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# PROPERTIES OF CHEMICALLY MODIFIED PORIN FROM *ESCHERICHIA COLI* IN LIPID BILAYER MEMBRANES

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**Purified porin OmpF from *Escherichia coli* outer membrane was chemically modified by acetylation and succinylation of amino groups and by amidation of the carboxyl groups. Native and chemically modified porins were incorporated into lipid bilayer membranes and the permeability properties of the pores were studied. Acetylation and succinylation of the porin trimers had almost no influence on the single channel conductance in the presence of small cations and anions and the cation selectivity remained essentially unchanged as compared with the native porin. Amidation had also only little influence on the single channel conductance and changed the pore conductance at maximum by less than 50%, whereas the cation selectivity of the porin is completely lost after amidation. The results suggest that the structure of the porin pore remains essentially unchanged after chemical modification of the pores and that their cation selectivity is caused by an excess of negatively charged groups inside the pore and/or on the surface of the protein. Furthermore, it seems very unlikely that the pore contains any positively charged group at neutral pH.**

## Introduction

The sieving properties of the outer membranes of Gram-negative bacteria are coupled to a class of major proteins, called matrix proteins [1] or porins [2]. These porins form usually trimers of three identical polypeptide subunits with molecular weights around 35 000 [3,4]. Reconstitution experiments with lipid vesicles in the presence of porins from *Escherichia coli* [2], from *Salmonella typhimurium* [5] and from *Pseudodomonas aeruginosa* [6] have shown that these vesicles are permeable for hydrophilic solutes with a well defined exclusion limit between 600 and 5000 daltons.

From these results one may conclude that these porins form simply a hydrophilic pathway in the outer membrane of Gram-negative bacteria. Lipid bilayer experiments have shown, however, that the porin pores are structured [7–10]. Most of the pores are cation selective [7–9], whereas only porin PhoE (Ic) from *E. coli* (Benz, Darveaux and Hancock, unpublished) and porin P from *Ps. aeruginosa* [10] have been shown to form anion selective channels. Both porins are induced together with the alkaline phosphatase, if the organisms grow on phosphate deficient media [11,12].

The cation selectivity of the porins OmpF (Ia) and OmpC (Ib) from *E. coli* outer membrane arises presumably from the presence of negatively charged groups in the pore or at the surface of the protein [7], which may be a common requirement for cation selective channels in biology. The chemical modification of the porin trimers of *E. coli* and the study of the ion selectivity of the chemi-

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Abbreviation: Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

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cally modified porins is therefore of special interest and will be described in this paper.

Recently, we have shown that chemical modifications did not change the structure of the OmpF trimers [13]. Furthermore, the chemically modified trimers remain active in the vesicle permeability assay [13] using ions of large molecular mass, several hundred daltons. The results of these studies suggest that the ionic interaction between the surface of the protein and/or the pore wall and the solutes play an important role for the diffusion of large ions.

In contrast to this we shall show here that acetylated and succinylated trimers do not show larger cation selectivity than the unmodified porin if ions with small molecular weight are used. This is consistent with the finding that amidated porin has only lost its cation selectivity and does not show anion selectivity at neutral pH. Furthermore, we shall show that the single channel conductance of the OmpF-porin from *E. coli* is only little affected by chemical modification. Our results provide additional support that the porin trimers from *E. coli* from large water filled pores and that one trimer contains presumably only one pore.

## Materials and Methods

### *Chemical modification of the porin trimers*

The porin trimers were isolated from the outer membrane of an *E. coli* strain producing one single porin (OmpF) [2]. The culture conditions and the purification of the protein has been described earlier in detail [13]. Acetylation and succinylation were performed as described in a previous publication [13] using acetyl anhydride at pH 7 and succinic anhydride at pH 8 (Fig. 1). 33 amino groups (from total 57 residues per trimer) were found to be acetylated, whereas the succinylation procedure changed 42 amino groups per trimer. The amidation of the porin trimers was performed in a two-step reaction [13]. The carboxyl groups were blocked with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide in the presence of glycinamide which neutralized finally one negative charge (Fig. 1). The native and the modified porin trimers were used as concentrated stock solutions containing either 0.4 M NaCl, 10 mM phosphate pH 7, 0.1% sodium dodecylsulfate and 3 mM sodium azide (native, acetylated and succinylated porin) or 0.4 M NaCl, 10 mM phosphate pH 7, 0.1% sodium

