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FLUORESCENT STUDIES ON THE INTERACTION BETWEEN A NOVEL Ca^{2+} ANTAGONIST, SR 33557, AND MEMBRANE LIPIDS

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Abstract—The fluorescent properties of SR 33557, a novel calcium entry blocker, have been characterized in solution and when interacting with phospholipid vesicles and natural membranes. The intensity and lifetime of fluorescence emission increased directly as a function of the decrease in the solvent dielectric constant (ε) with no change in the emission wavelength maxima. The quantum yield and the fluorescence lifetime in dioxane were 0.90 and 11.1 nsec, respectively. In various vesicles and in erythrocyte ghosts, SR 33557 was shown to be located in a lipidic environment corresponding to an ε of $30 \simeq 40$. At 25°, the dissociation constant (K_d) was $1.1–7.3 \times 10^{-6}$ M with a maximum of 15 SR 33557 molecules bound per 100 phospholipid molecules. When interacting with phospholipids, SR 33557 exhibited two fluorescence lifetimes (approximately 13 and 4 nsec) with the fractional contribution of 81 and 19% of the total decay, respectively. Binding of SR 33557 was enthalpy-driven. Both intensity of fluorescence emission and of fluorescence polarization of SR 33557 were indices of phase transitions in phospholipid vesicles. Thus, SR 33557 has fluorescent properties which may be of use in the study of its mode of action in biological media.

Drugs acting on calcium channels have been studied extensively following the initial observations of Fleckenstein *et al.* [1] with verapamil and Godfraind and Kaba [2] with cinnarizine. Although there are commonly considered to be three principal classes of drug binding site at the L-type calcium channel, there is now increasing evidence to suggest that there are a variety of binding sites [3]. These binding sites correspond to new ligands chemically unrelated to each other [3].

Among these new compounds, the indolizine sulfone SR 33557 has been shown to be a potent antagonist of the L-type calcium channel [4–7]. This compound is a potent antagonist of potassium-induced contraction in rat aorta with an IC_{50} value of approximately 6 nM [4, 6] and binds to the L-type calcium channels at a specific binding site with high affinity (K_d values between 0.08 and 0.037 nM) [4, 5, 7]. The pK_a of SR 33557 is 8.7.

This paper characterizes the fluorescence properties of SR 33557 in solution and in interaction with lipids. The fluorescent properties in solution are characterized mainly by a decrease in quantum yield and the absence of maximum emission shift as a function of the increase in the solvent dielectric constant. With regard to lipid vesicles and membrane, the fluorescence properties of SR 33557 permitted the determination of the localization of the compound in the bilayer, its affinity for phospholipids and the number of binding sites. The binding of SR 33557 is sensitive to temperature in that it can probe phase transitions of the bilayer.

MATERIALS AND METHODS

Phospholipids [egg phosphatidylcholine (PC†), dioleyl-DL-α-phosphatidylcholine (DOPC), dimyristoyl-DL-α-phosphatidylcholine (DMPC), dipalmitoyl-DL-α-phosphatidylcholine (DPPC)] were purchased from the Sigma Chemical Co. (St Louis, MO, U.S.A.). The fluorescent probes [1,6-diphenyl-1,3,5-hexatriene (DPH), 1-(4-trimethylammoniumphenyl)-6-phenyl-1,3,5 hexatriene (TMA-DPH), 1-anilino-naphthalene-8-sulphonic acid (ANS)] were purchased from either Sigma or Molecular Probes (Junction City, OR, U.S.A.). SR 33557 [2-isopropyl-1-((4-(3-(*N*-methyl-*N*-(3,4-dimethoxy-β-phenethyl)amino)propyloxy)benzenesulphonyl))-indolizine] (Fig. 1) was obtained from the Sanofi Chemical Department. All other chemicals were analytical reagent grade from commercial sources.

Vesicle preparation. Multilamellar vesicles (MLVs) were prepared by vortexing a film of dry lipids deposited on the wall of a glass vessel in the presence of the appropriate buffer (Tris–HCl, 20 mM, pH 7.4) at room temperature or at a temperature above the main phase transition temperature of the phospholipid [8]. The resulting MLV were then freeze-thawed five times employing alternative liquid nitrogen and warm water cycles [9]. The MLV were subsequently transferred in a device (Lipex Biomembranes Inc., Vancouver, Canada) which allowed the extrusion by eight successive passes through 0.1 μM pore size polycarbonate filters (Nucleopore) [9, 10].

