Physical and Conformational Properties of a Synthetic Leader Peptide from M13 Coat Protein

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According to the signal hypothesis proposed by Blobel and co-workers, nascent secretory proteins and membrane-bound proteins insert into the lipid bilayer via an N-terminal extension called the leader or signal peptide.[1] The molecular mechanisms by which leader peptides interact with membranes have been the subject of considerable discussion.[2-5] Most of our understanding of the features of signal peptides important in the translocation of peptides and proteins through membranes has been deduced from studying their primary structure.[6-8] Since signal peptides lack sequence homology among themselves but all are rich in nonpolar amino acids, the possibility exists that when they interact with membranes they have in common a lipophilic secondary structure.[3,9] In order to evaluate the aspects of their secondary structure that might be crucial in their function, we have chosen to synthesize leader peptides by chemical methods for the purposes of examining their conformational and physical properties in aqueous solution and in lipid and membrane mimetic systems.

For our first study of a signal region we used the Merrifield solid-phase method to synthesize the C-terminal carboxylamidated leader peptide from the M13 virus coat protein, Met-Lys-Lys-Ser-Leu-Val-Leu-Lys-Ala-Ser-Val-Ala-Val-Ala-Thr-Leu-Val-Pro-Met-Leu-Ser-Phe-Ala-NH_2.[10] This peptide has the general features of many leader peptides: there is a cluster of positively charged lysine residues at the N-terminus followed by a core of hydrophobic residues;[6] the presence of proline increases the probability of β-turn formation in the C-terminal region.[11,12] Although 70% of the peptide's residues are hydrophobic,[13] we hoped that the presence of lysines and serines would render the peptide soluble in aqueous solutions.

The M13 signal peptide was prepared on benzylhydrylamine-substituted divinylbenzene resin (1% cross-linked; Bio-Rad) by using blocking groups and HF cleavage methods previously described.[14] After HF cleavage, the peptide was extracted quantitatively from the peptide-resin mixture with 10-25% acetic acid. In order to prevent oxidation of the methionine residues during overnight extraction, dithiothreitol (5 mM) and degassed water were used. The peptide was chromatographed on Sephadex G25 SF with 1% acetic acid. Purification was achieved by semipreparative HPLC from a C18 column (Altex), eluting with a gradient of 40-70% CH_3CN in 0.02 M sodium phosphate buffer, pH 2.7, containing 0.10 M sodium perchlorate.[15] The major peptide peak eluted between 47% and 50% CH_3CN and accounted for ~40% of the total peptide peaks. Because the major peak was flanked closely on both sides by two other peaks, the yield on each HPLC run was sacrificed to 25% in order to assure greatest purity. To recover the peptide, acetic acid (20% by volume) was added prior to CH_3CN evaporation. Phosphate and perchlorate salts were then removed by chromatographing on G15 Sephadex in 1% acetic acid. The peptide in 1% acetic acid appeared as a single peak with good symmetry when subjected to isocratic HPLC with 48% CH_3CN on an analytical C18 column.

