Effect of Peptide PV
on the Ionic Permeability of
Lipid Bilayer Membranes

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ABSTRACT This paper reports the effects of peptide PV (primary structure: cyclo-(d-val-L-pro-L-val-d-pro)₃) on the electrical properties of sheep red cell lipid bilayers. The membrane conductance ($G_m$) induced by PV in either Na⁺ or K⁺ medium is proportional to the concentration of PV in the aqueous phase. The PV concentration required to produce a comparable increase in $G_m$ in K⁺ medium is about 10⁴ times greater than for its analogue, valinomycin (val). Although the selectivity sequence for PV and val is similar, K⁺ > Rb⁺ > Cs⁺ > NH₄⁺ > Tl⁺ > Na⁺ > Li⁺; the ratio of $G_m$ in K⁺ to that in Na⁺ is about 10 for PV compared to > 10⁴ for val. When equal concentrations of PV are added to both sides of a bilayer, the membrane current approaches a maximum value independent of voltage when the membrane potential exceeds 100 mV. When PV is added to only one side of a bilayer separating identical salt solutions of either Na⁺ or K⁺ salts, rectification occurs such that the positive current flows more easily away rather than toward the side containing the carrier. Under these conditions, a large, stable, zero-current potential ($V_m$) is also observed, with the side containing PV being negative. The magnitude of this $V_m$ is about 90 mV and relatively independent of PV concentration when the latter is larger than 2 × 10⁻⁶ M. From a model which assumes that $V_m$ equals the equilibrium potential for the PV-cation complexes ($MS^+$) and that the reaction between PV and cations is at equilibrium on the two membrane surfaces, we compute the permeability of the membrane to free PV to be about 10⁻⁶ cm s⁻¹, which is about 10⁻⁷ times the permeability of similar membranes to free val. This interpretation is supported by the fact that the observed values of $V_m$ are in agreement with the calculated equilibrium potential for $MS^+$ over a wide range of ratios of concentrations of total PV in the two bathing solutions, if the unstirred layers are taken into account in computing the $MS^+$ concentrations at the membrane surfaces.

INTRODUCTION

Mechanisms of carrier-mediated transport across artificial lipid bilayers and biological membranes have been studied extensively in recent years (1–5).
Valinomycin (val) is one of a number of macrocyclic compounds that have been shown to induce a highly selective increase in $\text{K}^+$ permeability in artificial and biological membranes (6–9). It is believed that val forms a complex with $\text{K}^+$ at the membrane surface, the charged complex moves through the hydrocarbon core and releases the $\text{K}^+$ to the opposite side. In the process of complex formation, val undergoes a change in conformation to form a bracelet-shaped cage surrounding the cation. In this cage structure, the inwardly directed ester carbonyl oxygen atoms replace completely the water oxygens in the hydration shell of the metal. The relatively nonpolar methyl and isopropyl side chains are directed outwardly either axially or equatorially. The bracelet conformation of the cation complex is stabilized by intramolecular hydrogen bonds involving all six peptide carbonyls and amide protons in the molecule.

The relationship between the molecular structure of val and its high specificity for $\text{K}^+$ is not fully understood. It has been shown that in the depsipeptide series (e.g., val and its analogs) the interactions of these compounds with $\text{Na}^+$ or $\text{K}^+$ are exquisitely sensitive to their primary structures (10, 11). An understanding of this sensitivity requires insight into the relationship between the primary structure of the macrocyclic compounds and their capacity to assume conformations suitable for complexation with ions. To this end, we have previously reported the synthesis of a cyclic dodecapeptide (PV) with the primary structure cyclo-(D-val-L-pro-L-val-D-pro)$_3$ which may be compared with val cyclo-(D-val-L-lac-L-val-D-hyv)$_3$ (12). PV contains only amino acids in contrast to the mixture of amino acids and hydroxy acids found in the parent compound. The compound was designed so that intramolecular hydrogen bonding was restricted to the amide protons of the $d$ and $l$ valine residues as it is in the parent molecule. We suspected that this constraint is important in promoting formation of the 10-membered rings which stabilize the “bracelet” conformation of the metal complexes of val (10). Proline was chosen to substitute for the hydroxy acids in val because it lacks an amide proton and thus, when in amide linkage, cannot participate in hydrogen bonding. Proton magnetic resonance and infrared spectroscopy studies show that PV forms 1:1 complexes with alkali metal ions and that the conformations of these complexes have a bracelet configuration similar to the cation complexes of val (10, 12, 24). However, the interaction with the cation is via coordination to the imide carbonyls of the proline residues in residues in contrast to the ester carbonyl-cation interactions of val. This substitution markedly alters the properties and specificity of PV for monovalent cations.

