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KINETIC PROCESSES IN *ESCHERICHIA COLI* MEMBRANES AND CELLS A LASER PHOTOLYSIS STUDY USING DERIVATIVES OF PYRENE*

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SUMMARY

Pyrene and several derivatives of pyrene are used to investigate photo-induced kinetic processes in whole cells and membranes extracted from *Escherichia coli*. A mutant of *E. coli* was used which, under appropriate growth conditions, produced a complete or incomplete lipopolysaccharide in the outer membrane. The pyrene derivatives used were: pyrene sulfonic acid, pyrene butyric acid and the ester of pyrene butyric acid and 10-hydroxydecanoic acid. The pyrene chromophore was excited by the ultraviolet pulse from a Q switch, frequency-doubled, ruby laser. The lifetimes of the pyrene fluorescence in the presence of the quenchers O_2, thallous ion (Tl^+), I^- and CH_3NO_2 were measured and tabulated as second order rate constants. For the most part the quenching rate constants were much lower than the corresponding values observed in simple nonviscous solution, e.g. ethanol. This is interpreted as being due to the location of the probe within the membrane. The membrane inhibits the movement of the quenchers to the excited state.

Cell membranes containing complete lipopolysaccharide showed significantly lower quenching rates for the probes pyrene and pyrene sulfonic acid than cell membranes with incomplete lipopolysaccharide. From an analysis of the kinetic data it is suggested that pyrene and pyrene sulfonic acid are located near and under lipopolysaccharide and close to membrane proteins. On the other hand, no effect of lipopolysaccharide composition was observed for the probes pyrene butyric acid and pyrene butyroyl decanoic acid. This may suggest that these probes are located primarily in the lipid part of the membrane. A simple model for the outer membrane of *E. coli* is suggested that accounts for the observed laser-induced kinetic processes.

INTRODUCTION

In earlier work a laser photolysis technique was used to study the photochemistry of pyrene in simple solvents such as methanol and cyclohexane. The singlet

Abbreviation: ANS, anilinonaphthalene sulfonate.

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excited state of pyrene was observed in these experiments, together with the complex excimer excited state [1]. In polar media, pyrene cations and solvated electrons were also observed [1]. The kinetics of formation of the excimer from the excited state of pyrene and the ground state were measured, and the rate constants for these processes calculated. Similar studies in more complex systems such as micellar aggregates [2–4] showed that pyrene singlet excited states could be used to measure the internal properties of these more complex systems. The pyrene excimer state was observed in these systems at higher pyrene concentrations [4]. The formation of the pyrene excimer state was studied in both spherical and rod-like micellar systems. The detailed kinetics of the process whereby pyrene excited states and pyrene diffuse through the interior of the structures to form excimers has been discussed [4, 5]. Similar studies have been used in systems of more biological interest [9, 10]. For example, the simple bile acids and mixtures of bile acids with detergents have been probed by means of pyrene excited states. These studies are similar to those used in the detergent micelles, given above. Other studies [11, 12] have been made with pyrene in phospholipid dispersions. The variation in the lifetime of monomeric singlet excited state, and the quenching rate of the state by additives external to the lipid, measure the permeability of the systems to various entities. An estimate of the lipid phase changes with temperature in these systems was also measured.

With this knowledge, it is possible to use the pyrene system to investigate the properties of membranes extracted from *Escherichia coli* cells. The phase changes with temperature for inner and outer membranes of *E. coli* are readily measured as a function of pyrene monomer emission lifetime [13]. It has been instructive to compare the pyrene data obtained in these membrane systems to data obtained using other techniques [14]. Recently the use of the pyrene excimer system has been extended [6, 11, 15] to various membranes and phospholipid dispersions. The excimer of pyrene is used to give some measure of lateral diffusion in these systems.