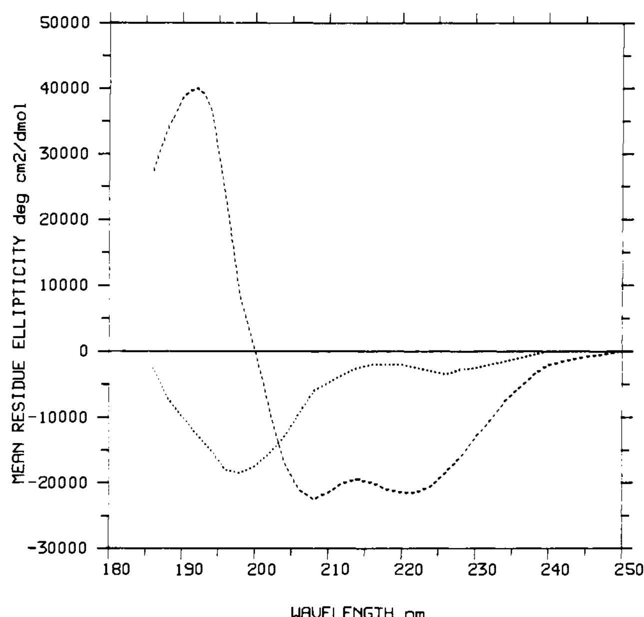

Figure 1. Circular dichroism spectra of M13 leader peptide. In 0.02 M sodium phosphate buffer pH 2.8, the synthetic M13 leader peptide (9.0 × 10^{-5} M) shows predominantly random coil structure (···). Upon addition of 33% hexafluoroisopropyl alcohol, the peptide (6.0 × 10^{-5} M) exhibits a helical structure (- - -). Peptide concentrations were determined by amino acid analysis.

The purified peptide, hydrolyzed in 6 N HCl at 110 °C for 20 h, showed the expected amino acid composition, except that the ratios of alanine and valine were ambiguous (3.5 mol of each per mol of peptide). When the peptide was hydrolyzed with HCl/TFA (2:1) at 166 °C for 30 min,[16] these hydrophobic residues then showed the expected composition of 4 mol each per mol of peptide, but with >20% loss of serine, threonine, and methionine.

As a further confirmation of the structure and purity, the FAB mass spectrum (m/z) from 5 μg of purified peptide in thioglycerol matrix was obtained using the VG-ZAB-2F high-field spectrometer.[17] The spectrum contained at least 30 sequence-related fragments (18 A′ series and 12 Z′ series), all of which are consistent with the expected primary structure. The most intense peaks appeared in the molecular ion region, representing m/z of $(M + H)^+$ and the isotopically enriched species. The accurate mass was measured as 2403.4178 for $(M + H)^+$, in excellent agreement with the calculated value of 2403.4234.

The synthetic signal peptide showed remarkable solubility properties both in aqueous and organic solvents. The peptide was readily soluble in acetate buffer, pH 4.7-5.6, at concentrations of 1 mg/mL (4 × 10^{-4} M) and in phosphate buffer pH 2.8 and 6.8 at 0.24 mg/mL. In 1% acetic acid, concentrations of 3-5 mg/mL could be attained. The peptide was also soluble in hexafluoroisopropyl alcohol, trifluoroethanol, and 70% CH_3CN (aqueous).

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Circular dichroism spectroscopy showed that the M13 leader peptide (10^{-5} M) changed conformation from >70% random coil in aqueous solution to >55% helix in 33% hexafluoroisopropyl alcohol[18] (Figure 1). Interestingly, application of the Chou–Fasman rules showed that $\langle P_\alpha \rangle$ and $\langle P_\beta \rangle$ were nearly equal.[12,19] The peptide showed the same CD spectrum characteristic of a predominantly random structure in 1% acetic acid, acetate buffer (pH 4.7), and phosphate buffer (pH 2.8 and 6.8) and became helical upon addition of hexafluoroisopropyl alcohol or trifluoroethanol.

The M13 signal peptide formed stable monolayers at the air–water interface in a Langmuir trough (0.0125 M Tris·HCl, pH 7.4, 0.10 M NaCl). The monolayer was slowly compressed and expanded repeatedly over several hours without showing hysteresis in the surface pressure vs. area curve. A discontinuity in π vs. A was observed at 32 ± 1 dynes/cm, indicating collapse of the monolayer. A high collapse pressure appears to be characteristic of surface-active peptides, which have been studied in this laboratory.[20] The π vs. A curve obeyed the equation $\pi[A - A_\infty(1 - k\pi)] = c$, where the compressibility constant k was determined as 9.9×10^{-3} cm/dyne. We calculated A_∞, the limiting molecular area extrapolated to 0 pressure, as 425 Å^2/molecule or 18.6 ± 1.4 Å^2/residue. This value for the limiting area per residue is consistent with a compactly folded α-helical structure,[21] where the nonpolar side chains are oriented toward air and the polar moieties seek the water surface. The molecular weights calculated from these studies were consistent with the monomeric form of the M13 signal peptide.

