supports the choice of the TIPS parameters for alkyl groups.

The computed distribution functions, $S(\phi)$, for the dihedral angle in the liquids are compared to the ideal gas results in Figures 1a and 2a. The increased gauche population in liquid DCE is dramatic, while the condensed phase effect for liquid *n*-butane is negligible. The dominance of the electrostatics and not the lower molar volume in producing the shift for DCE was proven by a Monte Carlo simulation with the Coulomb terms deleted.[7] When the charges are set to zero for DCE and all other parameters and conditions are unchanged, the computed $S(\phi)$ is nearly identical with the ideal gas result.[7] The trans populations are obtained by integrating $S(\phi)$ from 120 to 240°, the remainder being gauche. For DCE, the computed trans populations are 76.5% for the ideal gas and (43.5 ± 0.5)% for the liquid which agree well with the estimates from IR data of (77 ± 2)% and (35 ± 4)% at 25 °C.[10] For *n*-butane, the computed trans populations are 67.7% for the ideal gas and (67.1 ± 0.8)% for the liquid. The NPT simulation at 1 atm is in accord predicting (67.8 ± 1.3)% trans for liquid *n*-butane at −0.5 °C.[13] Thus, no statistically significant condensed phase effect on the conformational equilibrium for liquid *n*-butane at the standard density is revealed. This agrees with Flory's traditional position on polymer melts;[14] however, it contrasts some earlier, less extensive theoretical results which were interpreted as indicating a "significant" shift toward increased gauche population in the pure liquid.[3a,5] Finally, it is noted that correcting for the rigid constraints on the bond lengths and bond angles in a recent molecular dynamics simulation of *n*-butane in CCl_4 did not alter the computed gauche/trans ratio.[15]

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Design, Synthesis and Characterization of a Cytotoxic Peptide with Melittin-Like Activity

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Recently, we have been testing the hypothesis that the binding characteristics of certain proteins, such as those of apolipoproteins to interfaces, are determined by an important secondary structural feature, the amphiphilic α helix.[1-3] We have shown that a model docosapeptide, with minimum homology to apolipoprotein A-I (apo A-I), has the binding chracteristics of apo A-I by criteria of its binding to phospholipid single bilayer vesicles, surface activity, and ability to activate lecithin:cholesterol acyltransferase.[4,5] The main toxic component from bee venom, melittin[6] (Figure 1A) is a hexacosapeptide which has many properties in common with the apolipoproteins. It binds to phospholipid bilayers,[7-14] forms stable monolayers at the air–water interface,[7] and is capable of forming an α helix upon tetramerization or when bound to sodium dodecyl sulfate micelles or phospholipid bilayers.[13,14] This suggested to us that the amphiphilic α helix might be essential for the biological activity of melittin but only to the extent that it is providing a secondary structure. A second feature which appears to be necessary for the biological activity of melittin is the highly basic C-terminal hexapeptide, since synthetic melittin (1–20),[15] which lacks this hexapeptide, is as surface active as melittin but is not biologically active as measured by the erythrocyte lysis assay. In addition, the length of the amphiphilic helical segment appears important since segments with shortened N-terminal sequences such as melittin (8–26) are also very poor lytic agents.[14] Thus, the essential requirements for a lytic agent such as melittin appear to be an eicosapeptide segment capable of forming an amphiphilic α helix, followed by a highly basic C-terminal hexapeptide which is recognized by the cell. To test this hypothesis, we have synthesized peptide **1** (Figure 1A) which is homologous to melittin only in the C-terminal hexapeptide region. Peptide **1** has a higher potential to form amphiphilic α helices than melittin, and we will show that it epitomizes many of the biological and physical properties of melittin.

A. MELLITIN I

H_2N-GLY-ILE-GLY-ALA-VAL-LEU-LYS-VAL-LEU-THR-
THR-GLY-LEU-PRO-ALA-LEU-ILE-SER-TRP-ILE-
LYS-ARG-LYS-ARG-GLN-GLN-$CONH_2$

PEPTIDE I

H_2N-LEU-LEU-GLN-SER-LEU-LEU-SER-LEU-LEU-GLN-
SER-LEU-LEU-SER-LEU-LEU-LEU-GLN-TRP-LEU-
LYS-ARG-LYS-ARG-GLN-GLN-$CONH_2$

B.

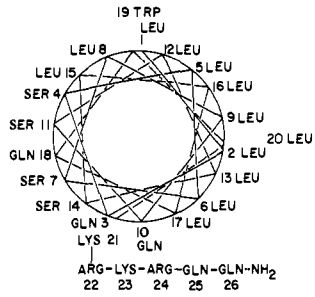

Figure 1. (A) Amino acid sequences of melittin I and peptide **1**. (B) Axial projection of the α-helical region of peptide **1** showing the relative location of the side chains with the segregation of the hydrophobic and hydrophilic residues.

