# Degree of Exposure of Membrane Proteins Determined by Fluorescence Quenching†

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ABSTRACT: Quaternary salts of 4-picoline are shown to act as efficient quenchers of tryptophan fluorescence in membrane proteins. Fluorescence quenching determinations of sarcoplasmic reticulum membranes from rabbit muscle and of human erythrocyte membranes of different cholesterol to phospholipid mole ratios (C/PL) were carried out with quaternary picolinium salts in phosphate-buffered saline (PBS) and in 2,2,2-trifluoroethanol (TFE)–water 2:1 (v/v), where the membrane is presumably completely disintegrated. In both solvent systems, the tryptophan quenching characteristics were typical of heterogeneous systems and were analyzed as such. The ratio of the fraction of fluorescence intensity available for quenching with *N*-methylpicolinium perchlorate in PBS and in 2:1 TFE–water, $\Sigma f_i^a/\Sigma f_i^\circ$, was taken as an index for the bulk degree of exposure of the membrane proteins to the aqueous surrounding. This value was found to increase with C/PL which is in line with the notion that increase in lipid microviscosity results in increase of exposure of membrane proteins. Analogous experiments were performed with *N*-hexyl- and *N*-benzylpicolinium, which can quench tryptophyl residues in both the aqueous phase and the hydrocarbon–water interface, and with *N*-hexadecylpicolinium which is dissolved in the membrane lipid layer and acts mostly as a static quencher of tryptophan at the hydrocarbon–water interface. With these quenchers the complementary indices $\Sigma f_i^b/\Sigma f_i^\circ$ and $\Sigma f_i^c/\Sigma f_i^\circ$, which represent the fraction of the protein mass located in the hydrocarbon–water interface and in the hydrocarbon layer, respectively, could be semiquantitatively resolved.

The availability of proteinic sites for interaction with their specific ligands, such as substrates, hormones, or nutrients, is one of the key parameters which determines the functional activity of most biological membranes. In some cell membranes the active expression of specific sites is modulated during cellular events which affects profoundly the physiological functions of the cell (Edelman, 1976; Raff, 1976; Nicolson, 1976a,b). We have recently proposed that one of the mechanisms by which the activity of membrane proteins is modulated involves changes in the microviscosity of the membrane lipid layer. According to this mechanism increase in lipid microviscosity will lead to exposure of membrane proteins, whereas decrease in microviscosity will result in protein masking (Shinitzky and Inbar, 1976; Shinitzky, 1976). This vertical displacement of membrane proteins has been already demonstrated in erythrocyte membranes of different cholesterol levels (Borochov and Shinitzky, 1976).

In the following we present a general method for determination of the degree of exposure of membrane proteins. The method is based on measurements of the availability of the natural tryptophan fluorescence generated from the membrane proteins to highly efficient collisional quenchers. Collisional quenching of the fluorescence of indole or tryptophan derivatives can be executed with either iodide (Lehrer, 1971; Burstein et al., 1973; Altekar, 1974), cesium (Burstein et al., 1973; Altekar, 1974), nitroxide radicals (Wallach et al., 1974; Bieri and Wallach, 1975), imidazoium or pyridinium ions (Shinitzky and Katchalski, 1968; Shinitzky, 1973; Pownall and Smith, 1974). These quenchers are virtually fully transparent at 290–300 nm, where tryptophans in protein can be selectively excited and could, therefore, be of practical use in studies of quenching of protein fluorescence. A comparison of the reported quenching efficiencies (Lehrer, 1971; Burstein et al., 1973; Altekar, 1974; Shinitzky and Katchalski, 1968) indicates that pyridinium derivatives, which quench the fluorescence of indoles by a charge-transfer interaction (Shinitzky and Katchalski, 1968), are more effective than the iodide, cesium, or imidazolium ions by several magnitudes. In addition, a series of quaternary pyridinium salts of different alkyl chains offers a set of quenchers which penetrate to a different extent into the membrane lipid layer (Klip and Gitler, 1974).