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† Abbreviations: SR 33557, 2-isopropyl -1- ((4-(3-(*N*-methyl-*N*-(3,4-dimethoxy-β-phenethyl)amino)propyloxy)-(benzenesulphonyl))indolizine; PC, phosphatidylcholine; DDPC, dioleyl-DL-α-PC; DMPC, dimyristoyl-DL-α-PC; DPPC, dipalmitoyl-DL-α-PC; DPH,1,6-diphenyl-1,3,5-hexatriene; TMA-DPH, 1-(4-trimethylammoniumphenyl)-6-phenyl-1,3,5 hexatriene; ANS, 1-anilino-naphthalene-8-sulphonic acid; MLV, multilamellar vesicles.

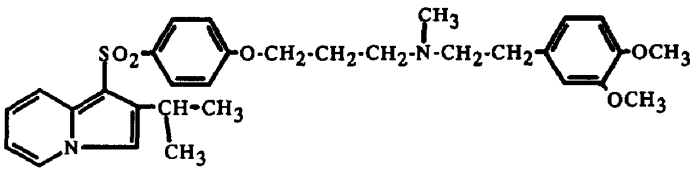

Fig. 1. Chemical structure of SR 33557.

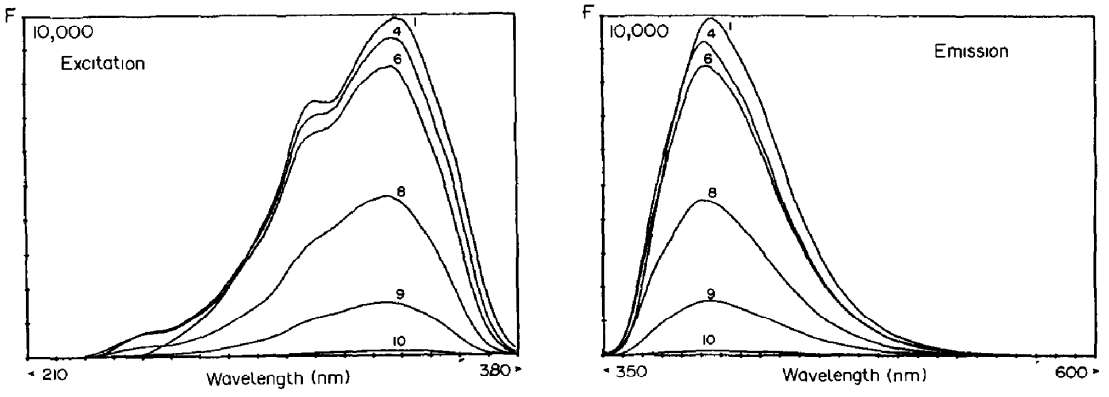

Fig. 2. Excitation (left panel) and emission (right panel) fluorescence spectra of SR 33557 solutions. The successive spectra correspond to: 1, dioxane; 4, *n*-propanol; 6, methanol; 8, ethanol/water 50/50; 9, ethanol/water 30/70; 10, water. The spectra were recorded under identical conditions using 10^{-5} M SR 33557 solutions.

Erythrocyte ghosts. Blood was collected in heparinized tubes from mongrel dogs. Erythrocyte ghosts were prepared according to Dodge *et al.* [11]. Total phospholipids were extracted [12] and vesicles were prepared as described above. Phospholipid content of the ghosts and the vesicles was determined [13] to adjust the final concentration to the values shown in Figs 4–6.

