Effects of Lipid Fluidity on Quenching Characteristics of Tryptophan Fluorescence in Yeast Plasma Membrane*

(Received for publication, March 5, 1982)

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Fluorescence characteristics of tryptophan residues in yeast plasma membrane indicate that the residues are buried. The fluorescence is fully quenchable by iodide with similar quenching kinetics at temperatures from 8 to 37 °C in oleate-enriched membranes and from 25 to 37 °C in palmitelaidate-enriched membranes. Substantial increases in lipid microviscosity in palmitelaidate-enriched membranes reduce the fraction of quenchable tryptophan fluorescence by about 40% and increase the effective quenching constant 3-fold. These observations indicate that at above 25 °C, proteins in this membrane undergo transient conformational changes and that freedom of conformational changes of the proteins is regulated by lipid microviscosity.

Tryptophan fluorescence and phosphorescence of some native soluble proteins are quenched by a number of quenchers even though fluorescence spectra and, where available, x-ray data suggest that the fluorophores are buried and are out of direct contact with the solvent (1-5). Consequently, it has been suggested that, in solution, proteins undergo structural fluctuations on the nanosecond scale that permit penetration of the quencher to the fluorophore(s) (1-5). There is no evidence to suggest that this is also true of membrane proteins in situ, and, if so, whether the conformational fluctuations are affected by lipid fluidity. The latter point is particularly important since, for optimal activity of some membrane-bound enzymes, lipids must be in the liquid-crystalline state (6-11). We have evaluated the effects of fatty acid composition and lipid microviscosity on the kinetics of iodide quenching of tryptophan fluorescence in yeast plasma membrane. The results indicate that membrane proteins undergo transient conformational changes and are in a "fluid" state at physiological temperatures. The results also indicate that freedom of conformational changes depends on lipid microviscosity.

MATERIALS AND METHODS

Fatty acids and DPH were obtained from Sigma. Plasma membrane was isolated, as previously described (12), from a fatty acid auxotroph of Saccharomyces cerevisiae grown at 30 °C in a medium containing 0.02% each of myristic and oleic or palmitelic acid (13). Tryptophan content of the membrane, determined according to Spande and Witkop (14), was found to be about 1.2% of the protein weight. Procedures for protein determination, lipid extraction, fractionation, and fatty acid analysis have been described (12).

Iodide quenching experiments were carried out essentially as described by Lehrer (15). Each assay mixture contained membrane (0.1 mg of protein) in 2 ml of 0.05 M potassium phosphate buffer, pH 7.2, and unless otherwise indicated, 0 to 0.5 M KI. Ionic strength was adjusted to 1 by addition of KCl. Iodide solutions contained 1 x 10^{-4} M S2O3^{2-} to prevent I_2 formation (15). Unless otherwise indicated, the reaction mixture was maintained for 15 min at 25 °C and tryptophan fluorescence emission was recorded at the same temperature.

DPH polarization was carried out, as described earlier (16), except that 0.1 mg of membrane protein was used. Spectra were recorded with a Perkin-Elmer MFP-44 fluorescence spectrophotometer. Temperature was maintained as described before (16). Excitation wavelengths were 290 and 368 nm for tryptophan and DPH, respectively. Quantum yield was determined according to Kirby (17).

RESULTS AND DISCUSSION

Fluorescence Spectra—The fluorescence emission spectra of the membrane excited at 290 nm exhibits a single broad maximum at 335 nm with a half band width of approximately 60 nm (Fig. 1). These are characteristics of tryptophan emission (18, 19) and remain the same when 1 M KCl or 1 M KI are added. In 6 M guanidine HCl, the emission max is shifted to 350 nm. These indicate the fluorescing tryptophyl residues are shielded from water and in 6 M guanidine HCl they are transfered to the aqueous environment (1, 4, 5, 15, 20, 21). It should be noted that iodide even at 2 M did not shift the \lambda_{max}.

Quenching by Iodide—Iodide is a general quencher of fluorophores (22). Quenching kinetic parameters can be calculated from a modified Stern-Volmer equation (15),

\[ F_0 = \frac{1}{1 + \frac{1}{\Delta F}} = f_kK_w(X) + \frac{1}{f_a} \]

where \( F_0 \) is fluorescence intensity in the absence of the quencher (X), \( \Delta F \) is the change in fluorescence intensity due to addition of a given concentration of X, \( K_w \) is a constant which is the product of the collisional quenching rate constant and fluorescence life-time in the absence of the quencher, and \( f_a \) is the effective fraction of the tryptophanyl fluorescence that is quenchable.

The effects of 1 M iodide on fluorescence intensity of tryptophan residues and of DPH incorporated into the membrane are shown in Fig. 2. DPH is not quenched. Since DPH is located in the paraffinic region of the lipid-bilayer (23) and in 60% ethanol is quenched by I_i, with a \( K_w \) of approximately 1 M^{-1}, then the quencher cannot diffuse through the lipid-bilayer. The kinetic parameters of tryptophan quenching in oleate-enriched membranes were calculated from modified Stern-Volmer plots (Fig. 3A). \( f_a \) was found to be 1 and \( K_w \) was 1.3 M^{-1}. These values changed little at assay temperatures ranging from 8 to 37 °C. Since \( f_a \) is 1, then either the fluorescence of each tryptophan residue is susceptible to quenching by iodide (15) or, alternatively, some may be accessible but all are connected by energy transfer networks. In either case, such assessability is feasible if transient conformational changes, similar to those proposed for soluble proteins (1-5), take place in membrane proteins.