## Materials and Methods

All quaternary salts of 4-picoline were prepared from freshly distilled materials. *N*-Methylpicolinium perchlorate was prepared by quaternization of 4-picoline with methyl iodide in benzene and crystallizing the iodide salt from ethanol–ether. The iodide was converted into the perchlorate by dissolving in minimum water and mixing with 10 M $NaClO_4$. The mixture was kept overnight at −20 °C, whereupon the *N*-methylpicolinium perchlorate precipitated. The salt was washed with minimum cold 2-propanol–water mixture and twice crystallized from 2-propanol. *N*-Hexylpicolinium chloride was prepared by refluxing a solution of 4-picoline in 1-hexyl chloride and crystallizing the precipitate from 2-propanol–ether. Attempts to prepare the related perchlorate yielded oily material. The *N*-hexadecyl and *N*-benzyl perchlorates of 4-picoline were prepared analogously by first quaternizing with hexadecyl bromide and benzyl chloride in benzene and then crystallizing the precipitate from ethanol–ether. The obtained salts were dissolved in minimum methanol and added to aqueous 1 M $NaClO_4$ to selectively precipitate the perchlorate salts. These were then crystallized twice from water. All the above quaternary picolinium salts were analytically and chromatographically pure.

Human erythrocytes of different cholesterol to phospholipid mole ratio, C/PL, were prepared from freshly drawn normal blood by treatment with lecithin–cholesterol liposomes according to Cooper et al. (1975), with modifications described

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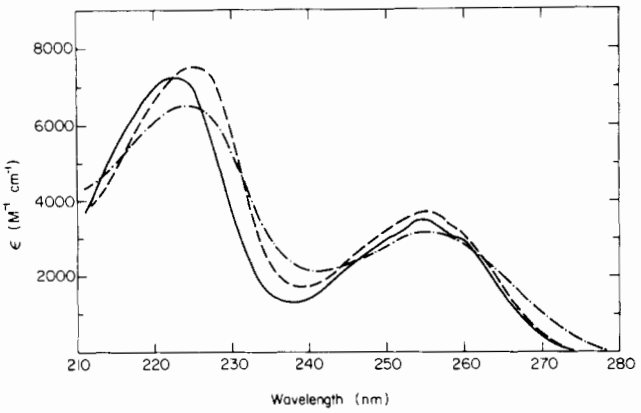


FIGURE 1: Absorption spectra in water of *N*-methylpicolinium perchlorate (—), *N*-hexylpicolinium chloride (- - -), and *N*-hexadecylpicolinium perchlorate (- · · · -).

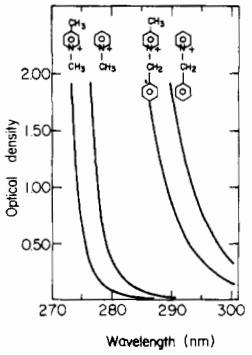


FIGURE 2: The long wavelength edges of the absorption spectra at 1-cm path length of four quaternary perchlorate salts 0.1 M in water.

in a previous publication (Borochov and Shinitzky, 1976). The hemoglobin-free leaky membranes were prepared from the treated erythrocytes by the method of Dodge et al. (1963). Sarcoplasmic reticulum membranes from rabbit muscle were isolated and purified as described by Meissner (1974).

Fluorescence quenching experiments with *N*-acetyl-L-tryptophanamide (NATA)[1] were carried out with an Aminco Bowman spectrofluorimeter by exciting at 290 nm and measuring the fluorescence intensity at 350 nm. The picolinium perchlorates or chloride quenchers were present at concentrations of up to 0.04 M. In the presence of 0.1 M $NaClO_4$ or NaCl, no quenching of NATA was detected.