As illustrated by the helical projection in Figure 1B, residues 1–20 of **1** have been chosen to form an amphiphilic helix with minimum homology to melittin while maintaining a hydrophobic–hydrophilic balance related to the two amphiphilic α helical segments of melittin (2–13) and (15–21). The hydrophobic side of the helix was composed of Leu residues, chosen for reason of their high helix-forming potential,[16] hydrophobicity, and electrical neutrality. While Gln seemed an ideal choice for the neutral hydrophilic residues, some Ser residues were included to increase the hydrophilicity of the model, allowing us to match the amphiphilicity of the native peptide. A Trp residue was left at position 19 for future studies of intrinsic fluorescence, and the C-terminal hexapeptide of melittin was retained as such.

Peptide **1** was synthesized by the solid-phase method on 1% cross-linked divinylbenzene–polystyrene by using the symmetric

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anhydrides[17] of BocLeu, BocGln, BocSer(Bzl), BocTrp(For), AocArg(Tos), and BocLys(2-ClZ) and a deprotection and coupling program similar to that employed by Yamashiro and Li.[18] The protected peptide was removed from the resin by ammonolysis using trifluoroethanol saturated with NH_3, and the remaining protecting groups were removed with liquid HF.[19] Purification of the deblocked peptide was accomplished by gel permeation chromatography using Sephadex G-25 SF with 10% acetic acid as the eluant, followed by partition chromatography[20] using Sephadex G-25 SF and the two-phase system: butanol/acetic acid/water (4:1:4, v/v/v). The peptide was further purified by partition chromatography using the same solvent system and a column of Sephadex G-50 SF (1.5 × 65 cm). After a second gel filtration and lyophilization, the peptide was obtained in 1.5% yield as calculated from the attachment of the first amino acid to the resin. In subsequent experiments the yield was increased to 5% by using Na in liquid NH_3 instead of HF to remove the protecting groups. The purified peptide had the expected amino acid composition within experimental error and showed single spots upon TLC in several solvent systems. Automated Edman degradation of 300 nmol of **1** confirmed its amino acid sequence and purity. No "previews" were detected over the entire sequence of 26 amino acids.

The circular dichroism (CD) of peptide **1** was measured in 0.16 M KCl–0.02 M phosphate buffer, pH 7.0. The concentration dependency of the mean residue ellipticity, θ, suggested that **1** might be aggregating, and by sedimentation equilibrium centrifugation,[21] peptide **1** indeed appeared to be tetrameric at 2.5×10^{-5} M. The concentration dependency of θ was consistent with a cooperative monomer–tetramer equilibrium with $K_{diss} = 4 \times 10^{-16}$ M^3 (at 222 nm, $\theta_{monomer} = -10\,600$ deg cm^2/dmol and $\theta_{tetramer} = -23\,900$ deg cm^2/dmol). Melittin also forms tetramers,[22] and in the above buffer we found $K_{diss} = 1.6 \times 10^{-12}$ M^3 (at 222 nm, $\theta_{monomer} = -3870$ deg cm^2/dmol and $\theta_{tetramer} = -15\,570$ deg cm^2/dmol). From these data we calculated the helical content of **1** to be 69% for the tetramer and 35% for the monomer; the corresponding values for melittin are 48% and 18%, respectively. The dissociation constant of **1** corresponds to a free-energy change of 5.25 kcal/mol of monomer or 0.40 kcal/mol of hydrophobic residue, suggesting that the main driving force for the reaction is the aggregation of the hydrophobic, leucine-rich, faces of the amphiphilic helices. The corresponding free-energy change for melittin is 4.02 kcal/mol of monomer, indicating that either a shorter sequence or a slightly less hydrophobic sequence is involved in the tetramerization process. Since the hydrophobicities of **1** and melittin are identical, the former explanation is the most likely.

