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Pyrene Excitation via Resonance Energy Transfer from Protein Tryptophan Reveals a Fluidity Gradient in Liver Microsomes

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Membrane fluidity measurements based on excimer formation of pyrene and pyrene derivatives as a measure of lateral diffusion yield a decreased fluidity in the presence of proteins [1,2]. It was the aim of our study to investigate whether the reduced excimer formation is due to a rigidifying effect of proteins on the whole membrane or if the fluorophore mobility is hindered mainly in the immediate protein environment. Resonance energy transfer in microsomal membranes between intrinsic tryptophan residues and pyrene was used to study the excimer formation rate in the vicinity of proteins. The excimer-to-monomer fluorescence ratio after excitation via resonance energy transfer decreased compared to that observed for the direct excitation. The results suggest that, because of the reduced fluidity in the neighborhood of proteins, pyrene and pyrenedodecanoic acid do not diffuse homogeneously in the membrane plane.

KEY WORDS: Pyrene excimer formation; resonance energy transfer; tryptophan; fluidity; liver microsomes.

INTRODUCTION

The presence of proteins decreases the ratio of the excimer-to-monomer fluorescence of pyrene and pyrene derivatives in a way which is related to the protein-to-lipid ratio. The protein-dependent decrease in pyrene collision frequency has been interpreted in different ways.

- Proteins may induce a rigidifying effect in the membrane as a whole or act as immobile obstacles which increase the average path for collisions of the diffusing molecules.
- Proteins are surrounded by a boundary layer. In the boundary layer pyrene diffusion is assumed to be slower than in the bulk lipid phase.
- Pyrene molecules may be bound to the protein–lipid interface, which effectively results in pyrene immobilization.

We used energy transfer from intrinsic tryptophan to pyrene or pyrenedodecanoic acid to differentiate membrane fluidity in the immediate protein neighborhood and in the bulk lipid. Tryptophan quenching in the presence of pyrene fatty acids of different chains can reveal an estimation of the transversal tryptophan density in the microsomal membrane.

METHODS

Microsomal tryptophan emission (donor) and pyrene absorption (acceptor) shows a broad spectral overlap. Energy transfer efficiencies (ET) from intrinsic tryptophans to pyrene are determined from the tryptophan fluorescence according to

$$ET = 1 - (I/I_O) \quad (1)$$

where I and I_O are the tryptophan emission intensities at 330 nm in the presence and absence of pyrene probes, respectively. The excitation wavelength is 292 nm.

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Sensitized pyrene fluorescence due to energy transfer from intrinsic tryptophan is calculated according to

$$I_{ET} = I - I_{Trp} \quad (2)$$

in which I_{ET} is due to nonradiative energy transfer, I is the total fluorescence intensity, and I_{Trp} is due to tryptophan emission background.

The excimer-to-monomer ratio (E/M) was determined from the monomer fluorescence intensity at 384 nm and the excimer fluorescence intensity at 470 nm under direct excitation at 336 nm or via energy transfer from tryptophan. E/M is correlated to the lateral diffusion coefficient [3].

RESULTS AND DISCUSSION

Pyrene Excimer Formation Under Direct and via Energy Transfer Excitation. Evaluation of the monomer and excimer fluorescence intensities reveals an increase in E/M with increasing pyrene and pyrenedodecanoic acid concentration (Fig. 1). Pyrenedodecanoic acid shows a lower E/M ratio than pyrene at the same molar label concentrations because it has a lower mobility in the membrane plane for structural reasons.

The slope of the E/M increase is higher for microsomes under direct excitation than via excitation with energy transfer from intrinsic tryptophan. As only the pyrene and pyrenedodecanoic acid molecules in the immediate protein environment can be excited via energy transfer, the lower E/M ratio after excitation via energy transfer indicates a possible immobilization of pyrene molecules located in this area (Förster radius). Comparison of the slope of E/M versus pyrene concentration upon direct excitation at 336 nm and upon excitation via resonance energy transfer in the microsomal membrane (Fig. 1) emphasizes that the immobilization of pyrene molecules does not occur over the whole membrane, but mainly in the protein environment. This means that proteins must not have a rigidifying effect on the whole membrane space.

An alternative explanation for the decrease in E/M is that adsorption of the probes at the protein surface occurs, completely immobilizing them. Pyrene binding to hydrophobic protein pockets results in an increased ratio of the fluorescence peaks ($I_{373\ nm}/I_{385\ nm}$) of the monomer spectrum due to an increased polarity of its environment [4]. In our measurements we observed no change in $I_{373\ nm}/I_{385\ nm}$. This results suggest that the reduced E/M cannot be interpreted in terms of total pyrene immobilization by protein binding.