The use of fluorescent probes in studying the structures of biological membranes is not a new innovation, and a significant amount of work has been carried out with various probes such as anilinonaphthalene sulfonate (ANS). One intriguing example of such work involves the construction of probes at the end of the long chain fatty acids [16]. A chromophore such as anthracene is synthesized at the end of the long hydrocarbon chain of a fatty acid, and this molecule is embedded in a membrane system. The polar carboxylate group normally associates with the polar head groups of the membrane surface and the anthracene is pressed into the interior of the membrane system. In the present study we have constructed several derivatives of pyrene in order to investigate (a) surface of the membrane, (b) positions just inside the membrane and (c) positions approximately 16 carbon atoms into the membrane and away from the membrane surface. Three different probes are used in these studies; pyrene sulfonic acid for the surface, pyrene butyric acid for probing just inside the surface, and the ester synthesized from pyrene butyric acid and the 10-hydroxydecanoic acid for probing further into the membrane. The kinetic processes observed with these three probes are compared with those observed with free pyrene, which may be dissolved in many different sites within the membrane. The biological systems used in these studies were *E. coli* inner and outer membranes and whole cells of *E. coli*.

EXPERIMENTAL

The laser equipment used in these studies has been described before [17, 18]. Basically, an ultraviolet pulse is developed in a Q-switched ruby laser by frequency doubling. The wavelength of the ultraviolet pulse is 347.1 nm, the duration of the pulse is 15 ns and the output of the pulse is 0.2 J. The ultraviolet pulse impinges on a rectangular quartz cell, which has short dimensions of 1 cm, or smaller if necessary. This cell contains the aqueous suspension of the membranes or whole cells. The pyrene chromophore, which is situated in the membrane, is excited by the ultraviolet laser pulse and the emitted fluorescence is subsequently monitored by fast spectrophotometric techniques. The net end point of the experimental measurement is a polaroid picture of the fluorescent intensity versus time. A typical trace is shown in Fig. 1. The ordinate in the insert shows the fluorescence intensity and the abscissa gives the time. The main body of the picture shows a typical computer plot of the data, which is usually first order. A rate constant or half-life for the decay of the pyrene excited state under the particular experimental condition used can be extracted from this first order plot. The samples are saturated with a suitable gas of interest by bubbling for prolonged periods of time. The *E. coli* strain, J5, used in these experiments is a galE mutant of *E. coli* 0111 : B4. When grown in the presence of galactose, the cells produce lipopolysaccharide containing a long carbohydrate chain (complete), while in the absence of galactose a short carbohydrate chain lipopolysaccharide (incomplete) is produced [7, 8]. The inner and outer membrane of *E. coli* are prepared according to procedures previously described [13]. Pyrene was purchased from the Aldrich Co. and purified chromatographically. Pyrene butyric acid was purchased from Pfatz and Bower Co. and the acyl chloride of this material was then synthesized. The acyl chloride was subsequently reacted with 10-hydroxydecanoic acid to produce the ester of pyrene butyric acid and hydroxydecanoic acid. This probe was kindly supplied to us by Mr. Kalyanasundaram [4]. Pyrene sulfonic acid was purchased from Eastman Kodak Co. The acidic probes were incorporated into the membranes

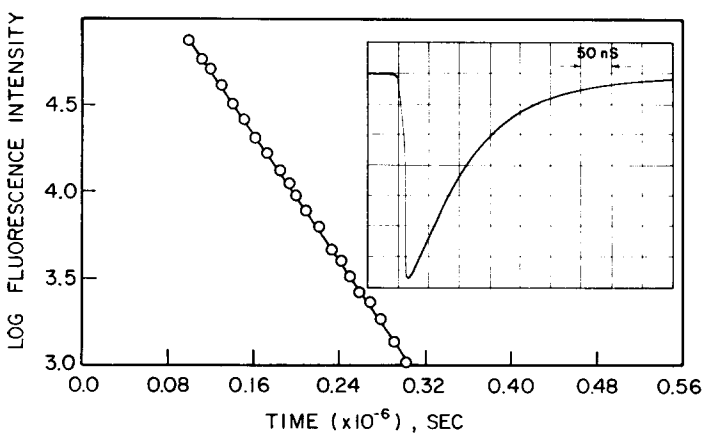

Fig. 1. The oscillographic trace of the decay of the pyrene butyric acid singlet excitated states and the corresponding semilogarithmic plot of the fluorescence intensity versus time for that fluorescence decay. The emission was monitored at 400 nm, 24 °C. [Pyrene butyric acid] = $2 \cdot 10^{-6}$ M.

by stirring a small quantity of the probe with the membrane suspension for 10 min. Pyrene was incorporated in the following manner. A solution of pyrene in hexane was evaporated on the inside of a small flask to produce a thin coating of pyrene on the flask wall. The membrane suspension was then agitated in this flask over a period of 1 h at 25 °C, when it was determined that sufficient pyrene had been incorporated into the membrane. The quantity of pyrene incorporated in the membrane was checked by its characteristic fluorescence on an Aminco-Bowman spectrofluorometer, or by its characteristic absorption spectrum on a Cary spectrophotometer. The probes were introduced into whole cells in a similar manner.