Measurement of fluorescence properties. All fluorescence measurements were obtained using either a scanning spectrofluorometer or a sub-nanosecond phase fluoremeter both from SLM Instruments Inc. (Urbana, IL, U.S.A.). These measurements were carried out using monochromators in both the emission as well as the excitation channels. Fluorescence lifetimes (τ) were calculated by the phase shift and modulation methods using modulation frequencies of 6, 18 and 30 MHz [14]. A solution of 2,2′ *p*-phenylenebis(5-phenyl)oxazole in absolute ethanol (τ = 1.35 nsec) or DPH in heptane (τ = 6.72 nsec) was used as reference. The quantum yields (Q) were determined according to Melhuish [15] using quinine sulfate (Q = 0.55) dissolved in 0.1 N sulfuric acid. The polarization measurements were performed as described previously [8] using the appropriate set of excitation and emission wavelengths (DPH and TMA-DPH: λ_{ex} = 356 nm, λ_{em} = 450 nm, ANS: λ_{ex} = 380 nm, λ_{em} = 471 nm). The steady-state fluorescence polarization (P) is defined as follows:

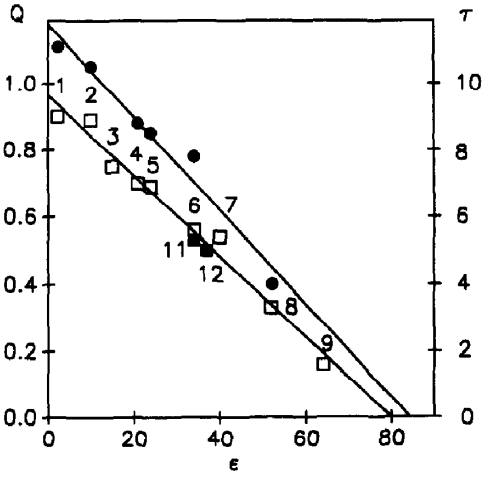

Fig. 3. Quantum yield Q (□, ■) and fluorescence lifetime τ (●) of SR 33557 as a function of the dielectric constant ε. The open squares correspond to quantum yield measured in the solvent defined in Table 1, the closed squares, 11 and 12, correspond to dog erythrocyte ghost and vesicles made of the total lipid extracted from the dog erythrocyte ghosts, respectively. The closed circles correspond to fluorescence lifetimes determined in the solvent defined in Table 1. The value of τ is expressed in nsec.

$$P = \frac{I_{\parallel} - I_{\perp}}{I_{\parallel} + I_{\perp}}$$

where $I_{\parallel}$ and $I_{\perp}$ are the intensities of the light emitted with its polarization plane parallel ($\parallel$) and perpendicular ($\perp$) to that of the exciting beam. All the fluorescence measurements were carried out in 1×1 cm quartz cuvettes fitted with teflon stoppers at 25° unless otherwise stated.

Calculations. The fluorescence lifetime (τ) of a fluorophore is defined by:

$$\tau = \frac{1}{k_e + k_{nr}}$$

where k_e is the emission rate constant and k_{nr} are the rate constants of all other non-radiative excited state deactivation processes. When $k_e \gg k_{nr}\tau$ approaches its upper limit defined as τ_o, the intrinsic lifetime, $\tau_o = 1/k_e$. The quantum yield (Q) of fluorescence is defined by:

$$Q = \frac{k_e}{k_e + k_{nr}}.$$

For substrates like SR 33557, which fluoresces much more intensely when bound than when free, the concentration of bound SR 33557 at each point was calculated using the following equation [16]:

$$\text{SR 33557}_b = \frac{F_o}{F_m} \times 30$$

where SR 33557_b is the concentration of bound SR 33557, F_o is the observed fluorescence and F_m is the maximum fluorescence of a solution containing 30 μM SR 33557. The maximum fluorescence of the solution of SR 33557 was determined by titration with the lipid vesicles at a wide range of concentrations (0.05–1.5 mg lipid/mL). Saturations isotherms were analysed by a computer-assisted method of non-linear regression, based on the Clark equation [17]:

$$[B] = \frac{B_{max}[F]}{K_d + [F]}.$$

The density of the sites (B_{max} expressed as the number of bound SR 33557 molecules per 100 lipid molecules) and the equilibrium dissociation constant (K_d expressed in molar terms) were calculated, taking into account the experimental values of B (specifically bound ligand) and of F (free ligand).

