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MECHANISTIC DIFFERENCES IN THE ENERGY-LINKED FLUORESCENCE DECREASES OF 9-AMINOACRIDINE DYES ASSOCIATED WITH BOVINE HEART SUBMITOCHONDRIAL MEMBRANES

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(1) The pH dependence of the fluorescence intensities of 9-aminoacridines associated with energized submitochondrial membranes suggests that a mechanism(s) other than protonation of the dye molecules, as is the case with quinacrine, is responsible for the energy-linked fluorescence decreases of 9-aminoacridine and 9-amino-3-chloro-7-methoxyacridine (9-ACMA). (2) That the fluorescence polarization of quinacrine associated with submitochondrial membranes more than doubles upon energization of the membranes is attributed to: (i) the bulky side chain at the 9-position of the acridine moiety which hinders the molecular rotation of quinacrine and (ii) electrostatic forces resulting from the protonation of quinacrine·H^+ which induce tight binding between the dye molecules and the membranes. (3) The protonation of quinacrine associated with energized membranes, from the monoprotonated to the diprotonated species, takes place in the membrane phase, as evidence by the observation of a 'break' in both the Arrhenius plot of the respiratory rate and the plot of fluorescence polarization as a function of temperature. (4) That the measured fluorescence polarization of both 9-aminoacridine and 9-ACMA associated with both energized and nonenergized membranes is nearly zero suggests that the emitting species of these dye molecules are those in the 'free' form and that the membrane-bound molecules have formed nonfluorescent complexes; consequently no polarization can be measured.

Introduction

One of the unique observations made when aminoacridines are associated with chloroplasts [1], bacterial membranes [2], submitochondrial particles [3,4], or chromatophores [5] is a significant decrease in the dyes' fluorescence intensities when these membranes are energized. This phenomenon has been widely reported and discussed (cf. Refs. 6 and 7). In 1972, Schuldiner and associates [8,9] first reported that the fluorescence quenching of 9-aminoacridine dyes could be used as a quantitative measure of the internal pH of chloroplasts, if one assumes that the 9-aminoacridine dye molecules are taken up into the interior of the vesicles and, as a result, their fluorescence is completely quenched. Without any direct experimental evidence, these investigators had assumed that the accumulated dye molecules were homogeneously distributed in the internal, aqueous phase of the vesicles; however, no precise mechanism for the fluorescence quenching was given. This method has subsequently been applied to several other systems (cf. Ref. 10) and has, in fact, been treated by some investigators as a convenient method for

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determining the internal pH of acidic vesicles.

Since the acridine derivatives tested thus far exhibit similar fluorescence decreases when associated with energized membranes and also possess similar chemical structures, some investigators [8,9,11,12] have treated these dyes collectively, assuming that their fluorescence properties result from identical interactions with the membranes for each of these compounds. As the mechanism of the energy-linked fluorescence decrease has not yet been fully elucidated, the biological significance derived from the study of membrane systems and their associated processes utilizing such dyes as probes may, therefore, be questionable and even misleading.

It was in this regard that we began the investigation of the spectroscopic properties of two types of aminoacridine molecules, with the emphasis on exploring their similarities and differences with regard to their interactions with an 'energizable' membrane system. The dyes employed in this study were 9-aminoacridine, 9-amino-3-chloro-7-methoxyacridine (9-ACMA), and quinacrine. Our data have indicated that the mechanism responsible for the fluorescence decrease of quinacrine associated with energized submitochondrial membranes is distinctly different from that of 9-aminoacridine and 9-ACMA; among other factors, the substitution on the 9-amino group may play an important role in determining the mode of the dye-membrane interaction. Portions of these data have been communicated briefly [13–15].

Materials and Methods

Submitochondrial particles derived from heavy bovine heart mitochondria by sonic disruption in the presence of EDTA were prepared as described previously [16,17]. The particle preparation was then treated with oligomycin and malonate [17]. All experiments were performed with the above preparation unless otherwise indicated. 9-ACMA was synthesized according to the method of Irwin and Irwin [18]. All other chemicals were of the highest purity available commercially. Glass redistilled water was used throughout the present study. The oxidase activities were assayed polarographically [17] with a Clark oxygen electrode. Protein was determined by the biuret method [19]. An Aminco DW-2 ultraviolet-visible spectrophotometer was used for absorption measurements.

The fluorescence emission and excitation spectra were recorded with a Hitachi-Perkin Elmer (Model MPF-2A) fluorescence spectrophotometer. The instrument filter appropriate for the excitation wavelength was used to minimize scattered light interference. A Forma Scientific constant temperature circulator was used to control the temperature of the sample to within 0.1°C; unless otherwise indicated, all experiments were performed at 25°C. Alternatively, fluorescence measurements were made with an SLM fluorescence spectrophotometer (model 4000) interfaced with a data processor (HP 9815A).