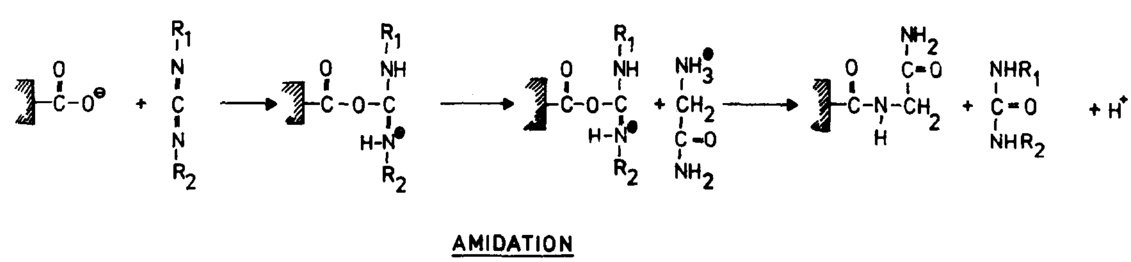

Fig. 1. Chemical modification of the porin.

dodecylsulfate, 5 mM ethylenediaminetetraacetic acid and 3 mM sodium azide (amidated porin). The protein concentration in the stock solutions was between 0.5 and 1.1 mg/ml. The native and modified porins remained active in these solutions for about 8 months.

### *Lipid bilayer experiments*

Artificial lipid bilayer membranes were obtained as described previously [14] from 1–2% (w/v) solution of two different lipids dissolved in *n*-decane (Fluka, Buchs, Switzerland, purum). Egg phosphatidylcholine was isolated in our own laboratory according to established procedures [15] and oxidized cholesterol was prepared as described earlier [14]. The chamber used for bilayer formation was made from Teflon. The circular hole in the wall separating the two aqueous compartments had an area of either 1.5 mm$^2$ (for the macroscopic conductance measurements) or 0.1 mm$^2$ (for the single-channel measurements). All salts were obtained from Merck (Darmstadt, F.R.G., analytical grade). The aqueous solutions were used unbuffered with the pH as indicated. The proteins were added to the aqueous salt solutions from the stock solutions either immediately before membrane formation or after the membrane had completely turned black in order to prevent protein inactivation.

For the electrical measurements, calomel electrodes with salt bridges were inserted in the aqueous compartments on both sides of the membrane. The macroscopic conductance measurements were performed with a Keithley 610 C electrometer. A Keithley 427 current amplifier was used for the single channel experiments. The amplified signal was monitored with a Tektronix 7633/7A22 storage oscilloscope and recorded with a strip chart recorder. The bandwidth of the single-channel experiments was between 100 Hz and 1 kHz. Zero-current membrane potentials were measured with a Keithley 610 C electrometer 10 min after application of a salt gradient to the membranes as has been described earlier [7].

## Results

### *Macroscopic conductance measurements*

The chemically modified porins were found to be active in lipid bilayer experiments. Similar as has been described earlier for the unmodified porin [14], the membrane conductance in the presence of the modified porins was found to be a function of the time after formation of the membranes (if the proteins were present prior to membrane formation) or after the addition of the proteins to a black membrane. In the latter case an initial lag of about 4 min was found, which was presumably due to diffusion of the protein through unstirred layers adjacent to the membrane. Then a rapid increase of the membrane conductance was observed during about 20 to 30 min. The absolute conductance level after that time was a linear function of the protein concentration in the aqueous phase up to about 1 $\mu$g/ml where saturation effects occurred. The conductance increase was dependent on the presence of the protein and was absent if only detergent was added in the same concentration to the aqueous phase.