Tryptophan fluorescence of membranes was measured with a fluorescence polarization instrument described elsewhere (Shinitzky, 1974). Excitation was performed with a horizontally polarized 298-nm Hg band and the emission was detected through a vertically oriented polarizer and a narrow band-pass filter with a transmission maximum at 340 nm (Corning UG-11). This set up was designed to reduce to a minimum the contribution of scattered light which in our experiments never exceeded 3% of the detected fluorescence signal. The determined membrane suspensions were 0.01 mg of protein/mL in isotonic media containing 0.01 M phosphate buffer (pH 7.4) and mixtures of NaCl and quencher salts of 0.14 M total concentration (PBS). Membrane disintegration was performed by mixing 1 volume of membrane suspension (0.03 mg of protein/mL) in 0.02 M phosphate buffer (pH 7.4) with 2 volumes of spectroscopic grade 2,2,2-trifluoroethanol (Merck "UVasol"). Quenching experiments with these mixtures were carried out by adding aliquots of concentrated aqueous solution of quencher.

## Results and Discussion

Preliminary experiments have shown that 10–50% quenching of trytophan fluorescence in biological membranes can be achieved in the presence of 0.01–0.1 M quaternary pyridinium quenchers. Among these the 4-picolinium derivatives were found most suitable since their absorption spectrum is shifted by about 4 nm toward higher energies without reducting their quenching capacity. The absorption spectra of three quaternary picolinium salts, which were used in the following study as tryptophan quenchers, are shown in Figure 1. The long wavelength edges of the absorption spectra of the *N*-methyl and *N*-benzyl derivatives of pyridine and 4-picoline, at a concentration of 0.1 M, are shown in Figure 2. This figure clearly demonstrates the advantage of quaternary picolinium derivatives over the analogous pyridinium derivatives as protein tryptophans quenchers, when concentrations around 0.1 M are used.

The protonated forms of pyridine and picoline are almost identical in physical and spectral properties with their analogous quaternary forms and as such can similarly act as fluorescence quenchers. However, since these are rather weak bases ($pK_a \sim 5.5$), they will remain essentially fully unprotonated at neutral pH and therefore their protonated salts cannot be used as quenchers at the physiological range of pH 7–8. Pownall and Smith (1974), however, have used pyridine hydrochloride as a quencher of tryptophan in lipoproteins, but the actual pH of the medium has not been reported. If in their study no buffer was used and the pH was deliberately kept acidic, the recorded quenching was indeed brought about by the protonated pyridine ion at a concentration which is pH dependent. The relevancy of such measurements to physiological pH conditions is obviously doubtful.

The degree of fluorescence quenching, owing to collisions between fluorophore and quencher molecules in solution, is described by the well-known Stern–Volmer equation (Stern and Volmer, 1919):

$$F_0/F = 1 + K_q[Q] \qquad (1)$$

$F_0$ and $F$ are the fluorescence intensities in the absence and presence of a quencher at a molar concentration of [Q], and $K_q$ is a constant which is the product of a collisional quenching rate constant and the excited state lifetime of the fluorophore in the absence of quencher. In a heterogeneous population of fluorophores, like tryptophan residues in a protein, the linear relation between $F_0/F$ and [Q] is generally not obeyed. In such systems the fluorescent species can be divided into $n$ groups according to their $K_q$ values and the fluorescence quenching can be described by the equation developed by Lehrer (1971):

$$\frac{F_0}{F_0 - F} = \left(\sum_i \frac{f_i K_{qi}[Q]}{1 + K_{qi}[Q]}\right)^{-1} \qquad (2)$$

where $f_i$ is the relative contribution of group $i$ to the fluorescence intensity in the absence of quencher.

In the general case a plot of $F_0/(F_0 - F)$ vs. $1/[Q]$ should yield a curve which at high quencher concentration extrapolates to $\Sigma f_i$. At low quencher concentration, when $K_{qi}[Q] \lesssim 1$, this curve assumes a linear shape which is expressed in the form

[1] Abbreviations used: NATA, *N*-acetyl-L-tryptophanamide; PBS, phosphate-buffered saline; TFE, 2,2,2-trifluoroethanol.