The fluorescence polarization was measured with the aid of a set of Polacoat lenses as polarizer and analyzer. The polarization (P) and the anisotropy (r) of the fluorescence were calculated according to the following equations:

$$P = \frac{I_{VV} - GI_{VH}}{I_{VV} + GI_{VH}}; \quad r = \frac{I_{VV} - GI_{VH}}{I_{VV} + 2GI_{VH}} \tag{1}$$

where I_{VV} and I_{VH} are the fluorescence light intensities parallel and perpendicular, respectively, to the vertically polarized exciting beam; and I_{HH} and I_{HV}, respectively, are those with respect to horizontally polarized excitation. The G factor ($G = I_{HV}/I_{HH}$) is an instrument correction factor [20] which was determined for each given measurement of P.

The microviscosity (η) of the dye environment was determined through a modified form of the Perrin equation [21]:

$$\frac{r_0}{r} = 1 + \frac{kT}{v}\frac{\eta_0}{\eta} \tag{2}$$

where r_0 is the r value in a medium of infinite viscosity, k the Boltzmann constant, T the absolute temperature, and v the effective rotational volume of the dye. The relative microviscosity of the environment in the vicinity of quinacrine associated with both the energized and nonenergized membranes was estimated by assuming that the ratio of the fluorescence lifetimes (ζ) of quinacrine associated with energized and nonenergized membranes is equal to the ratio of the quantum yields

of quinacrine associated with the membrane in the two, respective energy states [14,22], and r_0 and kT/v are constant, regardless of the energy state of the membrane.

Results and Discussion

pH effects on the absorption spectra

The absorption spectra of 9-aminoacridine and 9-ACMA in aqueous media at various pH are shown in Fig. 1. For 9-ACMA an isosbestic point is clearly seen at about 350 nm, indicating that two species of the molecule are present in this pH range (Fig. 1B). For 9-aminoacridine the isosbestic point appears at 330 nm and a red shift of 8 nm, for absorption peaks in the long-wavelength region, is observed at pH 11.5 (Fig. 1A).

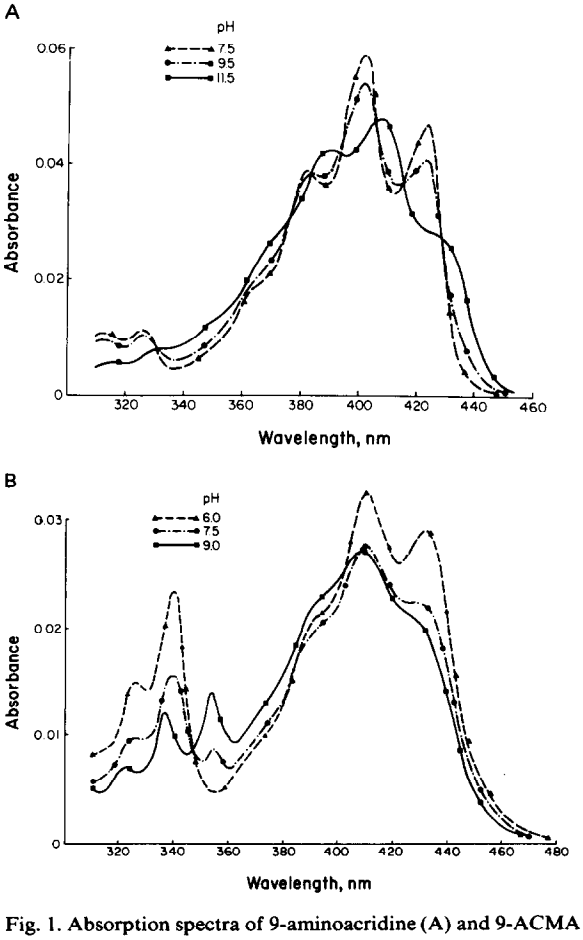

Fig. 1. Absorption spectra of 9-aminoacridine (A) and 9-ACMA (B). The reaction mixture consisted of 150 mM sucrose, 30 mM Tris-sulfate buffer, pH as noted, and 1.7 μM of the dye.

The relative abundances of the neutral (N) and protonated (NH^+) species of 9-aminoacridine and 9-ACMA are plotted as a function of pH (Fig. 2). The pK_a values derived are 9.75 and 8.80 for 9-aminoacridine and 9-ACMA, respectively. These values are close to those reported in the literature, which were determined at 20°C in aqueous solution [18,23,24].