To assess whether this synthetic leader peptide could bind spontaneously to lipid bilayer membranes, we prepared small unilamellar vesicles from egg lecithin by the ethanol injection method and chromatographed them on Cl-Sepharose 4B in either 0.16 M KCl or 0.01 M sodium phosphate buffer pH 6.8.[22,23] Upon addition of M13 peptide, the vesicle mixture became turbid. Vesicle aggregation was also indicated by an increase in light scattering. With phase-contrast microscopy, clumps of spherical particles resembling large vesicles with >0.5-μm diameter were observed. Because of the apparent fusogenic nature of this synthetic peptide, we are currently trying to determine K_D for the binding phenomenon by employing the recently developed lipid-coated polystyrene divinylbenzene bead technique.[24]

We have succeeded in synthesizing a complete, native leader peptide that is water soluble.[25,26] Our CD data show that this M13 peptide is capable of adopting different solvent-dependent conformational states. In fluorinated alcohol solutions, which promote intramolecular H bonding,[27] this leader peptide exhibits high helical content, suggesting that 13 out of 23 residues participate in an α-helix. At the air–water interface a stable monolayer is formed with a limiting molecular area which indicates that the peptide adopts a compact structure like that of a helix. These studies support the idea that this peptide most likely exists as some sort of helical structure in the membrane. We are now investigating the conformation and orientation of the M13 leader peptide when associated with polymeric lipid vesicles as well as synthesizing other leader peptides to further our knowledge of their secondary structure.

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Registry No. Met-Lys-Lys-Ser-Leu-Val-Leu-Lys-Ala-Ser-Val-Ala-Val-Ala-Thr-Leu-Val-Pro-Met-Leu-Ser-Phe-Ala-NH_2, 91178-69-7.

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A (Phosphavinylidene)molybdenum Complex. Two-Coordinate Phosphorus Involving Multiple Bonding to Both Carbon and Molybdenum

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Compounds with multiple bonds between main-group and transition elements are of interest because they feature markedly different centers of reactivity. We describe the synthesis of a new class of compound in which a two-coordinate phosphorus atom is engaged in multiple bonding to both carbon and a transition element.

In a typical preparation, 2.30 g (10.25 mmol) of $(Me_3Si)_2C{=}PCl$[1] was dissolved in 30 mL of THF and added slowly (25 °C) to a filtered solution of $K[Mo(CO)_3(\eta\text{-}C_5H_5)]$ prepared by treatment of 4.08 g (8.33 mmol) of $[Mo(CO)_3(\eta\text{-}C_5H_5)]_2$ with 0.70 g (17.45 mmol) of KH in 50 mL of THF.[2] After evacuation of the THF, 50 mL of n-hexane was added to the resulting brown residue. Concentration of the filtered n-hexane solution afforded bright orange crystals of $[Mo(CO)_2(\eta^1\text{-}P{=}C(SiMe_3)_2)(\eta\text{-}C_5H_5)]$ (**1**) (mp 98–105 °C dec). Preliminary identification of **1** was based on elemental analytical and spectroscopic data. Thus, the 70-eV MS revealed a parent peak at m/e 408 and peaks at m/e 380, 352, and 343 corresponding to the loss of one CO, two CO's, and C_5H_5, respectively. The presence of only two CO's was also indicated by FT-IR ($\nu_{C\equiv O}$ = 1882, 1940 cm^{-1}), and the low coordination number at phosphorus was apparent from the deshielded $^{31}P\{^1H\}$ NMR chemical shift for **1** (36.43 MHz, CH_2Cl_2, δ + 497).

The structure of **1** was confirmed by X-ray crystallography[3] and is illustrated in Figure 1 along with the atom numbering scheme. The solid state of **1** comprises isolated molecules with no short intermolecular contacts.

The geometry around the molybdenum is of the familiar three-legged "piano-stool" type for which the three "legs" comprise two carbonyl groups and the η^1-$PC(SiMe_3)_2$ ligand. The Mo–P

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