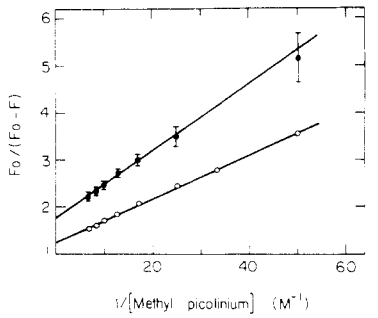


FIGURE 3: Quenching of tryptophan fluorescence of sarcoplasmic reticulum membranes (0.01 mg of protein/mL) with *N*-methylpicolinium perchlorate in PBS (●—●) and in 2:1 (v/v) TFE-water (○—○). The data are presented according to eq 3.

$$\frac{F_0}{F_0 - F} = \frac{1}{\sum_i f_i K_{qi}} \frac{1}{[Q]} + \frac{\sum_i K_{qi}}{\sum_i f_i K_{qi}} \quad (3)$$

From the slope of the linear region the value of the mean quenching constant, $\Sigma_i f_i K_{qi}$, is obtained, which together with the fraction of fluorescence intensity available for quenching, $\Sigma_i f_i$, describes the most important quenching characteristics of the system. From the intercept of this straight line the value of $\Sigma_i f_i K_{qi}/\Sigma_i K_{qi}$ can be obtained. This value is slightly greater than $\Sigma_i f_i$ and can serve as the upper limit value of the latter.

Four quaternary 4-picolinium perchlorates were selected as fluorescence quenchers. *N*-Methylpicolinium was used for quenching of tryptophans which are exposed to the aqueous environment. *N*-Hexyl- and *N*-benzylpicolinium, which can presumably accumulate at the hydrocarbon-water interface of biological membranes (Klip and Gitler, 1974), were used as quenchers for both the interface and the aqueous regions. *N*-Hexadecylpicolinium, like similar detergents, was assumed to intercalate into the lipid bilayer where its cationic head will be stationed at the interface region. This compound presumably acts mostly as a "static" quencher for tryptophan residues around the hydrocarbon-water interface (Shinitzky, 1973; Wallach et al., 1974; Bieri and Wallach, 1975).

The first three picolinium salts, as well as iodide and cesium ions, were found to quench NATA in water according to the relation described in eq 1. The $K_q$ values obtained from the plots of $F_0/F$ vs. [Q] are given in Table I. It should be noted that, since pyridinium derivatives can form static charge-transfer complexes with indole derivatives, the apparent $K_q$ values are combined of a dynamic collisional quenching constant and a static association constant (Shinitzky and Katchalski, 1968). Though no attempt was made to resolve these two constants (Vaughan and Weber, 1970), it can be inferred from a previous study (Shinitzky and Katchalski, 1968) that the contribution of the dynamic process to the quenching is predominant. Taking into account an excited state lifetime of 2.9 ns for NATA in water (Grinvald et al., 1975), the $K_q$ values obtained for the picolinium derivatives (see Table I) indicate a high quenching affinity toward excited indoles (Homer and Allsopp, 1976).

Fluorescence quenching studies were carried out simultaneously with intact and solubilized membranes. The membranes studied in detail were human erythrocyte membranes enriched with cholesterol, depleted of cholesterol, and untreated (see Materials and Methods), as well as untreated sarcoplasmic reticulum membranes. In all of these membranes quenching with the picolinium derivatives yielded characteristic profiles of heterogeneous populations. As an example Figure 3 represents plots of $F_0/(F_0 - F)$ vs. $1/[Q]$ according to eq 3 of protein fluorescence quenching by *N*-methylpicolinium obtained for intact and solubilized sarcoplasmic reticulum membranes.

TABLE I: Stern-Volmer Constants, $K_q$, for Quenching of *N*-Acetyl-L-tryptophanamide ($10^{-5}$ M) in Water by Iodide, Cesium, and Three Quaternary Picolinium Ions ($10^{-2}$ to $4 \times 10^{-2}$ M).

| Quencher | $K_q$ ($M^{-1}$) |
|---|---|
| NaI | 7 |
| CsCl | 4 |
| *N*-Methyl-4-picolinium perchlorate | 35 |
| *N*-Hexyl-4-picolinium chloride | 34 |
| *N*-Benzyl-4-picolinium perchlorate | 38 |