Fluorescence spectra and fluorescence yield

The effects of pH on the fluorescence excitation and emission of 9-aminoacridine and 9-ACMA are shown in Figs. 3 and 4. For 9-aminoacridine, the fluorescence spectra measured at pH < pK_a (pH 7.5 and 9.5) are similar; however, both a red shift and changes in band structure can be seen at pH 10.5 (Fig. 3B). The band structure and peak position remain essentially the same in the case of 9-ACMA as the pH of the medium varies (Fig. 4). In contrast to the fluorescence of quinacrine, the fluorescence intensities of both 9-aminoacridine and 9-ACMA decrease upon increasing the pH of the medium. The fluorescence quantum yields of 9-aminoacridine and 9-ACMA were estimated on the basis of their respective absorption and emission spectra obtained at various pH. The fluorescence yields at given pH values were normalized to those at lower pH (pH 7.5 and 7.0 for 9-aminoacridine and 9-ACMA, respectively) which correspond to those of the protonated species (Fig. 5). The fluorescence yield of 9-aminoacridine remains constant over the pH range 7.5–9.5, decreasing slightly as the pH is further increased; the fluorescence yield of the neutral species (pH 11.5) is approx. 70% that of the protonated species (pH

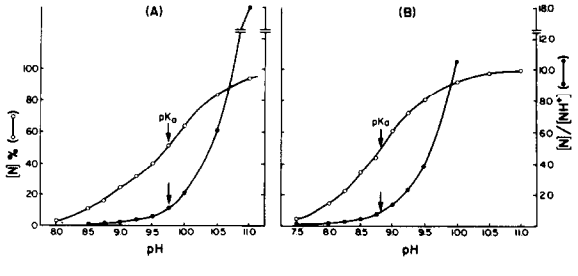

Fig. 2. Distribution of the neutral (N) and the protonated (NH^+) species, and the percent neutral species ([N]%) of 9-aminoacridine (A) and 9-ACMA (B). The reaction mixture was as indicated in Fig. 1.

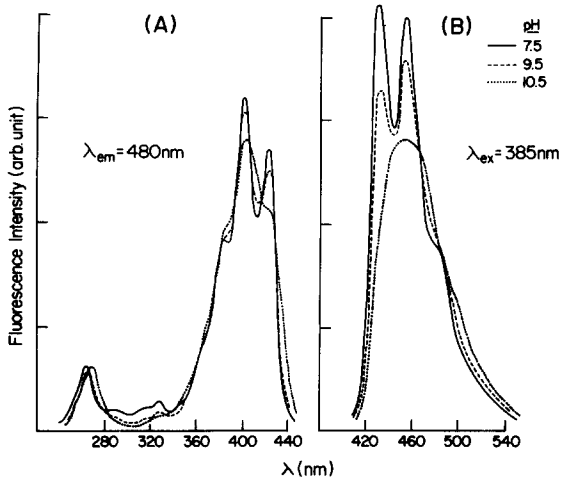

Fig. 3. Fluorescence excitation (A) and emission (B) spectra of 9-aminoacridine. The reaction mixture was as indicated in Fig. 1. The monitor emission and excitation wavelengths are indicated in the figure.

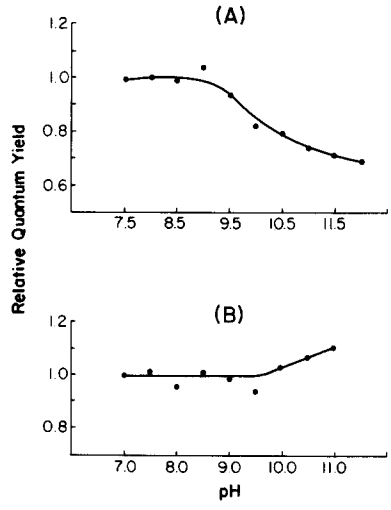

Fig. 5. The relative fluorescence quantum yield of 9-aminoacridine (A) and 9-ACMA (B) as a function of pH. The experimental conditions were as indicated in Figs. 1, 3 and 4.

7.5). The quantum yield of 9-ACMA does not change significantly between pH 7.0 and 9.0, with only a slight increase observed at higher pH. The fluorescence spectra and quantum yield of quinacrine in aqueous media of various pH were reported in our earlier work [22] which indicated that the quantum yield of quinacrine increases with increasing pH; the yield of the monoprotonated species of quinacrine is more than 3-times that of the diprotonated species.