EXPERIMENTAL DATA

The experimental measurements in each case involve exciting pyrene with the ultraviolet laser pulse and subsequently observing the fluorescence decay rate. The decay rate of the pyrene is increased if various additives are added to the aqueous phase. Rate constants for the quenching of the pyrene excited state by the added quenchers may be derived from plots [13] such as those shown in Fig. 1. The following Figs 2–5 show the rate constants for quenching of the pyrene excited singlet state by several quenchers for the four probes, pyrene sulfonic acid, pyrene byturic acid, the ester of pyrene butyric acid and 10-hydroxydecanoic acid and pyrene. Two uncharged quenchers, nitromethane and oxygen, and two charged quenchers, I^- and Tl^+, are used in the quenching experiments. For convenience, in Figs 2–5 the rate constants for the reaction of the quencher with the probe is plotted for all the different types of cell and membrane systems used against the particular probe. This is shown for each of the quenchers, Tl^+, O_2, nitromethane and I^-.

Quenching by nitromethane

Fig. 2 shows the rate constant for the quenching of the excited state of pyrene in each of the above probe molecules by nitromethane. Six different conditions are illustrated for each probe: (a) whole cell with complete lipopolysaccharide, (b) whole cell with incomplete lipopolysaccharide, (c) separated outer membrane with complete lipopolysaccharide, (d) separated outer membrane with incomplete lipopolysaccharide, (e) separated inner membrane from cells with complete lipopolysaccharide and (f) separated inner membrane from cells with incomplete lipopolysaccharide.

The presence of short carbohydrates chain (incomplete) or long carbohydrate chain (complete) lipopolysaccharide has little effect on the quenching rate constant for the whole cell systems (a) and (b). The absolute value of k is $(2\pm1) \cdot 10^9\ M^{-1} \cdot s^{-1}$ for pyrene sulfonic acid, pyrene butyric acid and pyrene butyroyl decanoic acid, but falls off dramatically for pyrene to $6 \cdot 10^7\ M^{-1} \cdot s^{-1}$. This indicates that the location of pyrene in the cell membranes is quite different from that of the other probes. All probes are screened by the membrane from the nitromethane, as the rate constant for quenching of excited states of pyrene by nitromethane is $8.1 \cdot 10^9\ M^{-1} \cdot s^{-1}$ in homogeneous solution [2].

The quenching rate constants show a continual decrease from pyrene sulfonic acid to pyrene for the outer membrane with incomplete lipopolysaccharide. The same behaviour is also illustrated by the outer membrane with complete lipopolysaccharide

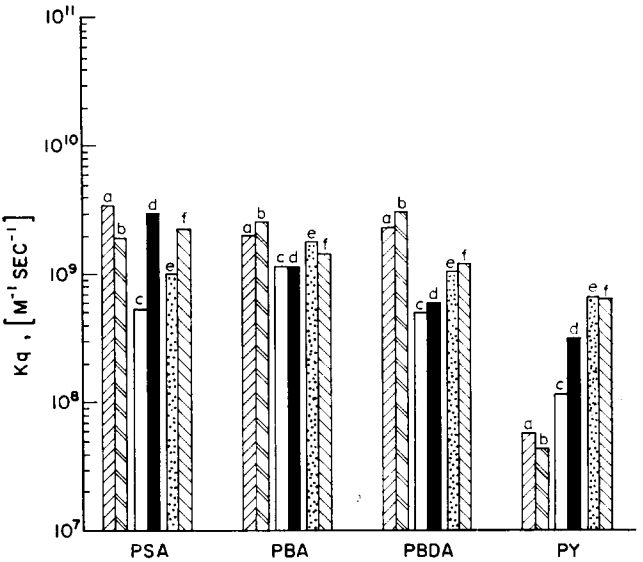

Fig. 2. Quenching rate constant of CH_3NO_2 with pyrene sulfonic acid (PSA), pyrene butyric acid (PBA), pyrene butyroyl decanoic acid (PBDA) and pyrene (PY) excited singlet states in cells and membranes. (a) +galactose, whole cell; (b) −galactose, whole cell; (c) outer membrane from +galactose cell; (d) outer membrane from −galactose cell; (e) inner membrane from +galactose cell; (f) inner membrane from −galactose cell.