Fig. 2 shows the dependence of the conductance 30 min after blackening of membranes (if the protein was present prior to membrane formation) or after addition of the protein as a function of the protein concentration in the aqueous phase. As can be seen from the results given in Fig. 2 there exist only little differences between the conduc-

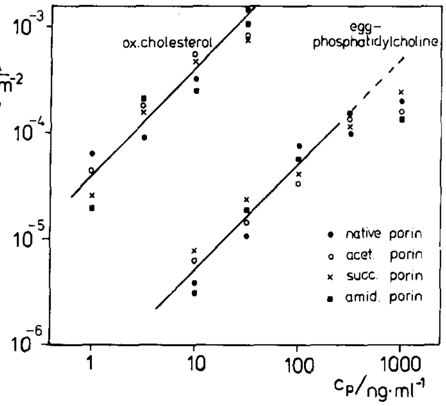

Fig. 2. Specific membrane conductance as a function of the concentration $c_p$ of the different porins in the aqueous phase. The membranes were formed from different lipids dissolved in *n*-decane. The aqueous phase contained 1 M KCl and less than 1 $\mu$g/ml dodecylsulfate. Each point represents the mean of at least three membranes 30 min after the blackening of the membranes or after the addition of the protein, $t = 25°C$; $V_m = 10$ mV.

tance values for the unmodified and the three modified porin trimers. This indicates that the hydrophobic nature, which seems responsible for the insertion of the porin trimers into the lipid bilayer membranes [8], remains essentially unchanged by the chemical modification of the molecules.

Fig. 3 shows current-voltage curves of two membranes from egg phosphatidylcholine/*n*-decane in the presence of 100 ng/ml succinylated porin (crosses) and 100 ng/ml amidated porin (full squares). As is shown by Fig. 3 the membrane current was also for the chemically modified porins a linear function of the applied voltage up to at least 150 mV. These results indicated that the properties of the single conductance unit are virtually independent of voltage even after chemical modification.

*Single-channel experiments*

When the chemically modified porins were added in small concentration (1 ng/ml) to aqueous solutions bathing a membrane, the membrane conductance started to increase in a stepwise fashion. An example for amidated porin is given in Fig. 4. The occurrence of these steps was specific for the presence of one of the porins and was not seen if only the detergent dodecylsulfate was added to the aqueous phase. Most steps were directed upwards, whereas terminating steps were only rarely observed. This behaviour has been found for most of the porins from Gram-negative bacteria [8–10]. Changes in the salt concentration and in the lipid composition had no influence on the lifetime of the pores, but considerably higher concentrations of the different porins were needed in order to induce pores in membranes from phospholipids as compared with those from oxidized cholesterol. This may be explained by the assumption that protein insertion into oxidized cholesterol membranes is facilitated because of a lower surface pressure or lower surface tension of these membranes as compared with phospholipid membranes [8]. It has to be noted, however, that the use of different neutral lipids had virtually no influence on the single-channel conductance.

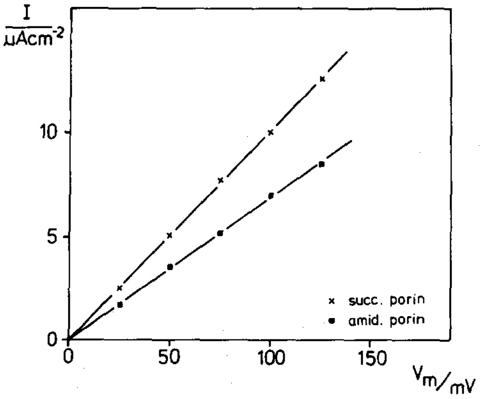

Fig. 3. Current-voltage curves of two membranes from egg phosphatidylcholine/*n*-decane doped either with succinylated or acetylated porin. The aqueous phase contained 100 ng/ml porins, 0.1 μg/ml dodecylsulfate and 1 M KCl; *t* = 25°C.