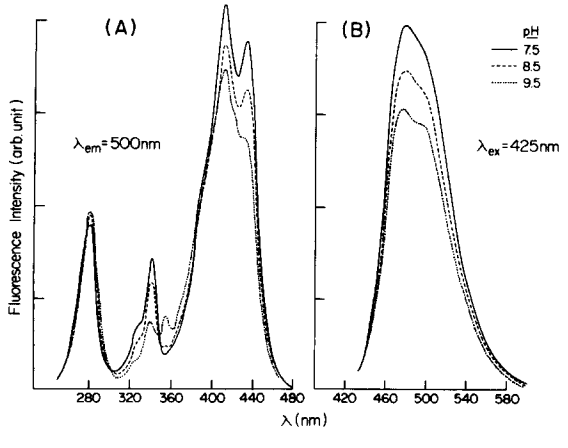

Fig. 4. Fluorescence excitation (A) and emission (B) spectra of 9-ACMA. The reaction mixture was as indicated in Fig. 1. The monitor emission and excitation wavelength are indicated in the figure.

Aminoacridines associated with submitochondrial membranes

When associated with bovine heart submitochondrial membranes, 9-aminoacridine, 9-ACMA, and quinacrine all exhibit an energy-linked fluorescence decrease (Fig. 6). The decrease in the fluorescence of quinacrine is attributed to the protonation of quinacrine molecules upon energization of the membranes, with the formation of the less fluorescent, diprotonated species [22].

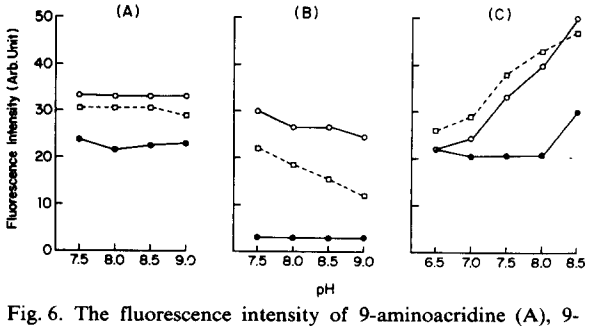

Fig. 6. The fluorescence intensity of 9-aminoacridine (A), 9-ACMA (B) and quinacrine (C) alone (○———○), associated with nonenergized (□———□) or energized (●———●) membranes, as a function of pH. The reaction mixture consisted of 150 mM sucrose, 30 mM Tris-sulfate buffer, 1.6 mM NaSCN, 1.7 μM dye and 0.3 mg submitochondrial particles/ml; pH as noted. 3.3 mM succinate was employed as the energy-yielding substrate. Quinacrine: λ_{ex} = 420 nm, λ_{em} = 510 nm. Others as in Figs. 3 and 4.

In contrast, the decreases in the fluorescence of both 9-aminoacridine and 9-ACMA, when associated with membranes in media of pH < pK_a, obviously do not result from a similar mechanism, since the protonated species, which possess equivalent or relatively higher fluorescence intensities, overwhelmingly predominate over the neutral species in this pH region (cf. Figs. 2 and 5).

Fluorescence polarization of aminoacridines associated with membranes

The degree of polarization of quinacrine, nearly zero in the absence of submitochondrial membranes, increases significantly when quinacrine is associated with nonenergized membranes, and further increases, by more than 2-fold, upon energization of the membranes [15]. These variations in polarization are observable over a pH range of 7.2–8.2 and at temperatures of 4–30°C. The fluorescence polarization of 9-aminoacridine and of 9-ACMA associated with submitochondrial membranes, in both the nonenergized and energized states, is, on the other hand, nearly identical to that of either dye alone in the medium, i.e., approximately zero [15].

The apparent zero fluorescence polarization of both 9-aminoacridine and 9-ACMA, when associated with either energized or nonenergized membranes, suggests that this polarization reflects only the fluorescence of those dye molecules that are 'free' in the medium and that any dye molecules associated with the membrane are nonfluorescent. The decreases in the fluorescence intensities of these dyes in the presence of submitochondrial membranes (cf. Fig. 6) must be due to the decreases in their effective concentrations in the external medium resulting from the binding of the dyes to the membrane. This binding increases dramatically upon energization (data not shown). Unlike quinacrine (see below), the membrane-bound 9-aminoacridine and 9-ACMA molecules do not exhibit fluorescence, perhaps as a likely result of the formation of nonfluorescent complexes between the dye molecules and some charged membrane components. Naturally, these membrane-bound dye molecules would not be reflected in the polarization data. Quinacrine, on the other hand, is in the fluorescent, diprotonated form when bound to the energized membranes [22] and thus shows a high degree of polarizability.

The polarization data for quinacrine associated with energized membranes correlate with the chemical and physical changes which occur in the microenvironment of the dye molecules as a result of probable, substantial alteration in the membrane structure which occurs upon energization. It is estimated (by Eqn. 2) that the viscosity of the microenvironment of quinacrine in energized membranes is increased nearly 2-fold over that in nonenergized membranes.