Our measurements reveal that in microsomal membranes excimer formation is heterogeneous in the lateral plane. The lateral heterogeneity is interpreted as a fluidity gradient from the membrane proteins to the bulk lipid regions.

Tryptophan Quenching in the Presence of Pyrene Fatty Acids. We determined the energy transfer efficiency from the quenched tryptophan fluorescence for pyrene and pyrene fatty acids of different chain lengths: pyrenebutanoic acid, pyrenedecanoic acid, and pyrenedodecanoic acid (Fig. 2).

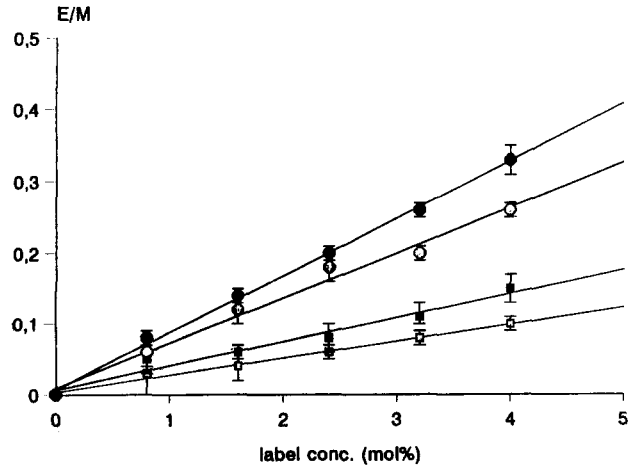

Fig. 1. Excimer-to-monomer fluorescence ratio (E/M) in pig liver microsomes plotted against pyrene (● ○) and pyrenedodecanoic acid (■ □) concentration (mole fractions relative to lipid). Pyrene was excited at 336 nm (filled symbols) or via energy transfer from intrinsic tryptophan (open symbols).

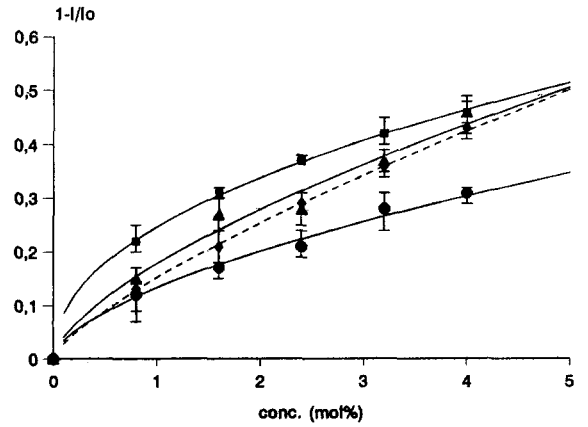

Fig. 2. Measured efficiencies of energy transfer [Eq. (1)] from intrinsic tryptophan to pyrene (♦), pyrenebutanoic acid (●), pyrenedecanoic acid (■), and pyrenedodecanoic acid (▲) plotted against concentration (mole fraction relative to lipid).

Table I. Energy Transfer from Microsomal Tryptophan to Pyrene Fatty Acids at Three Label Concentrations[a]

Label concentration	C	Za (Å)	Energy transfer efficiency 0.8 mol%	1.6 mol%	2.4 mol%
Pyrenebutanoic	4	7.3	0.12+0.05	0.17+0.02	0.21+0.03
Pyrenedecanoic	10	14.5	0.22+0.02	0.31+0.01	0.37+0.01
Pyrenedodecanoic	12	17.9	0.15±0.02	0.27+0.03	0.28+0.03

[a]The pyrene distance Za (Å) from the membrane water interface of the pyrene moiety is calculated from the acyl chain length: Za = 1.2·C + 2.5 Å, where 2.5 Å represents the diameter of the pyrene molecule and C represents the number of C–C bonds in the acyl chain.

While pyrene quenches the tryptophan fluorescence linearly in this concentration range, a saturation effect at higher pyrene fatty acid concentrations was observed. This effect is based on tryptophan residues in microsomal proteins, which are inaccessible to the quencher. Pyrene as an unpolar molecule enriches in both layers, while pyrene fatty acids are fixed in the glycerole backbone of the outer layer. Thus tryptophan residues located in the inner layer are quenched by pyrene only. According to a modified Stern–Volmer plot, only 70–80% of the tryptophans are quenched by pyrene fatty acids.

In microsomes it is impossible to determine the location of the intrinsic tryptophan, which is responsible for the energy transfer, because the microsomal membrane contains about 50 enzyme systems. The intrinsic tryptophan is quenched most efficiently for pyrenedecanoic acid (Table I), of which the pyrene moieties are located at a distance of about 10 Å from the bilayer center.

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