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Conformational Effects on the Fluorescence of Pyrene-Labeled Alkyldiacyl Glycerols in Different Model Membranes

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We synthesized two isomeric alkyldiacyl glycerols containing pyrene as a fluorescent reporter group bound to the omega end of both acyl chains. If located in the phospholipid monolayer of a vesicle both isomers showed intramolecular pyrene excimer fluorescence, indicating parallel orientation of both pyreneacyl chains in the lipid molecule. In micelles only pyrene monomer fluorescence was observed. Thus, in this system the labeled lipids adopt a conformation with both pyreneacyl chains extending into different directions. Using vesicles, lipase activities could be continuously determined from the increase of pyrene monomer fluorescence.

KEY WORDS: excimer fluorescence; ether lipids; vesicles; micelles; lipases.

Fluorescent triglycerides are useful tools for the quantitative determination of lipase activities. For this purpose, it is necessary that the fluorescence properties of the intact substrate and its degradation products are different. (Fluorogenic) substrates for lipases are lipophilic compounds and must therefore be solubilized in water before use, e.g., by appropriate amphiphiles (for example, phosphatidylcholine, Triton, and deoxycholate).

Lipases [1] are water-soluble proteins that catalyze lipid hydrolysis at the hydrophilic–hydrophobic interface of a lipid particle. The type of detergent thus may influence enzyme activity by interaction with the protein, or by affecting the accessibility and/or the conformation of the substrate. If the substrate is fluorogenic, its fluorescence properties may depend on the type of amphiphile that makes it suitable or unsuitable for a fluorogenic enzyme assay. Here we describe the use of two isomeric dipyrene-labeled alkyldiacyl glycerols for the fluorometric analysis of lipase activities.

We synthesized two isomeric alkyldiacyl glycerols [2] containing pyrene as a fluorescent reporter group bound to the omega end of both acyl chains. In position 1 or 2 we put a nonhydrolyzable alkyl chain. The re-

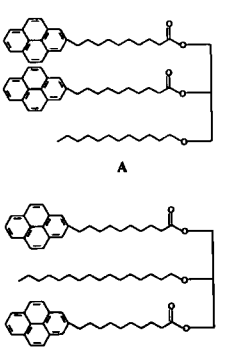

Fig. 1. Chemical structures of fluorescent alkyldiacyl glycerols. (A) 1,2-Di-pyrenedecanoyl-3-*O*-dodecyl-*sn*-glycerol. (B) 1,3-Di-pyrenedecanoyl-2-*O*-hexadecyl-glycerol.

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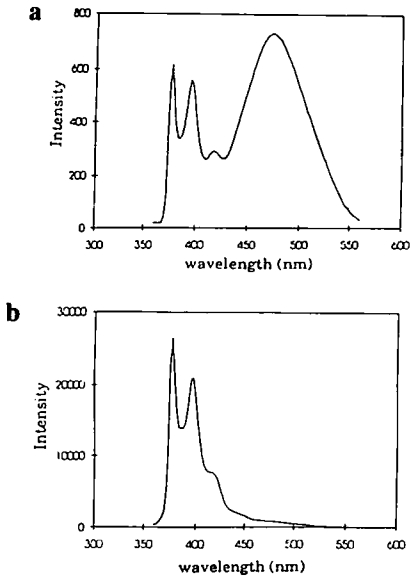

Fig. 2. (a) Emission spectra of fluorescent alkyldiacyl glycerol (1 μ*M*) in vesicles (45 μ*M* palmitoyloleoyl-phosphatidylcholine). Vesicles [3,4] were prepared by injecting an ethanolic solution of mixtures of the respective lipids into aqueous buffer (0.1 *M* Tris–HCl, pH 7.4, 0.1 *M* NaCl) at 37°C. (b) Emission spectra of fluorescent alkyldiacyl glycerol in micelles. The lipids were solubilized in buffer (0.1 *M* Tris–HCl, pH 7.4, 0.1 *M* NaCl), containing Triton or deoxycholate (2 mg/ml).

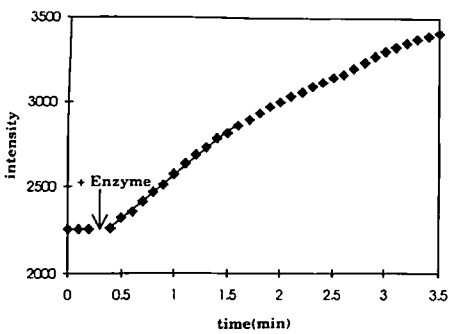

Fig. 3. Time-dependent increase in fluorescence intensity at 380 nm during hydrolysis of dipyreneacyl-alkylglycerol in phosphatidyl choline vesicles by lipase from *Rhizopus arrhizus* at 37°C.

spective compounds, namely, 1,2-di-pyrenedecanoyl-3-*O*-dodecyl-*sn*-glycerol and 1,3-di-pyrenedecanoyl-2-*O*-hexadecylglycerol (Fig. 1) were incorporated into vesicles of phosphatidylcholine and micelles of Triton or deoxycholate. The fluorescence of the labeled triglycerides served as a reporter signal for lipid conformation and/or degradation by lipases. If the dipyreneacyl glycerides were located in vesicles at sufficiently low concentrations, both isomers showed intramolecular pyrene excimer fluorescence (Fig. 2a), indicating a parallel orientation of both pyreneacyl chains in the lipid molecule.

In micelles, only pyrene monomer fluorescence was observed (Fig. 2b). Thus in this system the labeled lipids adopt a conformation with both pyreneacyl chains extending into different directions. This notion is supported by the fact that lipid pyrene fluorescence is more effectively quenched by potassium iodide in micelles as compared to phospholipid assemblies. Therefore, the triglyceride-bound fluorophores are more accessible to water in the former system.

In contrast, both pyreneacyl residues are better shielded from water in bilayers, due to their location in the hydrophobic membrane interior.

Dipyrene triglycerides cannot be used as fluorogenic substrates for lipases in micelles of Triton or deoxycholate since they show only monomer fluorescene as a consequence of an "unfavorable" substrate conformation in this systems.

Vesicles turned out to be more suitable for the continuous determination of lipase activities, if dipyreneacyl-alkylglycerols were used as fluorogenic substrates. In this case, enzyme activity could be estimated from the increase in pyrene monomer fluorescence due to hydrolysis of the substrate, followed by dilution of the labeled reaction products by unlabeled matrix lipids (Fig. 3).

The lipase-induced spectroscopic changes are in agreement with TLC analysis of lipid extracts, showing that the increase in pyrene monomer fluorescence is accompanied by the formation of hydrolysis products (nonfluorescent alkyl glycerol, fluorescent pyrene fatty acid).

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