Changes in the microenvironment of quinacrine associated with energized membranes are best demonstrated by a plot of P vs. $1/T$ (Fig. 7). The degrees of polarization of quinacrine in both medium alone and associated with nonenergized membranes are very similar, with $P = 0.01$ and 0.08, respectively, regardless of temperature (data not shown). Since the emitting species of quinacrine under these conditions are identical, as evidenced by their identical fluorescence spectra (data not shown), the increase in P upon lowering the temperature, observed with energized membranes, is mainly attributable to changes in the environment of quinacrine which exert differing degree of spatial hindrance on the molecular rotation of quinacrine, with a distinct 'break' observable at 15°C. Thus, unlike the polarization of quinacrine alone or that of quinacrine associated with nonenergized membranes, which are insensitive to temperature changes, the degree of polarization of

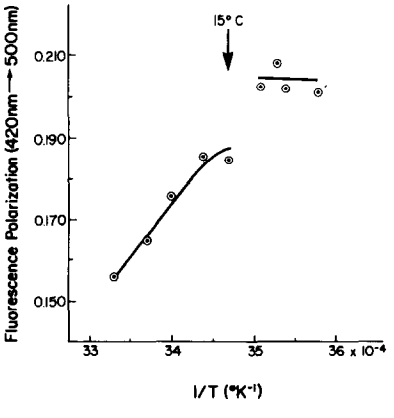

Fig. 7. Plot of P vs. $1/T$ (absolute temperature) for quinacrine associated with energized membranes. The experimental conditions were as indicated in Fig. 6, pH 7.5.

quinacrine associated with energized membranes exhibits a steady increase with respect to decreasing the temperature from 27 to 15°C; further decrease in temperature has little effect on *P* (Fig. 7). The appearance of a significant change in slope at about 15°C is suggestive of a phase change occurring in the microenvironment of the probe molecules. Similar observations made in several other membrane systems have been correlated with the occurrence of a phase transition in these systems [25–28].

That a considerable change does occur in the membrane environment around quinacrine, as a result of energization of the membrane, is further substantiated by the discontinuity in slope which occurs at the same temperature in an Arrhenius plot of the succinate oxidase activity catalyzed by submitochondrial membranes (Fig. 8). The Arrhenius activation energies calculated above and below 15°C are 8.2 and 18.3 kcal/mol, respectively. That such breaks at 15°C are observed in both the temperature profile of fluorescence polarization (Fig. 7) and in this Arrhenius plot are hardly coincidental, substantiating our contention that the emitting species of quinacrine, i.e., the

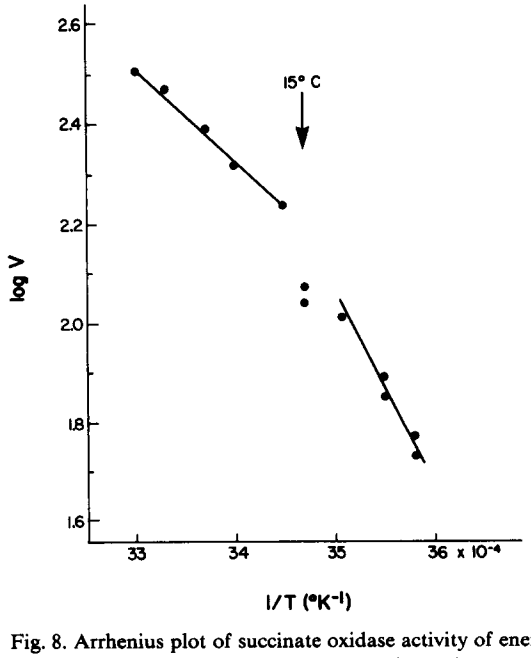

Fig. 8. Arrhenius plot of succinate oxidase activity of energized submitochondrial membranes. The reaction mixture was as indicated in Fig. 7.

diprotonated form, is located in the membrane phase and that these molecules are not readily equilibrated with the external medium.

This conclusion is further supported by other of our recent findings. Irradiation of a photoaffinity derivative of quinacrine (azidoquinacrine) in the presence of energized or nonenergized membranes results in the covalent binding of azidoquinacrine to the membranes [29], with the interaction dominated by the lipid components of the energized membranes [29,30]. In addition, we have shown that the anesthetic amines, chlorpromazine and butacaine, can effectively inhibit the energy-linked fluorescence quenching of quinacrine with submitochondrial particles [31]. The data indicate that there are specific binding sites for quinacrine with the membrane and that this effect is not due to uncoupling. In collaboration with Storey et al. [32], it has also been shown that submitochondrial particles derived from skeletal muscle mitochondria are also capable of supporting the energy-linked fluorescence decrease of quinacrine. However, these preparations apparently consist of open-membrane fragments which show energy coupling yet cannot sustain a transmembrane ion gradient; they are devoid of any isolated, vesicular space in which to accumulate dye molecules.