TABLE II: Parameters of Tryptophan Fluorescence Quenching by *N*-Methyl-4-picolinium Perchlorate.[a]

| Membrane | In PBS | | In 2:1 TFE-Water | |
|---|---|---|---|---|
| | $\Sigma f_i$ | $\Sigma f_i K_{qi}$ ($M^{-1}$) | $\Sigma f_i$ | $\Sigma f_i K_{qi}$ ($M^{-1}$) |
| Sarcoplasmic reticulum | 0.54 | 14 | 0.81 | 22 |
| Human erythrocyte (C/PL = 0.6-0.7 M/M) | 0.65 | 12 | 0.83 | 19 |
| Human erythrocyte (C/PL = 0.9-1.0 M/M) | 0.72 | 13 | 0.85 | 20 |
| Human erythrocyte (C/PL = 1.2-1.4 M/M) | 0.75 | 15 | 0.85 | 20 |

[a] The fraction of fluorescence intensely available for quenching, $\Sigma f_i$, and the mean quenching constant, $\Sigma f_i K_{qi}$, are given for intact membranes (0.01 mg of protein/mL) in PBS and for membranes dissolved in 2:1 (v/v) TFE-water.

The data obtained from the plots shown in Figure 3, as well as those obtained analogously for fluorescence quenching with *N*-methylpicolinium of the erythrocyte membrane series, are summarized in Table II.

In this study, 2,2,2-trifluoroethanol (TFE) was used as a membrane disintegrator. This solvent is very similar in solubilization properties to 2,2,2-trichloroethanol, a membrane solubilizer which is believed to preserve the tertiary structure of the isolated proteins (Zahler and Wallach, 1967; Zahler and Weibel, 1970) but has important advantages over the latter. TFE is stable in aqueous mixtures unlike trichloroethanol which decomposes with time while liberating HCl, that may substantially reduce the pH of the solution (Zahler and Wallach, 1967). In addition, spectroscopic grade of TFE is commercially available and is adequate for fluorescence measurements in the ultraviolet region as those performed in this study. Circular dichroism studies of proteins in TFE-water mixtures of 4:1 (v/v) have suggested that membrane proteins dissolved in such mixtures are likely to adopt the same conformation as in intact membranes (Urry et al., 1971; Pitner and Urry, 1972).

Upon addition of TFE to a membrane suspension, the solution clarifies and the obtained $\Sigma f_i$ value for quenching with *N*-methylpicolinium was always found to increase (see Figure 3 and Table II). This is expected since part of the tryptophan residues which were buried in the intact membrane become exposed to the solvent after solubilizing the membrane. The

TABLE III: Fraction of Protein Fluorescence Generated from the Aqueous Phase, $\Sigma f_i^a/\Sigma f_i^\circ$, the Hydrocarbon-Water Interface, $\Sigma f_i^b/\Sigma f_i^\circ$, and from the Hydrocarbon Core, $\Sigma f_i^c/\Sigma f_i^\circ$, of Biological Membranes.[a]

| Membrane | $\frac{\Sigma f_i^a}{\Sigma f_i^\circ}$ | $\frac{\Sigma f_i^b}{\Sigma f_i^\circ}$ | $\frac{\Sigma f_i^{a+b}}{\Sigma f_i^\circ}$ 1 | 2 | $\frac{\Sigma f_i^c}{\Sigma f_i^\circ}$ |
|---|---|---|---|---|---|
| Sarcoplasmic reticulum | 0.67 | 0.22 | 0.77 | 0.87 | 0.11–0.23 |
| Human erythrocyte (C/PL = 0.6–0.7 M/M) | 0.79 | 0.13 | 0.84 | 0.88 | 0.08–0.16 |
| Human erythrocyte (C/PL = 0.9–1.0 M/M) | 0.85 | 0.10 | 0.87 | 0.90 | 0.05–0.13 |
| Human erythrocyte (C/PL = 1.2–1.4 M/M) | 0.88 | 0.10 | 0.89 | 0.93 | 0.02–0.11 |