Determination of thermodynamic parameters. Equilibrium thermodynamic binding parameters were determined utilizing classical thermodynamic equations [18]. The standard Gibbs free energy change ($\Delta G°$) of association was calculated from the equation $\Delta G° = RT \ln K_a$, where R is the gas constant (8.31 J/mol.°K), T is the temperature in degrees Kelvin and K_a is the equilibrium association constant ($1/K_d$). The standard enthalpy change ($\Delta H°$) was calculated from van't Hoff plots of the dependence of K_a on temperature between 15 and 47° (288–320°K). The slope of a van't Hoff plot ($\ln K_a$ vs $1/T$) equals $-\Delta H°/R$. The standard entropy change ($\Delta S°$) was calculated from the equation

Table 1. Spectral properties of SR 33557 solutions

No.	Solvent	ε*	λ_{ex} (nm)	λ_{em} (nm)	Q	τ (nsec)	$k_e \times 10^7$ (sec^{-1})	$k_{nr} \times 10^7$ (sec^{-1})
1	Dioxane	2.2	338	406	0.90	11.1	8.11	0.90
2	*n*-Octanol	10	337	403	0.89	10.5	8.48	1.05
3	Cyclohexanol	15	340	403	0.75	ND	—	—
4	*n*-Propanol	21	340	402	0.71†	8.8	8.07	3.30
5	ETOH	24	336	403	0.69	8.5	8.12	3.65
6	Methanol	34	337	403	0.56	7.8	7.18	5.64
7	H_2O/ETOH (30/70)	40	336	402	0.54	ND	—	—
8	H_2O/ETOH (50/50)	52	336	400	0.33	4.0	8.25	16.75
9	H_2O/ETOH (70/30)	64	334	404	0.16	ND	—	—
10	H_2O	80	334	405	0†	ND	—	—

ETOH, ethanol.
* Taken from Ref. 19.
† Calculated.
ND, not determined.

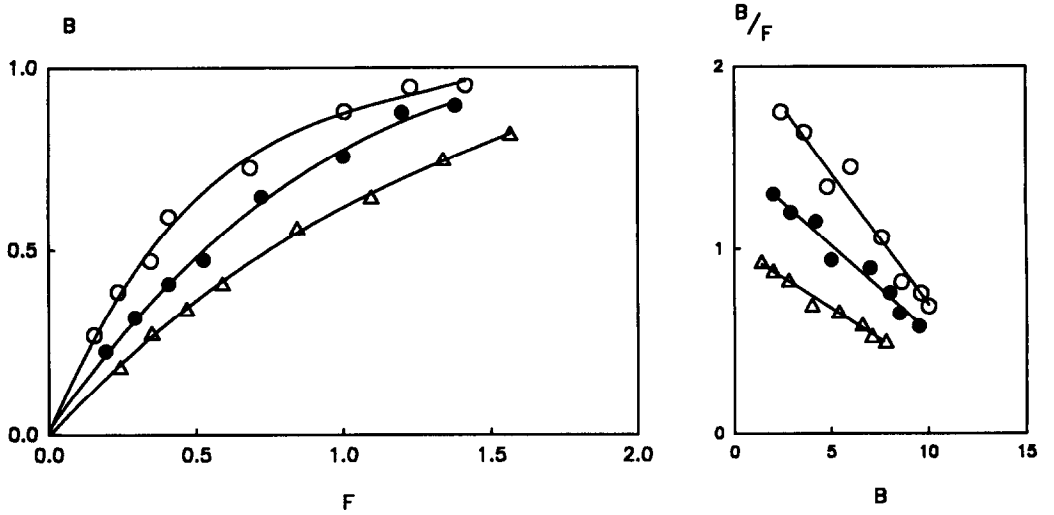

Fig. 4. Adsorption isotherm (left panel) and Scatchard representation (right panel) of the binding of SR 33557 to egg PC vesicles (○). Effect of NaCl 134 mM (●) or $CaCl_2$ 10 mM (Δ) on the binding parameters. Experimental conditions: SR 33557, 4×10^{-6}–3×10^{-5} M; egg PC, 1.3×10^{-4} M; buffer, Tris–HCl, 20 mM, pH 7.4; temperature 25°. The data are the means of 3–4 determinations.

Table 2. Effect of the phospholipid structure and temperature on the characteristics of binding of SR 33557

Phospholipids		Temperature (°C) 15	25	47
Egg PC	K_d*	5.0 ± 1.5	7.3 ± 0.3	10.0 ± 2.0
	N†	14.2 ± 2.9	14.9 ± 2.9	12.7 ± 0.9
DOPC	K_d	3.2 ± 0.3	4.7 ± 0.6	11.0 ± 1.0
	N	15.7 ± 0.7	15.9 ± 2.2	14.5 ± 1.5
DMPC	K_d	2.7 ± 0.3	4.8 ± 0.3	16.0 ± 3.0
	N	14.5 ± 0.1	12.4 ± 1.2	17.2 ± 2.4
DPPC	K_d	ND	ND	17.0 ± 4.0
	N	ND	ND	12.5 ± 1.2

* $K_d \times 10^{-6}$ M.