This paper reports in detail the effects of peptide PV on the electrical properties and ionic permeability of bilayers prepared from lipids extracted from sheep red blood cells. We show that PV has a lower potency in increasing
membrane conductance and a lower selectivity for $K^+$ over $Na^+$ than does its analogue val. Further, we show that addition of a relatively low concentration of PV, a neutral carrier, to one side of a membrane separating identical salt solutions can produce a large, stable, zero-current membrane potential. We present an analysis of this observation which leads to the conclusion that the permeability of bilayers to free, uncharged PV is extremely low, about $10^{-7}$ times the permeability to free val. This low permeability to free PV leads to the development of a substantial concentration difference across the bilayer when PV is added to only one side. This concentration difference for free uncharged PV produces a concentration difference for charged PV-cation complexes despite the equality of cation concentrations at the membrane surfaces. We propose that this difference in concentrations of charged PV-cation complexes produces the membrane potential observed when PV is added to one side of a bilayer.

**METHODS**

**Lipid Source and Membrane Formation**

Except when specified otherwise, membranes were formed with sheep red blood cell lipids extracted as described previously (13). The synthetic $\alpha$-dioleoyllecithin used in some experiments was kindly given to us by Dr. P. Läuger. Membranes were formed from lipids dissolved in $n$-decane (15-25 mg/ml). This lipid solution was applied with a brush across an aperture (2.9 mm$^2$ in area) in a polyethylene partition separating two chambers. Perfusion in each chamber was carried out by two matched, mechanically coupled syringes. Both chambers were stirred continuously with magnetic stirrers. All experiments were carried out at room temperature ($\approx 23^\circ$C) and the aqueous solutions were buffered at pH 7.4 with 1 mM phosphate.

**Electrical Measurements**

In some instances, membrane voltage ($V_m$) was measured with a Keithley model 602 electrometer (Keithley Instruments, Inc., Cleveland, Ohio) through a pair of calomel electrodes as described previously (13). In these cases, the membrane resistance was calculated, using Ohm's law, from the $V_m$ produced by applying a calibrated voltage pulse across the membrane plus a known resistance in series with the membrane. However, for the majority of the experiment, the $V_m$ and membrane current ($I_m$) were directly obtained with a four-electrode voltage clamp apparatus (14).

**Chemicals**

Val and PV were synthesized by Gisin and Merrifield (12, 15). Phloretin was purchased from K & K Laboratories, Inc., Plainview, N. Y. All inorganic salts were reagent grade. Decane was supplied by Eastman Organic Chemicals Div. Eastman Kodak Co. Rochester, N.Y.
RESULTS

Membrane Electrical Conductance

Fig. 1 shows the PV-dependent membrane conductance of bilayers plotted as a function of PV concentration in both bathing solutions which also contained either KCl or NaCl (1.0 M). The slopes of these lines do not differ significantly from unity. The scatter in the data at low PV concentrations is probably due to the small differences between the conductance in the absence and presence of the compound under these conditions. These data differ strikingly from those obtained with val in two ways (9). First, the concentration required to produce a comparable increase in membrane conductance in the presence of KCl is about $10^4$ times less for val than for PV, i.e. val is about $10^4$ times more potent than PV. Second, the ratio of membrane conductance in K$^+$ to that in Na$^+$ is about 10 for PV but greater than $10^3$ for val, i.e. val is more selective than PV for K$^+$ over Na$^+$. The membrane conductance is shown as a function of K$^+$ concentration at a constant total salt concentration of 1.0 M (Li$^+$ substitution) in Fig. 2. The slope of the log-log plot is about 0.5. Even at 1 M K$^+$, no saturation of the curve was observed.