Effect of Lipid Microviscosity on Iodide Quenching of Tryptophan Fluorescence—In order to investigate the effects of lipid fluidity on kinetics of I_i quenching, a comparison of

* This work was supported by United States Public Health Service Grant GM-26462. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 The abbreviation used is: DPH, 1,6-diphenyl-1,3,5-hexatriene.
The change in fluorescence life-time at 25 °C, and it was 0.04 at 25 °C, and 0.05 at 8 °C. Therefore, the increase in (25-27).

8 M guanidine hydrochloride were similar to those of the oleate-enriched membrane. However, at 15 °C, where polarization was 0.26, 0.28, and 0.3 respectively. In the palmitelaidate-enriched membrane at 25 and 37 °C, the quenching kinetic parameters were found to be linear at the assay temperature of 8 to 37 °C (Fig. 6). However, the plots deviated from linearity in the palmitelaidate-enriched membrane at 15 °C and below (Fig. 6). This indicates that at higher lipid viscosity the tryptophyl residues are no longer subject to a similar degree of fluorescence quenching (15) and must consist of a heterogenous population.

The possibility of a transition from liquid-crystalline to the gel phase leading to the observed changes in quenching kinetics of the palmitelaidate-enriched membrane at 15 °C and below should be considered. Such a phase change could conceivably inhibit possible penetration of I- to the level of glyceryl backbone and prevent quenching of the tryptophanyl groups that might be located in this region. However, neither in a previous report (16) nor in studies of Fig. 4 did we observe such a phase transition in the palmitelaidate-membrane. For such a phase change to occur and to explain our findings, we should have observed a change in slope of DPH polarization of the palmitelaidate-enriched membrane between 15 and 25 °C (23). In addition, the Arrhenius plots of data in Fig. 4 revealed a constant activation energy between 8 and 25 °C. Therefore, the above possibility is ruled out.

The constancy of $K_{av}$ in the oleate-enriched membrane (Fig. 5) is indicative of a very low energy of activation for quenching and a fluid protein matrix (5). Therefore, at the growth temperature of 30 °C both in this and in the palmitelaidate-enriched membrane, where $K_{av}$ was the same at 25 and 37 °C, there must be a substantial degree of mobility in the poly-

Fig. 1. Effect of guanidine hydrochloride on emission $\lambda_{max}$ of tryptophan fluorescence in oleate-enriched yeast plasma membrane. Membranes were suspended in 0.05 M potassium phosphate buffer, pH 7.2. (curve a) or the same buffer containing 6 M guanidine hydrochloride (curve b). The actual fluorescence intensity in a was approximately 12% smaller than that shown because the instrument sensitivity was set at 0.3 x 3.7 for curve a and 0.3 x 3 for curve b.

Fig. 2. Effects of iodide on fluorescence of fluorophores in oleate-enriched yeast plasma membrane. Tryptophan emission of plasma membrane (A) or DPH emission (B) of the membrane labeled with DPH was recorded as described under "Materials and Methods." The membrane was suspended in 0.05 M potassium phosphate buffer, pH 7.2, containing 1 M KCl (a) or 1 M KI (b).

Fig. 3. Modified Stern-Volmer plots of fluorescence quenching by iodide. Oleate-enriched membrane (A) and palmitelaidate-enriched membrane (B) were assayed at 25 °C (5) and 8 °C (6).
Lipid Fluidity and Membrane Tryptophan Fluorescence Quenching

The present studies give evidence for transient conformational changes in membrane proteins in situ that are consistent with the existence of protein matrices in a fluid state at growth temperature. They further show that protein fluidity is modulated by alterations in lipid fluidity and give evidence for conformational changes in proteins induced by increased lipid microviscosity. These observations provide a plausible explanation for previously reported effects of membrane lipid composition on activity of some membrane-bound enzymes (6-11).

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Fig. 4 (left). Effect of temperature on fluorescence polarization of DPH incorporated into yeast plasma membrane. Oleate-enriched membrane (O); palmitelaidate-enriched membrane (•). Bars show mean deviations.

Fig. 5 (center). Correlation between DPH polarization and plasma membrane tryptophan fluorescence quenching parameters. Iodide quenching of tryptophan fluorescence of the oleate-enriched membrane (O) and the palmitelaidate-enriched membrane (•) was determined at 8, 15, 25, and 37°C. Ksv and F values were determined and were plotted versus DPH polarization of the corresponding membrane at the same temperature (cf. Fig. 4). Bars show mean deviations.

Fig. 6 (right). Stern-Volmer plots of the quenching of tryptophan fluorescence by iodide in palmitelaidate-enriched membranes. The data of Fig. 3B were plotted according to the Stern-Volmer equation. Membranes assayed at 25°C (O) and 8°C (•).