† N, number of SR 33557 molecules bound per 100 phospholipid molecules.

The data are the means ± SD of 2 to 6 experiments.

ND, not determined.

$\Delta G° = \Delta H° - T\Delta S°$ after first determining $\Delta G°$ and $\Delta H°$ as described above.

RESULTS

The solubility of SR 33557 in media as different as dioxane and water is sufficient for spectroscopic studies. In Fig. 2, a series of technical fluorescence spectra shows that both excitation and emission intensity varies with the solvent without change in the position of the maxima. The effects of the solvent are summarized in Table 1. SR 33557 displays little fluorescence in water. For solvents of medium polarity, the quantum yield Q is slightly above 0.5. For the less polar solvent, the quantum yield Q is close to unity. The relationship between the quantum yield Q and the solvent dielectric constant ε corresponds to a straight line (Fig. 3) $y = 0.967 - 0.012x$, $r = 0.99$, N = 9. The fluorescence lifetime (τ) of the excited state of SR 33557 has been measured under the same experimental conditions (Table 1). In all the solvents, a single value for the fluorescence lifetime has been obtained. As illustrated in Fig. 3, the fluorescence lifetime increases with the decrease in the solvent dielectric ε according to a linear relationship ($y = 11.79 - 0.14x$, $r = 0.98$, N = 6). From these results (Table 1 and Fig. 3), it is clear that the quantum yield Q and the fluorescence lifetime τ are directly related to a factor which is close to unity.

Incubation of SR 33557 with lipid vesicles led to a rapid increase in fluorescence. Since the compound is not fluorescent in water (Fig. 2, Table 1), the fluorescence is due to the adsorption of SR 33557 to the vesicles. The shape and the position of the maxima of both excitation and emission spectra are not modified when compared to the reference spectra in organic solvent. From the comparison of the intensity of emitted fluorescence and of quantum yield recorded when SR 33557 is totally bound to the vesicles and in solution (Fig. 2), it can be deduced that SR 33557 is located in an environment with a dielectric constant of approximately 35.

Incubation of a fixed amount of vesicles with various concentrations (8×10^{-7} M – 3×10^{-5} M) of SR 33557 led to an adsorption isotherm (Fig. 4, left panel). Analysis of the isotherms indicated that SR 33557 binds to a single site as illustrated by the straight line in the Scatchard representation (Fig. 4, right panel). When the vesicles were composed of egg PC, the equilibrium dissociation constant K_d and density of sites B_{max} were 7.3 ± 0.3 10^{-6} M and 14.9 ± 2.9 molecules of SR 33557 bound per 100 phospholipids, respectively (mean ± SD, N = 6).

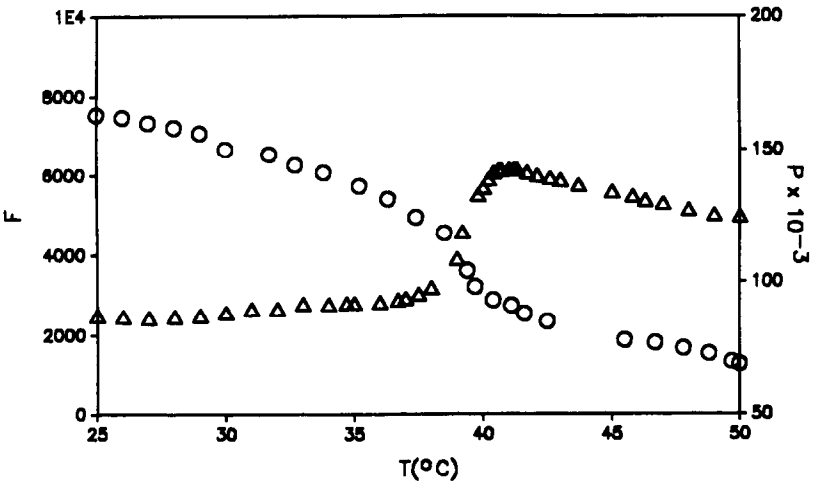

Fig. 5. Effect of the temperature on the intensity of fluorescence emission (F, left ordinate Δ) and the degree of fluorescence polarization (P, right ordinate O) of SR 33557 interacting with DPPC vesicles. Experimental conditions: SR 33557, 10^{-5} M; DPPC, 1.3×10^{-4} M; buffer, Tris–HCl, 20 mM, pH 7.4.