Fig. 9 shows the titration of the energy-linked response of 9-ACMA with chlorpromazine. Measurement of the respiratory rates of the oxidase activity indicates a lack of uncoupling by chlorpromazine over this concentration range (data not shown). These data indicate that, like quinacrine, there are indeed specific binding sites for 9-ACMA with the membrane and that chlorpromazine, which is structurally similar, is able to effect the release of the dye from its binding sites. This results in an increase in the amount of free 9-ACMA in solution, resulting in increased fluorescence. In addition, preliminary data (not shown) indicate a sharp decrease in quinacrine's polarization in the presence of chlorpromazine but little, if any, change in that of 9-ACMA, with either energized or nonenergized submitochondrial particles prepared as described in Materials and Methods. This further demonstrates that these aminoacridines are bound to the membranes and that those 9-ACMA molecules associated with the energized membranes are in a nonfluorescent form.

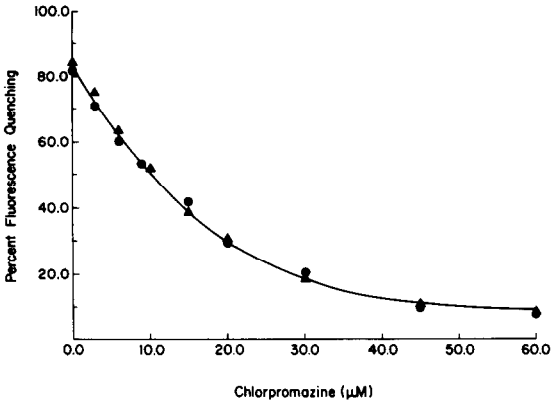

Fig. 9. Titration of the energy-linked fluorescence response of 9-ACMA with chlorpromazine. The reaction mixture consisted of 150 mM sucrose, 30 mM phosphate buffer (pH 7.5), 1.6 mM NaSCN, 2.0 μM 9-ACMA and 0.3 mg submitochondrial particles/ml. 135 μM NADH was employed as the energy-yielding substrate. The apparent K_i, the concentration of chlorpromazine which gives 50% inhibition of the energy-linked fluorescence response, is 15 μM. (●, ▲) Separate experiments. λ_{ex} and λ_{em} as in Fig. 4.

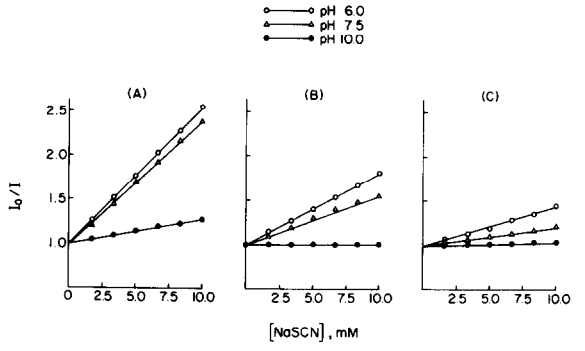

Fig. 10. Stern-Volmer plots of 9-aminoacridine (A), 9-ACMA (B) and quinacrine (C) at various pH, using SCN^- as the quencher. I_0 and I are the fluorescence intensities without and with quencher, respectively. The reaction mixture consisted of 150 mM sucrose, 20 mM phosphate buffer and 3.3 μM of the dye; pH as noted. λ_{ex} and λ_{em} as in Fig. 6.

The dye-membrane interaction

In the preceding discussion we suggested that the fluorescence decreases of 9-aminoacridine and 9-ACMA associated with submitochondrial membranes are due to the formation of nonfluorescent complexes, possibly through the interaction of the protonated species of the dyes with negatively charged membrane components, and/or the neutral species of the dye molecules with the membranes via hydrogen bonding. Such complex formation would be expected to occur readily with both 9-aminoacridine and 9-ACMA, since both lack a bulky side chain at the 9-amino group of the acridine nucleus. This mechanism may also account for the effective fluorescence quenching of aminoacridines by a variety of systems, such as nucleotides and polymers carrying charged components [33–39], and DNA molecules with large G-C contents [37–45].

To illustrate the characteristics of the formation of these nonfluorescent complexes Stern-Volmer plots, using the thiocyanate anion (SCN^-) as a quencher for aminoacridine fluorescence, in media of various pH values, are presented in Fig. 10. It is clearly illustrated that the quenching efficiency of SCN^- is greater at lower pH than at higher pH for all the aminoacridines tested; in addition, the quenching efficiency is significantly higher at pH $< pK_a$ than at pH $> pK_a$ for both 9-aminoacridine and 9-ACMA. Apparently, the protonated species of these dye molecules, being most abundant in the medium of lower pH, participate in the formation of nonfluorescent complexes with the quencher; the site of interaction is probably located on the ring nitrogen of the acridine moiety. It is worth noting that the presence of a charged, conjugated π-bonding system is essential for an efficient quenching effect.