Peptide **1** formed stable monolayers at the air–water interface. The surface pressure–area (π–A) curve showed a discontinuity at 45.5 dyn/cm, indicating collapse of the monolayer. Melittin also formed stable monolayers, but collapse occurred already at 22 dyn/cm. The π–A curves of the monolayers obey the equation $\pi[A - A_\infty(1 - \kappa\pi)] = nkT$ where κ is the compressibility and A_∞ is the limiting molecular area extrapolated to zero surface pressure.[4] The analysis yielded molecular weights of 3600 ± 300 for **1** and 3000 ± 250 for melittin, in good agreement with their monomeric molecular weights (3420 and 2840, respectively), indicating that there is no oligomerization induced by the air–water interface. Integration of the force area curve for **1** from 22 to 45.5 dyn/cm showed that the monolayers of **1** had about 4.8 kcal mol^{-1} additional stabilization with respect to the melittin monolayer at its collapse pressure. The limiting area was 368 $Å^2$ per molecule for melittin and 468 $Å^2$ for **1**. If the C-terminal hexapeptide is fully hydrated, this indicates the **1** is able to form a longer amphiphilic segment than melittin. This is reasonable since melittin has the helix breaker proline at position 14 in its sequence. Interestingly, the ratio of the free energies of tetramerization of the two peptides, $\Delta G_1/\Delta G_{melittin}$, and the inverse of the corresponding ratio of the surface areas, $A_{\infty_1}/A_{\infty_{melittin}}$, are the same within experimental error (1.30 and 1.27, respectively), indicating that the same segments are involved in the binding to the air–water interface and the self-association process. This is further supported in an indirect way by the ratio of the helicity of the two peptides (1.4) in the tetrameric state.

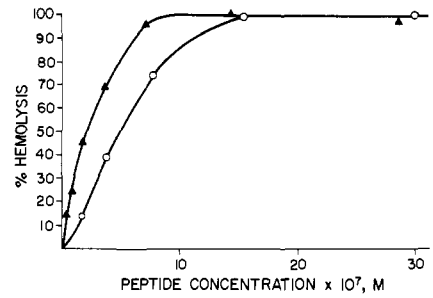

Figure 2. Dose–response curves for erythrocyte lysis:[23] ▲, peptide **1**: ○, melittin.

When erythrocytes were incubated in an isotonic solution with peptide **1** concentrations on the order of 10^{-7} M, rapid hemolysis occurred as assessed by the method of Habermann and Kowallek.[23] For 30-min incubations typical sigmoid curves were observed when the amount of solubilized hemoglobin was plotted as a function of peptide **1** or melittin concentration as shown in Figure 2. The concentration of peptide **1** required for 50% lysis is 2.5 times lower than that required for melittin. The shapes of the curves are, however, quite similar, and at high enough peptide concentration they reach the same plateau value. From these observations we conclude that the synthetic peptide effects the lysis of erythrocytes by the same mechanism as melittin. The amount of peptide required for total hemolysis is of the order of magnitude required to cover the surface of the erythrocytes. This, together with the higher surface affinity of peptide **1** with respect to melittin, would suggest that hemolysis requires binding of the peptide to the surface and proper binding depends mainly on the hydrophobic–hydrophilic balance in the amphiphilic α-helical segment. That surface binding is indeed involved in the hemolysis is also indicated by our observation that an amphiphilic α-helical synthetic docosapeptide model[4] of apolipoprotein A-I acts as a strong inhibitor of the hemolytic activity of melittin at concentrations comparable to those of the toxic peptide.

The model peptide and melittin bind readily to unilamellar egg lecithin vesicles,[24] and our experiments indicate that both peptides have a high potential to disrupt the phospholipid bilayers. Gel filtration of the vesicles incubated with peptide **1** showed the formation of large particles. Concomitant with the structural changes, glucose 6-phosphate and ATP were released from the captured volume. Electron microscopy showed the formation of multilamellar liposomes.

Our results thus show that the 20 N-terminal amino acid residues of melittin have a purely structural role which can be duplicated by a nonhomologous sequence of high α-helix-forming ability and of the proper hydrophobic–hydrophilic balance.[25] These observations strongly support the proposal that the N-

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terminal region of melittin binds to the phospholipid bilayer of cell membranes in the helical mode when the axis of the helix is parallel to the surface. In view of the absence of tertiary structure, it is not surprising to observe such a clear segregation of the structural and functional role of different segments of the peptide chain. The limited possibilities of well-defined secondary structures for peptides would then suggest that amphiphilic helical segments might be found in a variety of biologically important oligopeptides such as hormones. We hope that the results reported in this paper demonstrate the power of rational design of peptides based on secondary structural considerations for the investigation of structure–functional relationships in biologically active peptides.