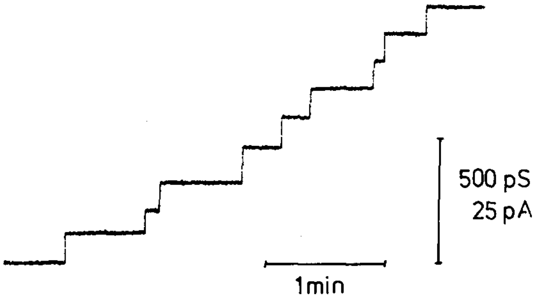

Fig. 4. Stepwise increase of the membrane current of a membrane from egg phosphatidylcholine/*n*-decane in the presence of 1 ng/ml amidated porin. The aqueous phase contained 0.1 M KCl; *t* = 25°C. The applied voltage was 50 mV, the current prior to the addition of the amidated porin was 0.8 pA.

From records of the membrane current over long periods of time and counting a sufficient number of single events a histogram of the fluctuations and the average single-channel conductance $\bar{\Lambda}$ may be obtained. Histograms for the native and amidated porins and a salt concentration of 0.1 M KCl are given in Fig. 5. The histograms for acetylated and succinylated porin were found to be very similar to that of unmodified porin (OmpF, not shown in Fig. 5), whereas the maximum for the amidated porin is shifted towards smaller conductance values. This has been found for all large alkali ions like $K^+$, $NH_4^+$, $Rb^+$ and $Cs^+$ (compare also Table I), whereas for lithium and sodium salts all porins have about the same single-channel conductance.

The shift of the single-channel conductance towards smaller values for the amidated porin and

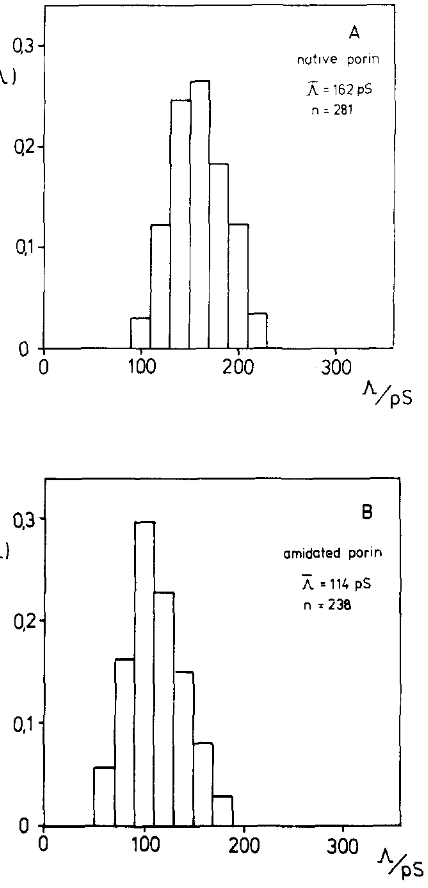

Fig. 5. Histogram of the conductance fluctuations observed with membranes from egg phosphatidylcholine/*n*-decane in the presence of (A) native porin, $n = 281$, $\bar{\Lambda} = 162$ pS; (B) amidated porin, $n = 238$, $\bar{\Lambda} = 114$ pS. The aqueous phase contained 0.1 M KCl; $t = 25°C$. The applied voltage was 50 mV.

chlorides with larger cations like $K^+$, $NH_4^+$, $Rb^+$ and $Cs^+$ was found to be significant. This can be seen from a statistical analysis of the data given in Fig. 5. The variance for both single channel distributions is about ±25 pS. The interval of 114 ± 25 pS contains about 62% of all observed events in the case of the amidated porin, whereas the interval 162 ± 25 pS contains about 58% of all observed single channels for the native porin. Similar considerations apply also to other histograms obtained from single-channel measurements with amidated porin and large monovalent cations as compared with the histograms observed with the other porins.

Table I contains the single-channel conductances for the four different porins and a variety of different salts. Again there exist a good agreement between the unmodified, the acetylated and the succinylated porin, whereas certain differences were observed between these porins and the amidated porin. In general, the single-channel conductance appears to be reduced for amidated porin in the case of large alkali ions and increased in the presence of divalent cations. It is obvious, however, that all these deviations of the single-channel conductances between the amidated porin and the others are small. This may be explained by the assumption that the formation of large water-filled pores by the porin OmpF of *E. coli* remains essentially unchanged even after chemical modification.