apart from the pyrene sulfonic acid probe, where complete lipopolysaccharide gives a low quenching rate constant. In micellar systems it has been shown [4] that the pyrene chromophore is located progressively further into the interior of the micelle with the series of probes pyrene sulfonic acid, pyrene butyric acid, pyrene butyroyl decanoic acid and pyrene. The decrease in k_q from pyrene sulfonic acid to pyrene can be attributed to a similar situation in membranes, where the pyrene chromophore is gradually located further into the membrane. If the nitromethane is located primarily in the aqueous phase, then it is progressively more difficult for it to penetrate into the membrane regions containing pyrene in the series pyrene sulfonic acid → pyrene. If pyrene sulfonic acid is on the surface of the membrane, then it is possibly surrounded by a greater amount of carbohydrate on the lipopolysaccharide in condition (c) than (d). This leads to a lower rate constant. The percentage of lipopolysaccharide in the surface could also affect the rate of penetration of nitromethane into the membrane. If pyrene is located deep into the membrane and under the lipopolysaccharide, then similar effects will be observed for pyrene. As the pyrene butyric acid and pyrene butyroyl decanoic acid do not show this effect, it is concluded that they are located in some other region of the membrane.

The inner membrane shows little effect of whether the cells had synthesized complete or incomplete lipopolysaccharide. This is to be expected as the lipopolysaccharide should not be on the inner membrane [19]. A decrease in k_q is noted from pyrene sulfonic acid to pyrene.

Quenching by I^-

Fig. 3 shows the quenching rate constant k_q for the reaction of I^- with the

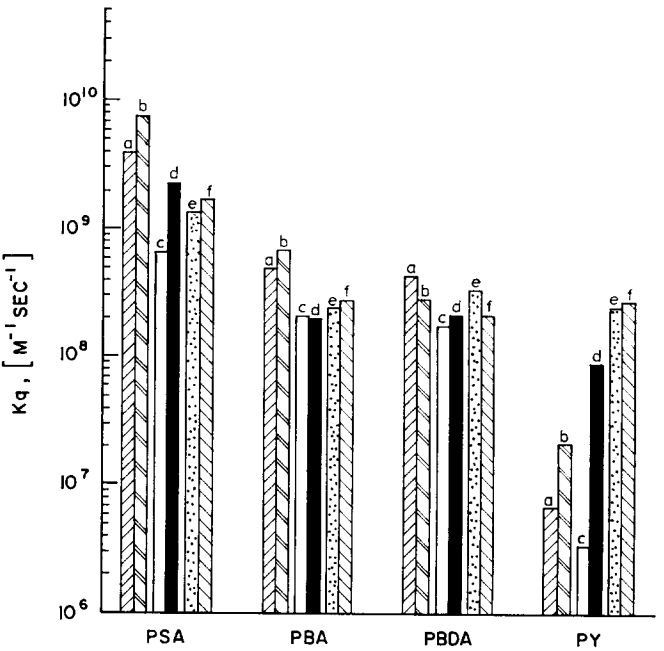

Fig. 3. Quenching rate constant of I^- with pyrene sulfonic acid (PSA), pyrene butyric acid (PBA), pyrene butyroyl decanoic acid (PBDA) and pyrene (PY) excited singlet states in cells and membranes. See explanation in Fig. 2.

probes pyrene sulfonic acid, pyrene butyric acid, pyrene butyroyl decanoic acid and pyrene in the systems (a) to (f).

With whole cells k_q decreases in going from pyrene sulfonic acid to pyrene. The parameter k_q for pyrene sulfonic acid and pyrene is slightly smaller for cells containing complete lipopolysaccharide compared to cells having incomplete lipopolysaccharide. The much lower k_q for pyrene compared to the other probes, again indicates that pyrene is shielded from I^- to a greater extent than the other probes. This might indicate that it is situated further into the membrane or in a different region of the membrane.