In the case of quinacrine, the extent of quenching at all three pH values is much less pronounced, indicating that nonfluorescent complex formation, if any, is not an important factor in the fluorescence decrease of quinacrine observed with energized membranes. This is in full agreement with the fact that the energy-linked fluorescence decrease is a quantitative measure of the extent of protonation of the monoprotonated species of quinacrine into the diprotonated form (cf. Table I of Ref. 22).

Submitochondrial membranes in the nonenergized state also quench the fluorescence of 9-aminoacridine and 9-ACMA, as illustrated in Fig. 6. Since the quenching efficiency of membranes is higher in media of higher pH, where the neutral species of the dye are more abundant, the nonfluorescent complex(es) might result from the interaction of the neutral species of the dye with

the nonenergized membranes, presumably via hydrogen bonding with the 9-amino groups of the dye molecules. As quinacrine molecules are less prone to complex formation they exhibit a high degree of fluorescence polarization when associated with membranes, in either the nonenergized or energized state.

Conclusion

From our earlier discussion there is little doubt that the fluorescence decreases of 9-aminoacridine and 9-ACMA are, indeed, related to complex formation between the dye molecules and charged components of the submitochondrial membranes. The number of charged components in the membrane has been shown to increase considerably upon energization [46]. The observed fluorescence decreases can, therefore, be interpreted as resulting from the increased internal conversion rates of the dye molecules, which, in turn, are a consequence of complex formation between the dyes and molecules in the membrane with conjugated π-bonding systems. The latter type of molecules is not uncommon in biological systems.

It appears that the radiationless transition rates from the first electronically excited singlet state of the dye molecules are enhanced through the formation of such complexes; this enhancement is considerably greater for 9-aminoacridine and 9-ACMA than for quinacrine, as the former have greater capability of forming complexes with membrane components. It has been shown [47–49] that complexation involving an N-heterocyclic molecule with a conjugated π-bonding system, via hydrogen bonding in polar solvents, usually has the effect of inducing a strong ($S_1 \rightarrow S_0$) internal conversion, resulting in the fluorescence quenching of the molecule.

In summary, we conclude that while quinacrine is primarily probing the H^+ content generated during the steady state of the energized submitochondrial membranes, 9-aminoacridine and 9-ACMA are probing charged components associated with a conjugated π-bonding system. Both events occur in the membrane phase and result in apparently similar decreases in fluorescence intensity. Thus, the estimation of the ΔpH across submitochondrial membranes [50] induced upon energization, based on the assumption that aminoacridines are taken up and homogeneously distributed in the internal vacuoles, would be severely overestimated. Whether our conclusion is applicable to other membrane systems remains to be elucidated, since the nature of the interaction of any specific 9-aminoacridine probe may differ considerably among the various membrane systems. These differences could be due, in large part, to the exact experimental conditions employed or the composition of the membrane system under investigation, e.g., chloroplasts [51], which are significantly different from submitochondrial particles in their composition of both lipids and proteins. It is clear, however, that under our experimental conditions the mechanisms of the observed energy-linked fluorescence decrease of quinacrine and those of 9-aminoacridine and of 9-ACMA, with submitochondrial particles, though different, are nevertheless the result of direct interaction with the energized membranes rather than so-called 'stacking or screening' effects [11,12,52].

Acknowledgements

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References

1 Kraayenhof, R. (1970) FEBS Lett. 13, 161–165
2 Eilermann, L.J.M. (1970) Biochim. Biophys. Acta 216, 231–233
3 Lee, C.P. (1971) Biochemistry 10, 4375–4381
4 Azzi, A., Fabbro, A., Santato, M. and Gherardini, P.L. (1971) Eur. J. Biochem. 21, 404–410
5 Gromet-Elhanan, Z. (1971) FEBS Lett. 13, 125–126
6 Lee, C.P. (1974) Biochim. Biophys. Acta Library 13, 337–353
7 Kraayenhof, R., Brockelhurst, J.R. and Lee, C.P. (1976) in Concepts in Biochemical Fluorescence (Chen, R.F. and Edelhoch, H., eds.), pp. 767–809, Marcel Dekker, New York
8 Schuldiner, S. and Avron, M. (1971) FEBS Lett. 14, 233–236
9 Schuldiner, S., Rottenberg, H. and Avron, M. (1972) Eur. J. Biochem. 25, 64–70
10 Rottenberg, H. (1979) Methods Enzymol. 55, 547–569
11 Dell'Antone, P., Collona, R. and Azzone, G.F. (1971) Eur. J. Biochem. 24, 553–565