Incubation with 134 mM NaCl or 10 mM $CaCl_2$ (Fig. 4) had no significant effect on the B_{max} values which were 16.9 ± 2.3 and 19.0 ± 2.2, respectively, but increased the K_d values which were 12.0 ± 0.5 ($P < 0.05$) and 21.0 ± 1.0 ($P < 0.01$), respectively. At a given temperature, within a homologous series of PCs, changing the chemical nature of the phospholipid had no effect on K_d and B_{max} values (Table 2). On the other hand, modification of the temperature had an influence on K_d while B_{max} remained fairly constant (Table 2). As shown from the K_d values determined at three different temperatures, K_d increased when the temperature increased: thermodynamic parameters could be calculated from the data shown in Table 2. A Van't Hoff plot of the dependence of SR 33557 affinity on temperature was linear over the temperature range examined for binding to vesicles made of a variety of PCs. The calculated values of $\Delta G°$, $\Delta H°$ and $\Delta S°$ were −30.0 kJ/mol, −29.4 kJ/mol and +2 J/mol.°K, respectively.

When phospholipid vesicles were incubated at various temperatures, a decrease in fluorescence intensity was observed with increasing temperature. This was expected from the data shown in Table 2 indicating a decrease in affinity and no change in the number of binding sites. In the case of phospholipids having no transition temperature in the interval studied, as is the case of egg PC and DOPC, the decrease in fluorescence intensity was monotonic. However, if the phospholipid showed a phase transition temperature due to its chemical structure (DMPC and DPPC), the decrease in fluorescence presented a discontinuity. This is shown in Fig. 5 in the case of DPPC. A sharp increase in fluorescence intensity is observed around 41° which corresponds (for that phospholipid) to the main phase transition temperature (T_m), i.e. from the gel to the liquid-crystalline phase. A small decrease in fluorescence is also seen around 34°. This corresponds to the so-called pretransition phase, i.e. a phase in which the surface of the bilayer is rippled and presents a wave-like appearance [20].

When SR 33557 is excited with polarized light, the degree of fluorescence polarization (P) which can be calculated from the emitted light had a similar behaviour (Fig. 5). However, below 40° when the phospholipids were in the gel state, P was low corresponding to a restricted motion. In contrast, above 42° when the phospholipids were in the liquid-crystalline state, P was high corresponding to more motional freedom. A slight variation in P was seen at approximately 34° corresponding to the pretransition phase discussed above. Thus, the evolution of P using SR 33557 as fluorescent probe permitted the detection of the main and pretransition of phospholipids vesicles. The degree of P for SR 33557 was compared to that of DPH, TMA-DPH and ANS in both liquid-crystalline and gel states using DPPC vesicles at 25° and 45° (Fig. 6). SR 33557 has a behaviour comparable to that of the other probes. The order of sensitivity was shown to be DPH > TMA-DPH > ANS > SR 33557. In the gel state, SR 33557, ANS and DPH display a comparable degree of P.

The study of the binding of SR 33557 was extended to natural membranes using erythrocyte ghosts as model membranes. Incubation of erythrocyte ghosts with increasing amounts of SR 33557 led to an absorption isotherm. Scatchard representation was linear, indicating one single binding site. At 25°, the calculated binding characteristics were $K_d = 1.1 \pm 0.4$ 10^{-6} M and $B_{max} = 15.8 \pm 1.5$ SR 33557 molecules bound per 100 phospholipids (mean ± SD, N = 3). Binding of SR 33557 to vesicles made of the total phospholipids extracted from the erythrocyte ghosts yielded similar binding characteristics ($K_d = 1.7 \pm 0.3$ 10^{-6} M, $B_{max} = 18.5 \pm 3.1$ SR 33557 molecules bound per 100 phospholipids, mean ± SD, N = 4). Compared to the binding characteristics of SR 33557