Ionic Selectivity

In order to estimate the ionic selectivity of bilayers exposed to PV, experiments were designed (a) to measure the steady-state membrane conductance
FIGURE 2. Membrane conductance ($G_m$) of sheep red cell lipid bilayers as a function of potassium concentration in the presence of $5 \times 10^{-6}$ M PV in both chambers. The ionic strength was maintained constant by substituting KCl for LiCl so that the total cation concentration was 1.0 M in all experiments, (the contribution of LiCl to the membrane conductance may be neglected, Tables I and II).

at zero current, potential difference, and frequency when the membrane separated identical solutions containing the test cation ($b$) to measure the electrical potential difference across a bilayer separating 0.01 from 0.001 M solution of the chloride salts of the test cation, and ($c$) to measure the so-called biionic potential ($V_{bi}$), i.e. the electrical potential difference at zero current across a membrane separating equimolar solutions of two different cation chloride salts. All measurements were made at pH 7.4 ($10^{-4}$M $XH_2PO_4$) where $X$ was a monovalent cation. Tables I and II present the results of such measurements in systems containing $10^{-1}$ and $10^{-2}$ M $XCl$ where $X$ was the test cation. Each figure in the tables is the mean of all observations on at least two different bilayers. By methods ($a$) and ($c$), the sequence was $K^+ \geq Rb^+ > Cs^+ > NH_4^+ > TI^+ > Na^+ > Li^+$. This selectivity sequence for PV is identical to that for val. However, the magnitude of the selectivity for $K^+$ over $Na^+$ was much less with PV ($10^{1-10^3}$) than it was with val ($10^2-10^3$) ($9$).

**Current-Voltage Relationship**

Fig. 3 illustrates the current ($I$)-voltage ($V$) relationships of lipid bilayers in KCl (1.0 M) with three concentrations of PV. For an unmodified bilayer, the $I-V$ characteristic was linear over the range $\pm 120$ mV ($16-19$). However, at all concentrations of PV tested, the $I-V$ curves were nonlinear and $I$ approached a maximum value ($I_{max}$) independent of $V$ when $V$ exceeded 100 mV (Fig. 3). $I_{max}$ increased progressively as KCl concentration was increased progressively from $10^{-2}$ to 1.0 M at constant PV concentration ($5 \times 10^{-6}$ M) (Fig. 4). When PV ($5 \times 10^{-6}$ M) was present on only one side of the
TABLE I
CATION SELECTIVITY OF PEPTIDE PV ON SHEEP RED CELL LIPOID BILAYERS

All measurements were made in 0.1 M XCl and 0.001 M XH2PO (pH 7.4) where X+ was a monovalent test cation. The biionic potentials (V_m) were the electrical potential difference at zero current across membrane separating equimolar solutions of K+ and test cation chloride salts. Each value represents the mean of observations on at least two membranes.

| PV (10^-6) | XCl (0.1 M) | XH2PO (0.001 M) | pH 7.4 | 23°C |
|------------|-------------|-----------------|--------|------|
| X+         | G_m         | V_m(X:X)        |        |
|            | 10^4 ohm^-1 cm^-2 | mV |         |
| Li         | 0.057       | -106            |        |
| Na         | 0.34        | -76             |        |
| NH4        | 0.86        | -39             |        |
| Cs         | 1.3         | -22             |        |
| Rb         | 3.5         | +2              |        |
| K          | 4.4         | 0               |        |

TABLE II
CATION SELECTIVITY OF PEPTIDE PV ON SHEEP RED CELL LIPOID BILAYERS

Similar experimental procedures as in Table I except the measurements were made in 0.01 instead of 0.1 M XCl. Each value represents the mean of observation on at least two separate membranes. For the column headed V_m(X:X/4.2) the concentration of XCl was 0.01 M on the inside and 0.001 M on the outside while the concentration of X+ as XHPO4-X2PO4 was 0.0018 M, yielding a concentration of ratio of X+ of 4.2. The equilibrium potential for X+ under these circumstances is -36 mV. t_2 is the transference number for X+ calculated from the measurement of V_m(X:X/4.2) and the assumptions that V_m = t_2E_x + t_1E_c and t_2 + t_1 = 1, where E_x and E_c are the equilibrium potentials for X+ and Cl-, respectively.

| PV (10^-6) | XCl (0.01 M) | XH2PO (0.001 M) | pH 7.4 | 23°C |
|------------|-------------|-----------------|--------|------|
| X+         | G_m         | V_m(X:X/4.2)    | t_2    | V_m(X:X) |
|            | 10^4 ohm^-1 cm^-2 | mV | mV | mV |
| Na         | 0.023       | -      | 0.94 | -66 |
| NH4        | 0.22        | -30    | 0.94 | -29 |
| Rb         | 0.35        | -28    | 0.92 | -5  |
| K          | 2.1         | -24    | 0.87 | +26 |
|            |             |        |      | 0   |

membrane, marked rectification occurred in the sense that positive current flowed much more easily away from rather than toward the side containing the carrier. Under these conditions, the potential difference at zero current was about 50-60 mV with the side containing PV negative (Fig. 5).
Figure 3. Current-voltage relations of lipid bilayers in the presence of indicated concentrations of PV. The aqueous solution contained 1.0 M KCl.