[a] The presented values were obtained by quenching with *N*-methylpicolinium perchlorate ($\Sigma f_i^a/\Sigma f_i^\circ$), *N*-hexadecylpicolinium perchlorate ($\Sigma f_i^b/\Sigma f_i^\circ$), *N*-hexylpicolinium chloride ($\Sigma f_i^{a+b}/\Sigma f_i^\circ$, 1), and *N*-benzylpicolinium perchlorate ($\Sigma f_i^{a+b}/\Sigma f_i^\circ$, 2). $\Sigma f_i^c/\Sigma f_i^\circ$ is given as a range obtained from $\Sigma f_i^c/\Sigma f_i^\circ = 1 - \Sigma f_i^a/\Sigma f_i^\circ - \Sigma f_i^b/\Sigma f_i^\circ$ and $\Sigma f_i^c/\Sigma f_i^\circ = 1 - \Sigma f_i^{a+b}/\Sigma f_i^\circ$.

increase in fraction of fluorescence intensity available for quenching with *N*-methylpicolinium leveled off at a value denoted here as $\Sigma f_i^\circ$, which for most membrane preparations was reached at 60–70% TFE. For determination of $\Sigma f_i^\circ$, we have therefore used mixtures of 2:1 (v/v) TFE and membrane suspension after a few minutes of incubation at room temperature.

The ratio of the fractions of fluorescence intensity available for quenching by *N*-methylpicolinium in the intact and the solubilized membrane, $\Sigma f_i^a/\Sigma f_i^\circ$, was taken as a measure for the overall degree of exposure of the membrane proteins. This ratio in principle extends between 0 and 1 which represent the extreme cases of fully masked and fully exposed proteins, respectively. The degree of exposure determined by this technique should be close to the geometrical reality if one presumes that the tryptophan fluorescence is evenly generated from the various regions of the protein matrix. The values of $\Sigma f_i^a$ and $\Sigma f_i^\circ$, obtained for quenching of membrane proteins with *N*-methylpicolinium, are given in Table II, and their ratios, $\Sigma f_i^a/\Sigma f_i^\circ$, are given in Table III.

The erythrocyte membranes analyzed in this study were not sealed and the quenching determined applies to both sides of the membrane. As demonstrated in Table III, the degree of exposure of membrane proteins, as presented by $\Sigma f_i^a/\Sigma f_i^\circ$, in erythrocyte membrane is rather high which presumably results from the major contribution of spectrin and the filament proteins to the fluorescence. Nevertheless, the bulk degree of exposure of proteins in this membrane varies with the cholesterol level of the membrane. Increase in C/PL, which increases the microviscosity of the lipid layer, increases the degree of exposure of the membrane proteins, and vice versa. Similar trends have been recently observed by us using an analogous method where fluorescent labels were attached to cysteines in erythrocyte membranes (Borochov and Shinitzky, 1976). The degree of exposure of proteins in the sarcoplasmic reticulum membrane is the lowest, which qualitatively agrees with the low C/PL of this membrane (Meissner and Fleischer, 1971). Values for the degree of exposure of $Ca^{2+}$-ATPase, the major protein of this membrane, which were estimated by various chemical methods (MacLennan and Holland, 1975), are of similar magnitude to the $\Sigma f_i^a/\Sigma f_i^\circ$ value.

By determining the $\Sigma f_i$ values for the three other picolinium quenchers, one can resolve, at least semiquantitatively, the fraction of the membrane protein fluorescence generated from the aqueous phase, the hydrocarbon phase, and the hydrocarbon-water interface. Such a classification can elucidate the overall degree of penetration of the membrane proteins into the lipid layer. The fractions of fluorescence intensity available for quenching with *N*-benzyl and *N*-hexylpicolinium, denoted as $\Sigma f_i^{a+b}$, were obtained from plots similar to those given in Figure 3 using quencher concentrations of up to 0.07 M. The results are given in Table III. The $\Sigma f_i^{a+b}$ values represent a sum of the fractions of fluorescence intensity available for quenching in the aqueous phase, $\Sigma f_i^a$, and in the hydrocarbon-water interface, $\Sigma f_i^b$. In the case of *N*-hexadecylpicolinium perchlorate incubation of membranes with concentrations of up to $4 \times 10^{-5}$ M, which is presumably below the critical micellar concentration of this detergent, resulted in a decrease of fluorescence intensity which leveled off at a concentration around $2 \times 10^{-5}$ M. The fractional decrease in fluorescence intensity after incubation of membranes with $2 \times 10^{-5}$ M *N*-hexadecylpicolinium perchlorate was taken as the $\Sigma f_i^b$ value. As shown in Table III, the predictable relation $\Sigma f_i^{a+b} \sim \Sigma f_i^a + \Sigma f_i^b$ indeed holds.