For the outer membrane (c) and (d) k_q falls substantially from pyrene sulfonic acid to pyrene butyric acid, is fairly constant from pyrene butyric acid to pyrene butyroyl decanoic acid and then falls on going to pyrene. There is little effect of lipopolysaccharide carbohydrate content on k_q for pyrene butyric acid and pyrene butyroyl decanoic acid. However, complete lipopolysaccharide with higher carbohydrate content leads to a smaller k_q for pyrene sulfonic acid and a much smaller k_q for pyrene. This latter point was noted previously [13]. It is suggested that negative charges built up on lipopolysaccharide repel I^- leading to lower k_q. Both whole cells and outer membranes show similar effects of lipopolysaccharide carbohydrate content on k_q for pyrene sulfonic acid and pyrene.

No significant effect of lipopolysaccharide carbohydrate content on k_q is observed for the inner membranes. The quenching rate constant decreases from pyrene sulfonic acid to pyrene butyric acid and is then more or less constant to pyrene.

The absence of any lipopolysaccharide effect is expected if lipopolysaccharide resides almost completely in the outer membrane.

Quenching by Tl^+

The quenching rate k_q for the reaction of Tl^+ with the excited states of the probes pyrene sulfonic acid, pyrene butyric acid, pyrene butyroyl decanoic acid and pyrene, in the systems (a) through (f) are shown in Fig. 4. The initial observation is that lipopolysaccharide structure has little effect on the data in the whole cell system and k_q decreases slightly from pyrene sulfonic acid to pyrene.

For the outer membranes, k_q also decreases only a little from pyrene sulfonic acid to pyrene and the effect of lipopolysaccharide structure is not large. For pyrene, decreasing the lipopolysaccharide carbohydrate content decreases k_q.

No effect of lipopolysaccharide carbohydrate content is observed for the inner membranes, again as would be expected. The quenching rate k_q increases from pyrene sulfonic acid to pyrene butyric acid and then decreases for pyrene butyroyl decanoic acid and pyrene.

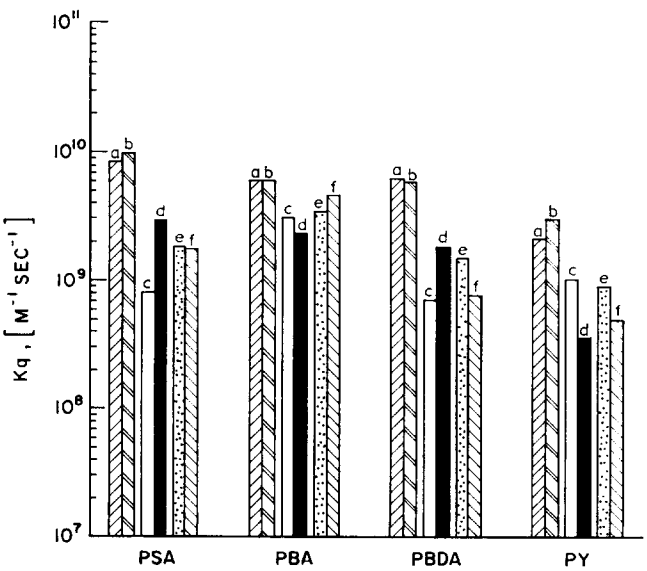

Fig. 4. Quenching rate constant of Tl^+ with pyrene sulfonic acid (PSA), pyrene butyric acid (PBA), pyrene butyroyl decanoic acid (PBDA) and pyrene (PY) excited singlet states in cells and membranes. See explanations in Fig. 2.

Quenching by O_2

The quenching rate constants k_q for the reaction of O_2 with the excited states of the probes pyrene sulfonic acid, pyrene butyric acid, pyrene butyroyl decanoic acid and pyrene in the system (a)–(f) are shown in Fig. 5. The rate constants k_q are all large, approx. 10^9–10^{10} $M^{-1} \cdot s^{-1}$ and independent of lipopolysaccharide carbohydrate content. For the whole cells, k_q is 10^{10}–$5 \cdot 10^9$ $M^{-1} \cdot s^{-1}$ and approaches the diffusion-controlled limit of $2 \cdot 10^{10}$ $M^{-1} \cdot s^{-1}$ observed in homogeneous and single