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Evidence for a Second Mode of Hydroxypalladation in Aqueous Solution

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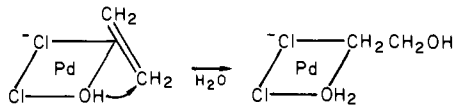
It is universally agreed that the oxidation of ethene to acetaldehyde by palladium(II) chloride in aqueous solution proceeds by a mechanism involving conversion of a π-bonded olefin to a palladium(II) β-hydroxylalkyl, a process called hydroxypalladation.[1] The rate expression for the oxidation (eq 1) has

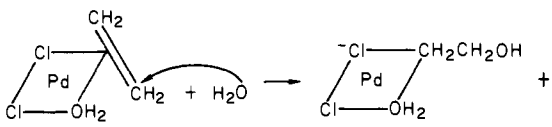
$$\frac{-d[C_2H_4]}{dt} = \frac{k[PdCl_4^{2-}][C_2H_4]}{[H^+][Cl^-]^2} \quad (1)$$

been interpreted in terms of formation of the hydroxypalladation intermediate, **1**, by either a cis attack of coordinated hydroxyl (eq 2)[2] or trans attack of external water (eq 3).[3-5] The first was



(2)

1

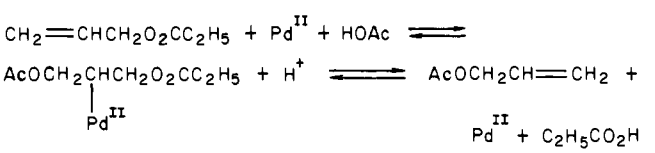


(3)

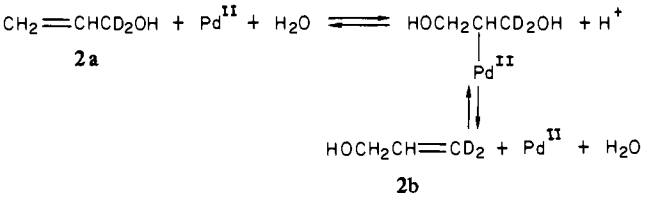
1

originally selected as most likely on the basis of isotope effects[2] but the latter has more recently been suggested because of stereochemistry studies which suggest hydroxypalladation occurs by attack of water from outside the coordination sphere of palladium(II).[3,4,6] The most convincing of these studies[4] involves the oxidation of ethene at high chloride (3.3 M) and high cupric chloride concentrations (2.7 M), conditions under which chloroethanol becomes the main product.[7] It was found that the configurations of the chloroethanol-d_2 products from oxidation of (*Z*)- and (*E*)-ethene-d_2 were consistent with trans hydroxypalladation. Assuming that $CuCl_2$ was intercepting the intermediate, **1**, a mechanism analogous to the second route (eq 3) was suggested.[4] This communication provides evidence that an intermediate other than **1** is actually being intercepted in the reaction forming chloroethanol.

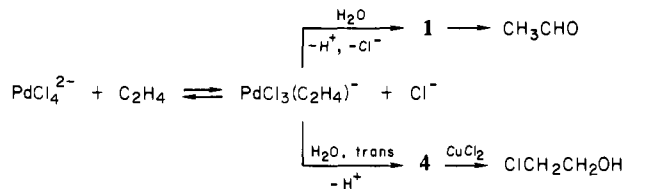
Scheme I



Scheme II



Scheme III



Allylic groups such as esters can be exchanged in a nonoxidative palladium(II)-catalyzed reaction.[8] An example is the exchange of allyl propionate with acetic acid solvent, a reaction which almost certainly occurs by the oxypalladation–deoxypalladation mechanism shown in Scheme I.[9,10]

In order to determine if other modes of hydroxypalladation might exist in aqueous solution, the isomerization of propenol-*1,1*-d_2 (**2a**) to a 50:50 mixture of **2a** and propenol-*3,3*-d_2 (**2b**) was studied by using H^1 NMR spectroscopy under conditions where the oxidation reaction, which gives acrolein as main product,[11] is slow.

The isomerization did not take place in the absence of palladium(II) chloride but did occur readily in its presence. The rate expression for isomerization is given by eq 4 ($C_3H_4D_2O$ = propenol-*1,1*-d_2) where $k_i = 1.5 \times 10^{-3}$ s^{-1}.

$$\text{rate} = \frac{k_i[PdCl_4^{2-}][C_3H_4D_2O]}{[Cl^-]} \quad (4)$$

The most plausible route for this isomerization is given by Scheme II which is analogous to that given in Scheme I for ester exchange. Since the rate expression for isomerization (eq 4) is quite different from that for oxidation, the modes of hydroxypalladation for the two processes must also be different. The first power chloride inhibition and lack of a proton inhibition term are consistent with the reaction scheme given in eq 5-7. The trans hydroxypalladation shown in eq 6 is consistent with the first power chloride inhibition since insertion of H_2O in the coordination sphere

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