Figure 4. Current-voltage relations of lipid bilayers in the presence of 5 × 10^-6 M PV as a function of potassium concentration.
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FIGURE 5. Current-voltage relations of lipid bilayers with bilateral (A) and unilateral (B) addition of PV. The aqueous solutions contained 1.0 M KCl.

Zero-Current Membrane Potentials Without Salt Gradients

Fig. 6 shows the zero-current membrane potential plotted as a function of PV concentration in the inside solution bathing bilayers formed from sheep red cell lipids. The outside solution contained no PV while both bathing solutions contained identical concentrations of either K+ or Na+ salts. The membrane potentials were much higher when K+ rather than Na+ was present at relatively low concentrations of PV. We ascribe this result to the greater affinity of PV for K+ than for Na+ which produced a greater concentration and thus a greater transference number for K+ than for Na+ complexes under these conditions. At high concentrations of free PV the membrane potential was the same for both cations. In both cases, the sign of the potential was negative on the side containing the carrier.

Fig. 7 is a graphic presentation of a model which we propose to account for the development of $V_m$. Here we consider two cases, in both of which carrier is added to the left or inside, but not to the right or outside solution. In case A, the permeability of the membrane to the carrier is high compared to that of the unstirred layers. During the steady state of diffusion, the concentration of the carrier decreases linearly with distance across the relatively
**Figure 6.** Effect of PV on electrical potential difference ($V_m$) across lipid bilayers. PV was added only to one side of the membrane. The aqueous solutions contained 0.1 M KCl (●) or 0.1 M NaCl (○).

**Figure 7.** A graphic presentation for membrane permeability to carrier (A) high permeability and (B) low permeability. $i_{CV}$ is concentration of carrier in the inside bulk solution; $i_{mCV}$ is concentration of carrier at the inside membrane surface, $i_{oCV}$ is the concentration of carrier at the outside membrane surface, and $i_{CV}$ is the concentration in the outside bulk solution.

thick (10^-2-cm) unstirred layers and there is no appreciable difference in concentration across the relatively thin (10^-4-cm) membrane. This situation obtains for val. In case B, the permeability of the membrane to the carrier is low compared to that of the unstirred layers. During the steady state of diffusion, the concentration of the carrier again falls linearly across each unstirred layer but there is an appreciable difference in the concentrations at the membrane surfaces. This situation obtains for PV. Since the potential difference with unilateral PV developed in the absence of current flow (zero flux of the charged complex), we argue that the reactions between free PV and cations must be at equilibrium at the membrane surfaces. Thus the equation

$$K = \frac{MS^+}{S \cdot M^+},$$

(1)
where \( K \) is the association constant and \( MS^+ \), \( S \), and \( M^+ \) are the concentrations of complex, free carrier, and free metal cation, must obtain at both membrane surfaces (see Fig. 8 for notation). If this condition holds, the ratio of the concentrations of PV-cation complexes at the two membrane surfaces must equal the ratio of the concentrations of free-uncharged PV, i.e.

\[
\frac{\frac{\text{im}MS^+}{\text{om}MS^+}}{\frac{\text{im}S}{\text{om}S}} = K
\]

(2)

If \( MS^+ \) complexes are the only significant charge carriers in the membrane, i.e. if the transference number for \( MS^+ \) in the membrane is one, then the membrane potential will equal the equilibrium potential for \( MS^+ \). If only chemical and electrical potential differences are significant driving forces for movement of \( MS^+ \), then

\[
V_m = \frac{RT}{F} \ln \frac{\text{om}MS^+}{\text{im}MS^+}.
\]

(3)