*Zero-current membrane potentials*

Further information on the changes of the porin pores induced by chemical modification may be obtained by the study of zero-current membrane potentials. These experiments were carried out in the following way. The membranes were either formed from oxidized cholesterol/*n*-decane or from egg phosphatidylcholine/*n*-decane in $10^{-2}$ M salt solution. After blackening of the membranes, the porins were added to the aqueous phase in a concentration around 100 ng/ml. 20 to 30 min. after addition of the proteins most of the conductance increase was over. Subsequently, the salt concentration on one side of the membrane was raised by addition of small amounts of concentrated solution while stirring. The membrane potential reached its final value within about 10 min after establishing the salt gradient across the membranes.

The observed membrane potentials $V_m$ in the presence of the different modified and unmodified porins can be very well fitted to the Goldman-Hodgkin-Katz equation:

$$V_m = \frac{RT}{F} \ln \frac{P_c c'' + P_a c'}{P_c c' + P_a c''} \qquad (1)$$

with $c'$ and $c''$ being the salt concentrations on both sides of the membrane. $P_c$ and $P_a$ are the permeabilities of the cations and the anions through the pores, respectively. $R$ is the gas constant, $T$ the absolute temperature and $F$ the Faraday constant. Fig. 6 shows membrane potentials

TABLE I

AVERAGE SINGLE-CHANNEL CONDUCTANCES $\bar{\Lambda}$ FOR THE DIFFERENT MODIFIED AND UNMODIFIED PORINS AS A FUNCTION OF DIFFERENT SALT SOLUTIONS

The aqueous solutions contained 1 ng/ml porin and about 1 ng/ml sodium dodecylsulfate; $t = 25°C$. The pH of the aqueous solutions was between 6 and 7 if not otherwise indicated. The membranes were made from egg phosphatidylcholine/*n*-decane; $V_m = 50$ mV. $\sigma$ is the specific conductance of the corresponding aqueous salt solution and was taken from Ref. 7. Succ., succinylated; Acet., acetylated; Amid., amidated.

| Salt | Concn. (M) | $\bar{\Lambda}$ (nS) Native porin | Succ. porin | Acet. porin | Amid. porin | $\bar{\Lambda}/\sigma$ ($10^{-8}$ cm) Native porin | Amid. porin |
|---|---|---|---|---|---|---|---|
| LiCl | 1 | 0.62 | 0.65 | 0.66 | 0.58 | 0.87 | 0.82 |
| NaCl | 1 | 1.1 | 1.0 | 1.1 | 0.93 | 1.3 | 1.1 |
| KCl | 0.1 | 0.16 | 0.17 | 0.16 | 0.11 | 1.1 | 0.73 |
|  | 1 | 1.7 | 1.6 | 1.7 | 1.2 | 1.5 | 1.1 |
| $NH_4Cl$ | 1 | 1.7 | 1.8 | 1.6 | 1.1 | 1.5 | 0.98 |
| RbCl | 1 | 1.6 | 1.7 | 1.7 | 1.2 | 1.4 | 1.0 |
| CsCl | 1 | 1.7 | 1.8 | 1.7 | 1.2 | 1.5 | 1.0 |
| $MgCl_2$ | 0.5 | 0.42 | 0.45 | 0.46 | 0.65 | 0.65 | 1.0 |
| $CaCl_2$ | 0.5 | 0.40 | 0.41 | 0.43 | 0.70 | 0.51 | 0.90 |
| $BaCl_2$ | 0.5 | 0.41 | 0.46 | 0.40 | 0.72 | 0.53 | 0.93 |
| $Tris^+$ $Hepes^-$ (pH 8) | 0.5 | 0.069 | 0.063 | 0.065 | 0.068 | 0.96 | 0.94 |
| $N(CH_3)_4^+$ $Hepes^-$ (pH 8) | 0.5 | 0.082 | 0.078 | 0.079 | 0.081 | 0.55 | 0.54 |
| $N(C_2H_5)_4^+$ $Hepes^-$ | 0.5 | 0.035 | 0.042 | 0.038 | 0.036 | 0.73 | 0.75 |

observed with the different modified and unmodified porins for a KCl-gradient across membranes from egg phosphatidylcholine at pH 6.0. The experimental values for $V_m$ could be fitted either with $P_c/P_a = 3.6$ (acetylated, succinylated and native porin) or with $P_c/P_a = 1.1$ (amidated porin). This indicated that acetylation and succinylation did not change the ionic selectivity of the porin pore whereas the cation selectivity is completely lost after amidation of the protein.