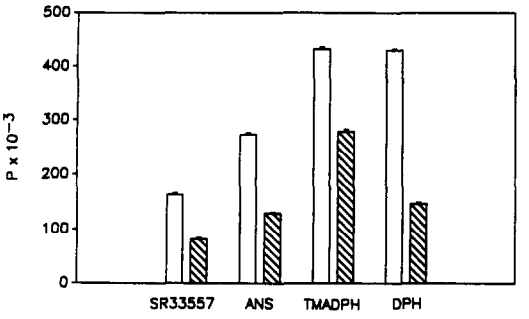

Fig. 6. Comparison of the degree of fluorescence polarization (P) of SR 33557, ANS, TMA-DPH and DPH in the gel phase (open column) and the liquid-crystalline phase (hatched column). The phospholipid used is DPPC at the final concentration of 1.0×10^{-4} M at 25° (gel state) and 45° (liquid-crystalline state). The data are the means ± SD of 3–5 determinations.

to PCs (Table 2), these results indicated that SR 33557 binds mainly, if not only, to the phospholipid component of the membranes since the number of bound SR 33557 molecules per 100 molecules of lipid was similar. However, the dissociation constant was lower suggesting an increased affinity for the phospholipid component of the erythrocyte ghosts. When SR 33557 is totally bound, it can be deduced from the intensity of emitted fluorescence and of quantum yield that SR 33557 is located in an environment of ε of 34 and 36 in the case of erythrocyte ghost membranes and of vesicles made of extracted phospholipids, respectively (Fig. 3). In the same membrane preparations, the degree of fluorescence polarization of SR 33557 at 25° was $P = 0.184 \pm 0.001$ and $P = 0.151 \pm 0.002$ (mean ± SD, N = 3). For comparison, the degree of fluorescence polarization in egg PC vesicles was $P = 0.118 \pm 0.002$ (mean ± SD, N = 5). When the fluorescence intensity or the degree of fluorescence polarization was recorded as a function of temperature, a decrease in intensity was observed as the temperature increased. The decrease was monotonic, there was no evidence for a phase transition in the lipid matrix in the dog erythrocyte ghost nor in the vesicles made of extracted phospholipids.

The fluorescence lifetimes of SR 33557 bound to lipid vesicles made of either egg PC or the total phospholipids extracted from the erythrocyte ghost and to erythrocyte ghosts has been determined. In the three membranous systems, the results obtained are comparable. The fluorescence lifetime of SR 33557 had two well-defined components (Table 3). The predominant fluorescence lifetime component (τ_1), of approximately 13 nsec, represents approximately 85% to the time resolved decay. The second fluorescence lifetime component (τ_2), of 3–5 nsec, represents approximately 15% to the time resolved decay.

DISCUSSION

The results presented in this study demonstrate the fluorescence properties of the newly synthesized Ca^{2+} antagonist SR 33557. In addition, this study provides information on the usefulness of the fluorescence properties to characterize the interaction with the lipid component of biological membranes.

The centres of the excitation and emission bands of SR 33557 are well separated. However the Stokes shift is small (approximately 66 nm). In addition there is no displacement of the maximum of the emission band as a function of the solvent dielectric constant. Both sets of data strongly suggest that the fluorescent moiety of SR 33557 has a rigid structure. This suggestion is reinforced by the calculation of rate constants of deactivation processes. The emission rate constant k_e remains constant in all solvents while the non-radiative rate constant k_{nr} increases as expected directly as a function of solvent polarity so that in non-polar solvents k_e largely exceeds k_{nr}. From the values of k_e, the intrinsic or radiative lifetime of SR 33557 can be calculated according to:

$$\tau_0 = \frac{1}{k_e}.$$

Table 3. SR 33557 lifetimes in phospholipid vesicles and erythrocyte ghost

Sample	Heterogeneity analysis				
	τ_1	α_1	τ_2	α_2	χ^2
Egg PC (N = 3)	13.6 ± 1.7	0.81 ± 0.19	5.0 ± 4.3	0.19 ± 0.19	0.73
Phospholipids from erythrocytes (N = 3)	13.4 ± 1.3	0.82 ± 0.03	5.0 ± 1.6	0.18 ± 0.03	0.35
Erythrocyte ghosts (N = 6)	13.3 ± 0.4	0.91 ± 0.04	2.9 ± 2.5	0.09 ± 0.06	0.49

τ_1 and τ_2 are the lifetimes of SR 33557 expressed as nsec.