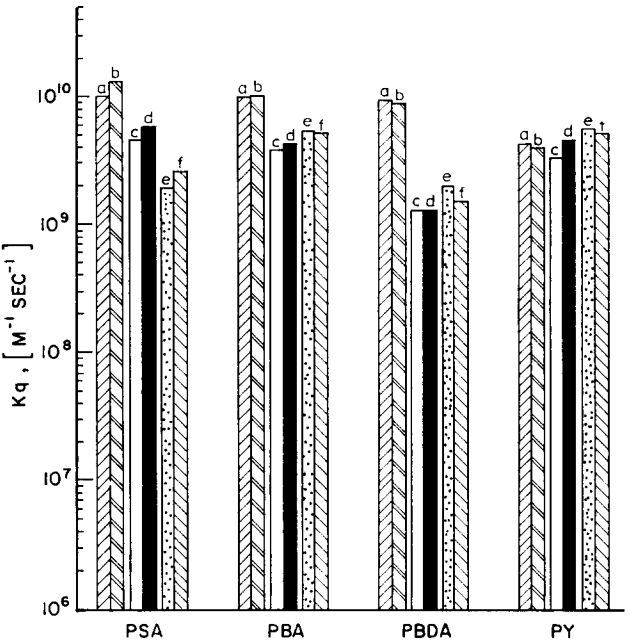

Fig. 5. Quenching rate constants of O_2 with pyrene sulfonic acid (PSA), pyrene butyric acid (PBA), pyrene butyroyl decanoic acid (PBDA) and pyrene (PY) excited singlet states in cells and membranes. See explanations in Fig. 2.

solution. This suggests a high degree of permeability of the cells to O_2, or more probably a situation where O_2 is already in the membrane.

The k_q in the outer membrane shows an initial decrease from pyrene sulfonic acid to pyrene butyric acid to pyrene butyroyl decanoic acid, followed by an increase to pyrene. The absolute values of k_q in the outer membranes are lower than in the whole cells for pyrene sulfonic acid, pyrene butyric acid and pyrene butyroyl decanoic acid. For pyrene the values are constant in whole cells, outer and inner membranes. These data suggest that O_2 is already dissolved in the outer membrane, and that quenching of the excited states occurs by the movement of the membrane oxygen to the probe. The probes pyrene sulfonic acid, pyrene butyric acid and pyrene butyroyl decanoic acid are not as mobile as pyrene, owing to the interaction of the acid groups with polar groups on the membrane surface. Pyrene, however, is not restricted in this way. The quenching rate constant may decrease from pyrene sulfonic acid to pyrene butyric acid to pyrene butyroyl decanoic acid owing to an oxygen gradient across the membrane. The increase to pyrene is possibly due to the additional mobility of this probe.

Similar effects are observed in the inner membrane for pyrene butyric acid, pyrene butyroyl decanoic acid and pyrene and are explained in a manner similar to that used in the outer membrane system. However, pyrene sulfonic acid is not quenched as rapidly as the other probes. This can be explained by a lower $[O_2]$ close to the membrane surface for the inner membrane. This effect is not observed in the outer membrane, as the cell surface has lipopolysaccharide, which may also have a higher $[O_2]$.

DISCUSSION AND CONCLUSIONS

Trauble and Overath [21] have suggested a model for the inner membrane of *E. coli*. The model is in accord with the Davison-Danielli-Robertson-Benson-Singer statements on membranes. A lipid bilayer is suggested, with proteins on the surface as well as protruding through the membrane. For *E. coli* inner membrane, Trauble and Overath are able to give the fraction of the lipid in the bilayer compared to that associated with the protein. The lipid in the region of the protein will have disrupted structure lacking the order of the bilayer. They can also give a measure of the relative amount of protein on the surface. We propose a similar picture for the outer membrane and have interpreted our data accordingly. A diagram of the model for the outer membrane is shown in Fig. 6. The protein is shown in three different locations in a matrix of phospholipid. The lipopolysaccharide is shown to be associated with the lipid near the protein [22]. Regions of disorder are indicated near the protein. The probes are also shown at various selected sites in the membrane.

