence emission is blue-shifted 740 wavenumbers and is accompanied by a decrease in the fluorescence bandwidth of 470 wavenumbers. These large shifts in the spectral properties of 6-propionyl-2-(dimethylamino)naphthalene (Prodan) in actin provide a simple method for obtaining a spectral discrimination between the G- and F-actin populations during the polymerization reaction. Steady-state fluorescence techniques were used to study the environment of the fluorophore in the monomeric and polymeric forms of actin. Fluorescence emission spectral analysis and quenching and polarization studies of G-actin—Prodan indicated that the fluorophore lies immobile on the protein surface but with one of its faces in full contact with the solvent. In F-actin, the fluorophore has a limited exposure to the solvent and is located in a dielectric environment similar to those seen for Prodan in polar, aprotic solvents or buried within a protein matrix [Macgregor, R. B., Jr., & Weber, G. (1986) Nature (London) 318, 70–73]. Additionally, our results demonstrate that the Prodan molecule conjugated to F-actin is completely immobile during its fluorescence lifetime, exhibits an increase in the resonance energy transfer (RET) from tryptophan residues compared to that observed in G-actin, and shows evidence of homologous RET within the polymer.

Numerous fluorescence spectroscopic techniques have been used to study structure–function relationships of actin and actin binding proteins (Cooper & Pollard, 1982; Frieden, 1985). Conformational transitions in the actin monomer during polymerization or ligand binding are often accompanied by a change in some fluorescence property of an intrinsic or extrinsic fluorophore. Virtually all fluorescence properties can be affected: the fluorescence quantum yield; the polarization of the fluorescence; the decay rate and/or rotational rate of the...