If these assumptions are correct, the value of \( V_m \) can be used to estimate the relative magnitudes of the rate constants and permeability coefficients in the model for carrier-mediated transport shown in Fig. 8. This model is similar to that proposed by Läuger et al. (1, 2) and identifies three distinct steps in the translocation of components across the membrane system, i.e. movement across the unstirred layers, the membrane surfaces, and the membrane interior. Steady-state transport of free carrier (\( S \)) across these three regions can be described by the following set of equations:

\[
J_s = uP_s(S - \text{im}S)
\]

(4 a)

\[
J_s = m_s k_s (\beta_s S - \text{im}S),
\]

(4 b)

\[
J_s = m_s k_s (\text{im}S - \text{om}S)
\]

(4 c)

where \( J_s \) is the flux of free carrier, \( uP_s \) is the permeability of the unstirred layer to free PV, \( m_s k_s \) is the rate coefficient for exit of free PV from the membrane surface, \( \beta_s \) is the partition coefficient of free PV, and \( k_s \) is the rate coefficient for translocation of free PV across the membrane interior (see legend of Fig. 8). Solution of this set of equations leads to the expression

\[
V_m = \frac{-RT}{F} \ln \left(1 + \frac{uP'_s}{\beta_s m_s k_s}\right),
\]

(5 a)

where

\[
\frac{uP'_s}{\beta_s m_s k_s} = \left(\frac{1}{\beta_s m_s k_s} + \frac{1}{uP_s}\right)^{-1}.
\]

(5 b)
FIGURE 8. Proposed model for carrier-mediated ion transport. $M^+$, $S$, and $MS^+$ indicate concentrations of free metal ion, free carrier, and cation-carrier complex, respectively. The superscripts $i$, $ia$, $im$, $oa$, and $o$ represent the inside bulk aqueous solution, the aqueous phase adjacent to the inner membrane surface, the inner membrane surface, the outer membrane surface, the aqueous phase adjacent to the outer membrane surface and the outer bulk aqueous solution, respectively. The symbols $^uP_m$, $^uP_s$, and $^uP_{ma}$ denote the permeability coefficients of the unstirred layers to free metal, free carrier, and complex, respectively. The symbols $^uP_m$, $^uP_s$, and $^uP_{ma}$ indicate rate coefficients for translocation of free carrier and complex across the membrane interior, $^uP_m$, $^uP_s$, and $^uP_{ma}$ are rate coefficients for exit of free metal, free carrier, and complex from the membrane surface. $\beta_m$, $\beta_s$, and $\beta_{ma}$ are the partition coefficients of free metal, free carrier, and complex (e.g. $^oS/\alpha S$ where $^oS$ is the surface concentration in mol cm$^{-2}$ and $\alpha S$ is the concentration in the adjacent aqueous phase in mol cm$^{-3}$. Thus $\beta$ has the unit centimeter.) $k_D$ and $k_B$ are the rate coefficients for dissociation and formation of the cation-carrier complex where the superscripts $m$ and $a$ indicate membrane surface and aqueous phase, respectively.

If the translocation of free carrier across the membrane-aqueous solution interface is rapid compared to transport across the membrane interior (i.e. if $^uP_m > ^uP_s$), $^uP_s = ^uP_m$ and $5 a$ becomes

$$ V_m = -\frac{RT}{F} \ln \left( 1 + \frac{^uP_s}{\beta_s ^uP_m} \right) \quad (6). $$

Eq. 6 can be used to evaluated $\beta_s ^uP_s$ if $V_m$ and $^uP_s$ are known. $^uP_s$ can be estimated by taking into account that

$$ ^uP_s = \frac{D_s}{\Delta} \sim \frac{4.3 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}}{10^{-2} \text{ cm}} \sim 4.3 \times 10^{-4} \text{ cm s}^{-1}, \quad (7) $$

where $D_s$ is the diffusion coefficient of free PV in water estimated from the Stokes-Einstein equation and $\Delta$ is the thickness of the unstirred layers. When the two sides of a bilayer are bathed with identical salt solutions, the magnitude of $V_m$ with unilateral PV ($2 \times 10^{-4}$ M) is about 90 mV (Fig. 6). The computed value for $\beta_s ^uP_s$ equals about $1.3 \times 10^{-4}$ cm s$^{-1}$. Since this com-
putation of $\beta_s = k_s$ depends on the assumption that PV is distributed at equilibrium between the membrane surfaces and the adjacent aqueous solutions, it is a maximum estimate (see Eq. 5 b and Discussion).