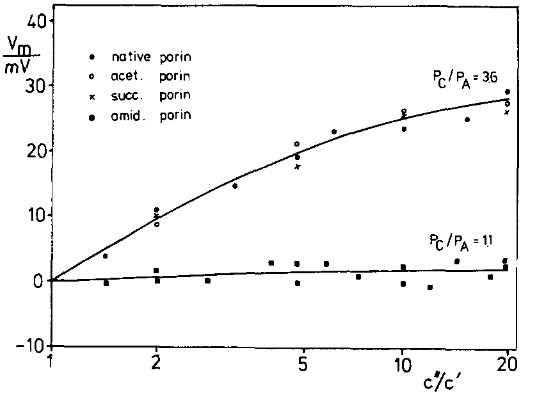

Fig. 6. Zero-current membrane potentials $V_m$ across membranes from egg phosphatidylcholine/*n*-decane in the presence of the different porins as a function of the KCl-gradient $c''/c'$ across the membranes. $c'$ was kept constant at $10^{-2}$ M and $c''$ was varied by adding increasing amounts of concentrated KCl solution. The aqueous phase contained 100 ng/ml of the different porins and 0.1 µg/ml dodecylsulfate; $t = 25°C$. The full lines were drawn according to Eqn. 1 with the specified values of the permeability ratio $P_c/P_a$.

In earlier studies with the porin pore we have found that the ionic selectivity is pH-dependent [7]. This indicated that the ionic selectivity is presumably a result of charges in (or near) the pore. In order to test this for the chemical modified porins a number of zero current measurements were performed at different pH-values. Table II shows the values for the zero-current membrane potentials derived for 10-fold salt gradients across membranes from oxidized cholesterol/*n*-decane and egg phosphatidylcholine/*n*-decane in the presence of the different native and modified porins. The permeability ratio $P_c/P_a$ was calculated according to the Goldman-Hodgkin-Katz equation. As can be seen from Table II there exists even after chemical modification a change of the selectivity dependent on the pH in the aqueous phase. It is obvious, however, that chemical mod-

TABLE II

ZERO-CURRENT MEMBRANE POTENTIALS FOR THE DIFFERENT MODIFIED AND UNMODIFIED PORINS FROM *E. coli* IN THE PRESENCE OF A 10-FOLD KCl GRADIENT

$V_m$ is the electrical potential of the dilute side ($10^{-2}$ M) minus the potential of the concentrated side ($10^{-1}$ M). The membranes were either formed from egg phosphatidylcholine/*n*-decane or from ox cholesterol/*n*-decane; $t = 25$°C. The ratio of the permeabilities $P_c/P_a$ was calculated from the Goldman-Hodgkin-Katz equation (Eqn. 1).

| Porin | pH | $V_m$ (mV) | $P_c/P_a$ |
|---|---|---|---|
| Egg phosphatidylcholine | | | |
| Native porin | 6 | 26 | 3.6 |
| | 3 | 0 | 1.0 |
| | 9 | 30 | 4.7 |
| Acetylated porin | 6 | 27 | 3.8 |
| | 3 | 2 | 1.1 |
| | 9 | 30 | 4.5 |
| Succinylated porin | 6 | 26 | 3.7 |
| | 3 | 4 | 1.2 |
| Amidated porin | 6 | 2 | 1.1 |
| | 3 | −13 | 0.53 |
| | 9 | 7 | 1.4 |
| Ox cholesterol | | | |
| Native porin | 6 | 25 | 3.5 |
| Acetylated porin | 6 | 27 | 3.8 |
| Succinylated porin | 6 | 26 | 3.7 |
| Amidated porin | 6 | 0 | 1.0 |

ification causes a shift of ionic selectivity as a function of pH. Whereas acetylated, succinylated and normal porin have lost their selectivity at pH 3, the pores formed by the amidated porin show a certain anion selectivity at this pH. This result shows that at low pH there exist presumably an excess of positively charged groups in or near the pores formed by this modified porin.