The site of location of the probe is only partly understood in membranes as described above. If the probes pyrene sulfonic acid, pyrene butyric acid, pyrene butyroyl decanoic acid and pyrene reside in the lipid bilayer, then the acid groups of the first three molecules will associate with the polar surface of the membrane. As in a micellar system, the pyrene absorption of pyrene sulfonic acid will be close to the surface, that of pyrene butyric acid further into the membrane and that of pyrene butyroyl decanoic acid considerably further into the membrane. In the latter probe, the pyrene absorption should be close to the center of the membrane. Pyrene will move throughout the membrane, but will tend to reside in regions of least lipid restriction, i.e. away from the polar head groups and towards the center of the membrane. The movement

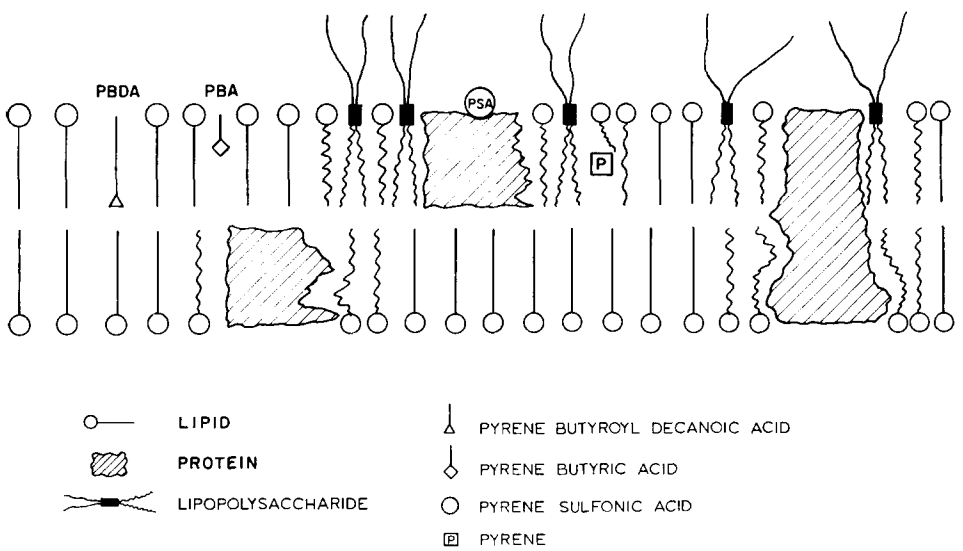

Fig. 6. A simplified model of the outer membrane of *E. Coli* with the proposed sites of different probes.

of pyrene within lipid structures has already been illustrated [3, 10, 11, 9, 5].

In the presence of a quencher which is mainly in the aqueous phase, k_q should decrease from pyrene sulfonic acid to pyrene butyric acid to pyrene butyroyl decanoic acid. The site of pyrene butyroyl decanoic acid is similar to that of pyrene, but the mobility of pyrene in the membrane is greater than that of pyrene butyroyl decanoic acid, hence an increase in k_q might be expected from pyrene butyroyl decanoic acid to pyrene. For nitromethane, a smooth decrease in k_q is noted from pyrene sulfonic acid to pyrene. With I^-, k_q decreased from pyrene sulfonic acid to pyrene butyric acid and is constant to pyrene. With Tl^+, k_q increases to pyrene butyric acid, decreases to pyrene butyroyl decanioc acid and shows a further decrease to pyrene. The data indicate that the site of pyrene is different from that of pyrene in pyrene butyroyl decanoic acid.

This is best explained if we consider the effect of protein on the membrane. For example, the strongly lipid nature of pyrene butyric acid and pyrene butyroyl decanoic acid will still tend to locate these molecules in the lipid bilayer. However, pyrene may prefer the less restricted and disordered region of the lipid in contact with protein (Fig. 6). Pyrene sulfonic acid, being a strong acid, may also associate with polar groups in the protein, but is still located toward the polar surface. This model provides an explanation for the differing site of pyrene and pyrene butyroyl decanoic acid, which is indicated by the CH_3NO_2 and Tl^+ data. It also explains the decrease in k_q for pyrene sulfonic acid compared to pyrene butyroyl decanoic acid. Pyrene sulfonic acid will associate with a positively charged region of the protein causing a repulsion of Tl^+ and decrease in k_q and an enhancement of the I^- quenching reaction.

Oxygen is a special case and is best explained if the O_2 is also present in the membrane and not only in the aqueous phase, as for the other quenchers. The k_q values are then all very large, being close to the values for diffusion-controlled rates in simple solution. If an O_2 gradient exists in the membrane from the surface to the center, then k_q will decrease from pyrene butyric acid to pyrene butyroyl decanoic acid as observed. However, the site of pyrene is less restricted than that of pyrene butyric acid and pyrene butyroyl decanoic acid and the $[O_2]$ may be higher here than in the lipid bilayer portion of the membrane. Pyrene sulfonic acid is more closely associated with the protein and the O_2 content could well be smaller in that region.