The validity of this interpretation of $V_m$ is supported by the results of the experiments shown in Fig. 9. Here, a value of $\beta_s = k_s$ was computed from the value of $V_m$ when PV was present only in the inside solution using Eq. 6 and the above estimate of $\delta P$. Subsequently, $V_m$ was measured after successive additions of PV to the outside solution. These measured values of $V_m$ were then compared to values of the equilibrium potential for $MS^+$ computed either from the total PV concentrations in the bulk bathing solu-

![Figure 9](image_url)

**Figure 9.** Correlation between the calculated $V_m$ and the observed $V_m$ (A) uncorrected for unstirred layers and (B) corrected for unstirred layers. The points represent four separate membranes. For experimental details and calculations, see Results.

...tions (A) or from the calculated concentrations of $MS^+$ at the two membrane surfaces (B). The concentrations at the membrane surfaces were computed using the values for $\beta_s = k_s$ and $\delta P$, estimated above and in Eqs. 4. The fact that the calculated equilibrium potential for $MS^+$ agrees with the measured membrane potential in B but not in A is consistent with the arguments that $V_m$ is, in fact, equal to the equilibrium potential for $MS^+$ under these conditions, that $\beta_s = k_s$ is independent of PV concentration over the range tested, and that the unstirred layers do offer an appreciable resistance to diffusion of the carrier (20–22).

If the occurrence of the $V_m$ is due to the low permeability of the membrane to free PV, then changes in the magnitude of the potential can be interpreted in terms of changes in the membrane permeability. The data presented in Figs. 10 and 11 are of interest in this respect. When synthetic dioleoyllecithin was used instead of sheep red cell lipids to form bilayers, the observed $V_m$ was lower. The molar ratio of cholesterol to phospholipids of lipids extracted from sheep red cells is about 1 (13) and cholesterol is known to decrease the
FIGURE 10. Effect of unilateral PV on the electrical potential difference across bilayers formed from sheep red cell lipids (●) and synthetic L-dioleoyllecithin (○). The aqueous solutions contained 0.1 M KCl.

FIGURE 11. Effect of PV and phloretin on electrical potential difference across sheep red cell lipid bilayers. The aqueous solutions contained 1 M KCl.

Phloretin has been shown by Cass et al. (29) to increase carrier-mediated cation and decrease carrier-mediated anion transport across bilayers. They have interpreted these data to mean that phloretin reduces the magnitude of the electrical potential difference which renders the hydrocarbon interior of the membrane several hundred millivolts positive to the membrane surfaces. The data shown in Fig. 11 suggest that phloretin increases membrane permeability to uncharged molecules like free PV. Cass et al. (29) have shown that phloretin also increased bilayer permeability to the nonelectrolyte acetamide. However, the magnitude of the increase in acetamide permeability is considerably less than the increase in PV permeability produced by phloretin. Furthermore, the fact that phloretin is much more effective in reducing the potential when it is added to the same rather than to the opposite side to PV suggests two conclusions. First, the permeability of sheep red cell lipids bilayers to phloretin is low. Second, the major resistance to PV movement involves entrance rather than exit from the membrane.

DISCUSSION

The results presented in this paper further support our earlier observations that the substitution of carbonyl oxygens in amide linkage as in PV for those
in ester linkage as in val alters the interactions of the compound with metal ions (12, 23, 24). Despite the greater affinity of PV for cations in two-phase extraction system (23, 24) this compound is much less potent than val in inducing cation permeability across bilayers (Figs. 1 and 2). Furthermore, we have shown that in contrast to val, PV is much less selective for K\(^+\) over Na\(^+\) (Tables I and II) though the selectivity sequence is similar to that for val, K\(^+\) \(\simeq\) Rb\(^+\) \(>\) Cs\(^+\) \(>\) NH\(_4^+\) \(>\) Tl\(^+\) \(>\) Na\(^+\) \(>\) Li\(^+\). However, the observed values of \(V_m\) show that the quantitative interpretation of the measurement of \(V_m\) is difficult. The failure of \(V_m\) to equal the equilibrium potential for X\(^+\) can be interpreted in at least two different ways. First, PV could induce permeability of the bilayers to chloride. Second, complexation in the aqueous phases and low membrane permeability leading to concentration differences of free PV could produce a deviation of the ratio of the concentrations of PV-cation complexes at the membrane surfaces from the ratio of salt concentrations in the aqueous phases. The positions of NH\(_4^+\) and Tl\(^+\) in the sequence of selectivity (Table II) are of interest because of the suggestion of Eisenman and Krasne (25) that these ions can be used to test the number and field strength, respectively, of ligands involved in the interaction between cations and carriers. They predicted that carriers in which the ligands interacting with the metal ion are carbonyl oxygens in amide linkage, Tl\(^+\) would be preferred to K\(^+\). PV, like val, preferred K\(^+\) and Rb\(^+\) to both NH\(_4^+\) and Tl\(^+\) in bilayers. Thus, conversion of ligands from ester carbonyls (val) to amide carbonyls (PV) did not alter the K\(^+\)-Tl\(^+\) selectivity. The failure of this result to agree with the prediction of Eisenman and Krasne could be due to the fact that the field strength of carbonyl oxygens in imide linkage with proline nitrogen atoms is different from the field strength of carbonyl oxygens in amide linkage with amino acids which have a proton available for hydrogen bonding when in amide linkage.