Further measurements of zero-current membrane potentials were performed in the presence of a salt with large cation and large anion ($Tris^+Hepes^-$) to study if the size of the ions had an influence on the selectivity of the pore before and after chemical modifications. The results of these experiments (given in Table III) show that the size of the ions had only a small influence if any on the permeability ratio $P_c/P_a$. It has to be noted, however, that the combination of large cations with small anions and vice versa causes large effects on $P_c/P_a$ presumably because of the different mobility of both ions in the aqueous phase and possibly also in the pore.

TABLE III

ZERO-CURRENT MEMBRANE POTENTIAL FOR THE DIFFERENT MODIFIED AND UNMODIFIED OmpF PORIN TRIMERS FROM *E. coli* IN THE PRESENCE OF A 10-FOLD GRADIENT OF $Tris^+$ $Hepes^-$

$V_m$ is the electrical potential of the dilute side ($10^{-2}$ M) minus the potential of the concentrated side ($10^{-1}$ M). The pH of the aqueous solutions was kept at pH 8; $t = 25$°C. The membranes were formed from egg phosphatidylcholine/*n*-decane. The ratio of the permeabilities $P_c/P_a$ was calculated from the Goldman-Hodgkin-Katz equation (Eqn. 1).

| Porin | $V_m$ (mV) | $P_c/P_a$ |
|---|---|---|
| Native porin | 30 | 4.5 |
| Acetylated porin | 31 | 4.7 |
| Succinylated porin | 28 | 4.3 |
| Amidated porin | 7 | 1.4 |

## Discussion

The results of the studies with lipid bilayer membranes in the presence of the different native and modified porins are two-fold. First of all we found that the ion transport through the modified porin pores is not very much changed as compared with the native porin pore. Secondly, the ionic selectivity of only one of the modified porin pores is changed, whereas the selectivity of the others remains essentially unchanged as compared with the native porin pore.

Let us consider first the almost unchanged ion transport through the porin pores after chemical modification. This may be explained on the basis of the formation of large water filled pores by the porins of *E. coli*, a conclusion which has already been derived earlier from the permeation of solutes and large organic ions like $Tris^+$ and $Hepes^-$ through the porin pores [2,3,7]. It is obvious from the results of the vesicle permeability assay [13] and the lipid bilayer experiments given here that the basic structure of this wide channel is not changed by the different chemical modifications. This is also not expected because the groups attached to the protein during the modifications are by far too small to block the channel. Nevertheless, one would expect at least in the case of the amidation of the protein a more dramatic change of the permeability properties of the pore than we have observed in lipid bilayer experiments (see below). The group attached to the protein during amidation has a length of about 0.5 nm and converts one negative charge in a neutral group (Fig. 1) so that positive charges on the surface of the protein and/or in the pore interior could show a larger effect on the ion transport through the pore.

Nevertheless, there exist small but distinct differences of the single-channel conductances of the amidated porins as compared with the others. This is given especially for the single-channel conductance in the presence of chlorides with large alkali ions in the aqueous phase. The single channel conductance is reduced for the amidated porin by about 50% in this case. Simultaneously, the single channel conductance of the amidated porin is increased for salts with divalent cations by about 40%. This can also be derived from Table I, which includes also the ratio $\bar{\Lambda}/\sigma$ for the native and amidated porin (with $\sigma$ specific conductance of the corresponding aqueous salt solution taken from Ref. 7). $\bar{\Lambda}/\sigma$ varies about 4-fold for the native porin (and also for the acetylated and succinylated porin), whereas $\bar{\Lambda}/\sigma$ varies much less for the amidated porin.