The fraction of fluorescence intensity available for quenching in the hydrophobic core of the membrane, $\Sigma f_i^c$, is approximately equal to $\Sigma f_i^\circ - \Sigma f_i^{a+b}$ or $\Sigma f_i^\circ - \Sigma f_i^a - \Sigma f_i^b$. The mass portion of the proteins situated in the lipid core of the membrane can thus be represented by $\Sigma f_i^c/\Sigma f_i^\circ$. The occupancy fraction of the membrane proteins in the aqueous layer, the hydrocarbon-water interface, and the hydrocarbon core, as represented by $\Sigma f_i^a/\Sigma f_i^\circ$, $\Sigma f_i^b/\Sigma f_i^\circ$, and $\Sigma f_i^c/\Sigma f_i^\circ$, are summarized in Table III.

The mean quenching constant $\Sigma f_i K_{qi}$ obtained from the slope of the plots of $F_0/(F_0 - F)$ vs. $1/[Q]$ (see Table II) can, in principle, add subtle information to that obtained from the various $\Sigma f_i$ values. However, the quenching constant $K_{qi}$ in the above experiments combines three independent parameters: the quencher diffusion constant, the excited state lifetime of the *i* population, and the average charge density around its tryptophyl residues (Lehrer, 1971; Homer and Allsopp, 1976). Resolution of these parameters requires more elaborate techniques than the one used here which is beyond the scope of this method.

The series of erythrocyte membranes of diffrent C/PL (see Tables II and III) can serve as an elucidative example for changes in the position of proteins when the membrane microviscosity is increased (Shinitzky and Inbar, 1976). An increase in membrane microviscosity reflects an overall increase in the lipid–lipid interaction and packing density of the lipid layer. Such a change will perturb the original thermodynamic equilibrium of the membrane and will shift it to a new state of minimum free energy. The observed increase in degree of exposure of the membrane proteins mediated by increase in the lipid microvicosity could, in principle, originate from a series of rearrangements other than the vertical displacement, or a combination of these. Such rearrangements may include reduction in the lateral interaction between the membrane pro-

teins, dissociation of their surface bonds with oligosaccharides or changes in their tertiary and quaternary structure. Unlike these processes, which may occur in particular instances, the vertical displacement of membrane proteins can be explained by direct and simple thermodynamic arguments (Borochov and Shinitzky, 1976). Membrane proteins, as typical amphipathic structures, will generally partition between the aqueous surroundings and the lipid layer according to the free energies of solubilization in these regions. Changes in the molar free volume of the lipid core, which is reflected in microviscosity changes, will affect its solubilization capacity and hence the equilibrium position of the membrane proteins. This physical mechanism, which may also control the exposure of membrane receptors and antigens, can play a significant role in modulation of membrane functions in vivo (Edelman, 1976; Raff, 1976; Shinitzky, 1976).

The above described method offers a simple means for determining the overall positioning of the membrane proteins. The method is especially adequate for following changes in the degree of exposure of proteins which are associated with the physiological activities of the membrane. Like most other methods available for membrane studies, the fluorescence quenching method obviously lacks specificity and the results obtained give an overall view of the bulk protein population. However, in membranes which contain one major protein, like the $Ca^{2+}$-ATPase in the sarcoplasmic reticulum (see Table III), the results may be related to this specific protein and can be correlated with its biochemical and physiological functions.

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