One striking difference in the effect of PV and val on the electrical properties of lipid bilayers is the development of a large, stable, zero-current potential difference across a bilayer separating identical salt solutions when PV was present on only one side of the membrane (Fig. 6). No such effect was observed with val in K\(^+\) Medium. Electrical potential differences at zero current have also been observed across bilayers separating identical solutions of NaCl with different concentrations of val (9). However, the sign of the potential difference was opposite in the two cases. In contrast to the situation with PV, the side of the membrane exposed to val was positive. We argue that the zero-current membrane potential between identical salt solutions upon unilateral addition of PV (\(V_m\)) arises from the establishment of a concentration difference for the cation complexes of PV. The concentration difference for MS\(^+\) develops because the permeability of bilayers to the free, uncharged form of PV is extremely low. This explanation for the \(V_m\) produced
by unilateral addition of PV cannot account for the $V_m$ produced by adding val to one side of a bilayer separating identical NaCl solutions.

Assuming that the rate coefficient for desorption from the membrane surface ($\beta N k_s$) much exceeds both the permeability of the unstirred layer ($P_u$), and the rate constant for translocation across the membrane ($N k_s$) i.e. under conditions of equilibrium at the membrane surfaces, we have estimated a value for $\beta N k_s$ of $1.3 \times 10^{-6}$ cm$^2$ s$^{-1}$ for PV. Läuger et al. (1, 2) have measured values of $2 \times 10^{-2}$ cm and $2 \times 10^{4}$ s$^{-1}$ for $\beta N k_s$ for val. This yields a calculated value of $2 \times 10^2$ cm s$^{-1}$ for $\beta N k_s$ for val, about $10^7$ times greater than the value for PV. The physicochemical basis for this profound difference in the permeability to compounds as similar as free PV and free valinomycin is not clear. It could be due to a low value either of $\beta N k_s$ or of $N k_s$ or both. It may be related to the different conformations of free PV and free valinomycin in nonpolar solvents as revealed by proton NMR (23, 24, 26) and infrared spectroscopy (12) studies.

The "saturating" current-voltage relations shown in Fig. 3 might also be due to low membrane permeability to free PV. However, if the value of $\beta N k_s$, estimated above from $V_m$ (ca. $10^{-4}$ cm s$^{-1}$) is correct, the saturation current at high values of membrane voltage would be less than $\frac{1}{10}$ that observed (Figs. 3 and 4). Furthermore, if this were the case, increasing the salt concentration would be expected to decrease the maximum current. Fig. 4 shows the opposite to be the case. Therefore, the rate of dissociation and/or formation of $X^+$.PV complexes is probably rate limiting under these conditions.

The occurrence of an electrical potential difference between identical salt solutions is of interest to electrophysiologists who often attempt to determine ionic transference numbers from measurements of zero-current potential differences when different concentrations of salts are in the solutions bathing the membrane. Implicit in this approach is the assumption that no potential difference develops when the concentrations of all ions are equal on the two membrane surfaces. We have shown that this assumption is not valid for membranes exposed unilaterally to low concentrations of PV. This effect will occur whenever the membrane permeability to the uncharged form of a carrier is sufficiently low and when the transference number of the charged form of the carrier is sufficiently high. The presence of low concentrations of such uncharged ion carriers in the cytoplasm of some kinds of living cells cannot be excluded at present.

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