The variations may be explained on the basis of the following. The native OmpF channel is slightly cation selective. This means that most of the current through the pore is carried by cations and that the mobility of the cations within the pore has a large influence on the single-channel conductance. The results of this study and those of earlier publications [7,14] suggest that the pore contains one or several negatively charged groups. The interactions of these groups with the cations, i.e. a binding could increase or decrease their mobility in the pore as compared with that of the aqueous phase. The amidation reactions blocks presumably the negatively charged groups and inhibits thus the interaction of the cations with pore interior. Furthermore, the pore becomes unselective and the pore conductance behaves as the sum of the mobilities of the ions in the aqueous phase which is given by the specific conductances of the corresponding salt solutions. In this case $\bar{\Lambda}/\sigma$ should vary much less for the amidated porin than for the others as was in fact observed (Table I).

The changes in the $\bar{\Lambda}/\sigma$ values after amidation on the OmpF-protein are of considerable interest. For instance, it could be argued that the variations between the $\bar{\Lambda}/\sigma$ ratios observed with the native porin pore reflect simply the interaction of the ions or of their hydration shell with the interior of the pore. This would favour the existence of three smaller rather than one large pore in one trimer [16,17]. The disappearance of the variations of $\bar{\Lambda}/\sigma$ after amidation, however, shows that we are simply dealing here with an effect of negatively charged groups, present on the surface or inside the OmpF-pore. These charges (responsible for the cation selectivity under normal conditions) become neutralized after amidation.

Simultaneously the pore looses its larger single channel conductance for some alkali cations, whereas the conductance for divalent ions is increased. Thus the variation of the ratio $\bar{\Lambda}/\sigma$ cannot be used as an argument for the existence of three pores in a porin trimer. Therefore we still believe that our results are better consistent with one pore in a trimer although the other possibility cannot completely be ruled out on the basis of our results.

The results obtained from the vesicle permeability assay in the presence of the modified porins [13] contradict to a certain extent the lipid bilayer experiments presented here. Positively charged solutes have an increased permeability in vesicles doped with acetylated or succinylated porin and a reduced permeability for amidated porin, whereas it is the opposite for negatively charged solutes [13]. The effect of amidation can be understood on the basis of the results presented here. Amidation of the porin trimers increases the permeability of anions, whereas it decreases the permeability of cations. This is also consistent with data obtained with lipid bilayers and vesicles in the presence of PhoE porin from *E. coli* (Ref. 18–20 and Benz, Darveaux and Hancock, unpublished data). Acetylation and succinylation of the porin have on the other hand no effect on the results of lipid bilayer membranes. The reason for this difference is presumably caused by the size of the probe molecules although even large cations show no increase of the permeability as compared with large anions (compare Table III). Charged solutes of considerable molecular weight have been used in the vesicle permeability assay [13], whereas the lipid bilayer experiments were performed with smaller ions. If we assume that the positively charged amino groups are only located on the surface of the trimer and not inside the pore (see below) then the diffusion of large organic anions could be reduced after acetylation and even more after succinylation. The permeation of small anions through the pore could be less affected, since the pore area seems fairly large as compared with the size of the ions and the pore is already cation selective before the blocking of the amino groups.

Sequence and amino acid analysis of the porin from *E. coli* shows that this protein is not particular hydrophobic [1,21,22]. This can be explained by the fact that a large number of polar amino acid residues are needed to form a channel of 7.5 nm length and a diameter around 1.2 nm [7]. The protein trimers have a fixed structure which makes the channel virtually independent of composition and thickness of the lipid bilayer membranes [7]. Lipid bilayer experiments with the modified porins showed that only amidation is able to change the ion transport properties of the porin pores. This result allows us to speculate about the structure of the pore and its environment. The porin trimer has under normal conditions 147 carboxyl groups and 57 amino groups. The excess of negatively charged groups leads to a certain cation selectivity. The acetylation of 33 amino groups or the succinylation of 42 groups does not increase the cation selectivity. This leads to the assumption that the pore interior does not contain positive charges, otherwise we would expect a larger effect on the selectivity. Carbodiimide-promoted amide formation converts 60 carboxyl groups into 60 neutral groups and the pore looses only the cation selectivity and does not become anion selective. This is consistent with the assumption made above that the pore contains presumably no positive charged groups, otherwise the pore should become anion selective after amidation. From these considerations it seems very likely that the pore formed by OmpF porin from *E. coli* outer membrane contains at least one negative charge.

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