12 Dell'Antone, P., Collona, R. and Azzone, G.F. (1971) Eur. J. Biochem. 24, 566–576
13 Huang, C.S. and Kopacz, S.J. (1977) Fed. Proc. 36, 904
14 Huang, C.S. and Lee, C.P. (1977) Biophys. J. 17, 250a
15 Huang, C.S. and Lee, C.P. (1978) in Frontiers of Biological Energetics, Vol. 2 (Dutton, P.L., Leight, J.S., and Scarpa, A., eds.), pp. 1285–1292, Academic Press, New York
16 Lee, C.P. and Ernster, L. (1967) Methods Enzymol. 10, 543–548
17 Lee, C.P. (1979) Methods Enzymol. 55, 105–112
18 Irwin, J.M. and Irwin, E.N. (1950) J. Am. Chem. Soc. 72, 2743–2749
19 Gornall, A.G., Cardawill, C.J. and David, M.M. (1949) J. Biol. Chem. 177, 751–766
20 Chen, R.F. and Bowman, R.L. (1965) Science 147, 729–732
21 Weber, G. (1953) Adv. Protein Chem. 8, 415–459
22 Huang, C.S., Kopacz, S.J. and Lee, C.P. (1977) Biochim. Biophys. Acta 459, 241–249
23 Albert, A. and Goldacre, R. (1946) J. Chem. Soc. 706–713
24 Albert, A. (1965) J. Chem. Soc. 4653–4657
25 Vanderkooi, J., Fischkoff, S., Chance, B. and Cooper, R.A. (1974) Biochemistry 13, 1589–1595
26 Lentz, B.R., Barncholz, Y. and Thompson, T.E. (1976) Biochemistry 15, 4521–4528
27 Lentz, B.R., Barneholz, Y. and Thompson, T.E. (1976) Biochemistry 15, 4529–4537
28 Faucon, J.F., Dufourcq, J., Lussan, C. and Bernon, R. (1976) Biochim. Biophys. Acta 436, 283–294
29 Mueller, D.M., Hudson, R.A. and Lee, C.P. (1982) Biochemistry 21, 1445–1453
30 Mueller, D.M. (1981) Ph.D. Dissertation, Wayne State University, Detroit, MI
31 Mueller, D.M. and Lee, C.P. (1982) FEBS Lett. 137, 45–48
32 Storey, B.T., Scott, D.M. and Lee, C.P. (1980) J. Biol. Chem. 255, 5224–5229
33 Kubota, Y. (1973) Chem. Lett. (Jap.) 299–304
34 Kubota, Y. (1977) Chem. Lett. (Jap.) 311–316
35 Kubota, Y. and Fujisaki, Y. (1977) Bull. Chem. Soc. (Jap.) 50, 297–298
36 Georghiou, S. (1975) Photochem. Photobiol. 22, 103–109
37 Thomas, J.C., Weill, G. and Daune, M. (1969) Biopolymers 8, 647–659
38 Weisblum, B. and De Haseth, P.L. (1972) Proc. Natl. Acad. Sci. U.S.A. 69, 629–632
39 Pachman, U. and Rigler, R. (1972) Exp. Cell Res. 72, 602–608
40 Lerman, L.S. (1961) J. Mol. Biol. 3, 18–30
41 Lerman, L.S. (1963) Proc. Natl. Acad. Sci. U.S.A. 49, 94–102
42 Weil, G. and Calvin, M. (1963) Biopolymers 1, 401–417
43 Tubbs, R.K., Ditmars, W.E. and Van Winkle, Q. (1964) J. Mol. Biol. 9, 545–557
44 Weil, G. (1965) Biopolymers 3, 567–572
45 Ellerton, N.F. and Isenberg, I. (1969) Biopolymers 8, 767–786
46 Singer, I. and Tasaki, I. (1968) in Biological Membranes (Chapman, D., ed.), pp. 347–410, Academic Press, New York
47 Mataga, N. (1958) Bull. Chem. Soc. (Jap.) 31, 481–486
48 Mataga, N. (1958) Bull. Chem. Soc. (Jap.) 31, 487–491
49 Lim, E.C. and Yu, M.H. (1966) J. Chem. Phys. 45, 4742–4743
50 Rottenberg, H. and Lee, C.P. (1972) Biochemistry 14, 2675–2680
51 Haraux, F. and De Kouchkovsky, Y. (1980) Biochim. Biophys. Acta 592, 153–168
52 Massari, S., Dell'Antone, P., Colonna, R. and Azzone, G.F. (1974) Biochemistry 13, 1038–1043