α_1 and α_2 are the fractional contribution to the time-resolved decay of the component with a lifetime τ_1 and τ_2, respectively.

The data are the means ± SD of N independent experiments. In each experiment, the results were obtained from at least 10 measurements, each of which is an average of 10 single measurements. All measurements were performed at 3 modulating frequencies: 6, 18 and 30 MHz.

This has permitted a value of $\tau_o = 12.5 \pm 0.7$ nsec (mean ± SD, N = 6) to be obtained. Due to the wavelength region in which SR 33557 absorbs, all these results suggest that the fluorescence properties arise from a limited part of the molecule which includes the indolizine ring system, the sulfonyl group and the alkyl substituent (Fig. 1). The precise origin of the fluorescence has not been investigated in this study but it must be emphasized that the nature of the alkyl substituent has little influence on the fluorescence. On the contrary, the sulfonyl group is of prime importance since its substitution by the carbonyl, the sulfoxide and/or the sulfur function led to a non-fluorescent compound in the case of the carbonyl substitution and to a drastic decrease in fluorescence in the other cases.

When interacting with phospholipids in vesicles and in natural membranes, a single binding site has been identified. The characteristics (K_d and B_{max}) are fairly constant. This suggest that, under the present experimental conditions, SR 33557 binds in the first place to the phospholipid matrix. A van't Hoff plot of the dependence of SR 33557 affinity on temperature was linear over the temperature range examined for binding to vesicles made of PC. This implies that the enthalpy changes ($\Delta H°$) were independent of temperature and that there is no evidence for change in heat capacity during the binding reaction. The calculated values of $\Delta G°$, $\Delta H°$ and $\Delta S°$ indicate a large decrease in enthalpy and no modification to entropy.

The affinity of SR 33557 is sensitive to ions like Na^+ and Ca^{2+}. The degree of fluorescence depolarization and its variation is similar to that of ANS and different from that of TMA-DPH and DPH. From the known position of these fluorescent probes in the lipid matrix: ANS in the polar head [21], TMA-DPH in the glycerol backbone [22] and DPH in the acyl chains [22], it can be proposed that SR 33557 is mainly located in the polar head region. In this case, SR 33557 would lie parallel to the interface. However, estimation of the dielectric constant of the environment of SR 33557 indicates that the compound is located in the lipid matrix in a region of intermediate dielectric value. This region includes the phospholipid polar head, the glycerol backbone and the ester functions [23]. The exact localization of SR 33557 within these limits is difficult to assess. SR 33557 is an elongated molecule of which the longest distance determined by X-ray* extends over 19 Å. Thus, SR 33557 could well cover the entire region from the polar head to the first methylene of the acyl chain of the phospholipid. Due to its amphiphilic nature, the molecule would lie perpendicular to the interface, the amino group close to the phosphorylcholine group and the indolizine ring system buried in the hydrocarbon chains.

In solution, a single fluorescence lifetime has been obtained whatever the solvent. However, with phospholipids, two values for SR 33557 fluorescence lifetime have been determined. These two fluorescence lifetimes do not correspond to two binding sites since, under the same experimental conditions, only one permanent binding site has been observed. We have no explanation for the origin of these two fluorescence lifetimes. However, since the pK_a of SR 33557 is 8.7, one could propose that the two fluorescence lifetimes reflect the difference in interaction between the protonated and neutral forms of the drug and the phosphate group of PC.

In conclusion, some of the basic fluorescent properties of SR 33557 in solution have been established. As a result of its useful properties in terms of quantum yield, we have been able to characterize the interactions with phospholipids as a first approach to the study of SR 33557 in biological media by means of its fluorescence. Although some aspects, for example the exact position of SR 33557 in the bilayer, have not been resolved in this study, the data presented here are complementary to the results obtained previously with the radiolabelled molecule [5]. Thus, results reported here provide the basis for further studies on the interactions with membranes and plasma proteins as well as on its dosage in clinical trials.

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