To a first approximation it is expected that the kinetic behavior in whole cells and separated outer membranes will be similar. The probes will probably not penetrate beyond the outer membrane of the whole cells. In one series of experiments, pyrene was incubated with whole cells and the inner and outer membranes were separated, pyrene was found to reside exclusively in the outer membrane of whole cells, in agreement with the preceding suggestion. A major difference of the inner membrane from the outer membranes is the presence of lipopolysaccharide in the outer membranes. We can take the preceding model membrane structure, with the modification that the outer membrane surface will also have a coating of lipopolysaccharide. In the present experiments the carbohydrate content of the lipopolysaccharide was varied by using a galE mutant as described in the Methods section.

A comparison of the quenching data in membranes with complete and incomplete lipopolysaccharide is advantageous at this stage for the outer membrane the nitromethane and I^- data, Figs 2 and 3, indicate that lipopolysaccharide carbohydrate content does not play a significant role for the kinetic quenching of the probes

pyrene butyric acid and pyrene butyroyl decanoic acid. However, for both pyrene sulfonic acid and pyrene the incomplete lipopolysaccharide gives rise to a larger k_q, which is interpreted as being due to a larger permeability of the membrane to CH_3NO_2 and I^-. This effect is also observed for the whole cells with I^-, but not for whole cells with CH_3NO_2. The Tl^+ (Fig. 4) shows no effect of lipopolysaccharide carbohydrate content on the whole cells, and little effect for the outer membrane for the probes pyrene sulfonic acid, pyrene butyric acid, pyrene butyroyl decanoic acid and pyrene. The influence of lipopolysaccharide is larger for pyrene, where complete lipopolysaccharide leads to a large k_q.

The effect of lipopolysaccharide in I^- and Tl^+ mobility is best explained by the presence of a negative charge on the lipopolysaccharide. The larger negative charge from the higher carbohydrate content of complete lipopolysaccharide repels I^- and decreases k_q in Fig. 3, while Tl^+ is attracted and k_q increases in Fig. 4. This is the same for the probes pyrene sulfonic acid and pyrene and indicates that they are situated in a similar environment of the membrane. The CH_3NO_2 data also indicate similar effects of lipopolysaccharide carbohydrate content. The absence of any effect of lipopolysaccharide with the probes pyrene butyric acid and pyrene butyroyl decanoic acid indicates that these probes are in a region of the membrane that is different than pyrene sulfonic acid and pyrene as shown in Fig. 6. Pyrene butyric acid and pyrene butyroyl decanoic acid are located in the lipid away from the protein. These data are reminiscent of that for inner membranes. Pyrene sulfonic acid and pyrene again may be located close to or on the protein, while pyrene butyric acid and pyrene butyroyl decanoic acid are in the lipid bilayers. If this model is correct then it suggests that the lipopolysaccharide must reside mainly in or near a region where the protein is situated.

The whole cell data do not show any significant effect of lipopolysaccharide carbohydrate content, and is an indication that the outer membrane may be altered by the separation procedures.

The oxygen data in Fig. 5 for the whole cells and outer membranes show no effect of lipopolysaccharide carbohydrate content. The quenching constants k_q for the whole cell show ready access of the membrane for O_2, which is to be expected. A smaller affinity for O_2 is illustrated by the outer membrane as k_q values are for the most part lower than the corresponding whole cells. The data are best interpreted in a manner similar to that outlined for the inner membrane. Oxygen is already present in the membrane and diffuses to the probes pyrene sulfonic acid, pyrene butyric acid and pyrene butyroyl decanoic acid. In the case of pyrene this may also diffuse and the k_q for pyrene tends to be higher than that for pyrene butyroyl decanoic acid.

The present data illustrate kinetic information on membranes that are available from pulsed laser techniques. The discussion indicates the necessity of obtaining a clear picture of the structure of the membrane in order to interpret the kinetic data unambiguously. Conventional and accepted membrane models have been chosen to explain the data. However, other models might be equally successful. It is hoped by the use of more specific probes, which are now being developed, to rule out some of the ambiguity regarding the location and environment of the probe, and to enhance our understanding of membrane structure.

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