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Prediction of the three-dimensional structure of the leader sequence of pre-κ light chain, a hexadecapeptide

(conformational energy/hydrophobic sequence/nondegenerate minima)

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ABSTRACT **The three-dimensional structure of the signal sequence for murine κ light chain has been calculated by using conformational energy calculations. These calculations, based on tested and reliable potential energy functions, employ a novel global search technique to identify the lowest energy structures for the hexadecapeptide signal sequence, Glu-Thr-Asp-Thr-(Leu_3-Trp-Val)$_2$-Pro-Gly. It has been found that the core hydrophobic sequence, Leu_3-Trp-Val-Leu_3, adopts an α-helical conformation that is terminated by chain reversal conformations for the four residues, Trp-Val-Pro-Gly. The amino-terminal four residues adopt low energy conformations that are fully compatible with the succeeding α-helix. The immediately neighboring sequence, Asp-Thr, exists in a single lowest energy double-equatorial conformation, whereas the first two residues, Glu-Thr, can adopt a variety of low energy conformations. The calculations arrive at a highly structured and specific model for the conformation of a leader sequence, compatible with recent experimental data.**

Over the past several years, it has been shown that all known secreted proteins (except ovalbumin) contain a sequence of between 15 and 30 amino acids attached to their amino termini called the leader or signal sequence, whose function is to enable translocation of the newly synthesized polypeptide chain across the microsomal membrane (1, 2). These sequences are unique in that they all contain long stretches of hydrophobic amino acid residues thought to be crucial in interacting with the membrane and possibly with a membrane protein receptor (1, 2). Unfortunately, leader sequences themselves have not been isolated (all sequence analysis has been performed on the signal sequences attached to their parent pro-proteins) because presumably they are rapidly degraded by proteases (1, 2) so that most structural data have been inferential (1–3). Recently the signal peptide of preproparathyroid hormone has been synthesized. Circular dichroism data on this peptide indicate a high α-helical content in nonpolar solvents and little or no α-helix but substantial β-structure in polar solvents (4). Secondary structure prediction schemes (4, 5) show both high α- and β-structure probabilities for this and other leader sequences. Despite these studies on secondary structures, there has been no effort to determine or to predict the actual three-dimensional or tertiary structures of these peptides.

In this paper, we present an analysis based on conformational energy calculations of the three-dimensional structure of a leader sequence for murine pre-κ light chain (6). This peptide contains 16 amino acids with a long repeating hydrophobic amino acid sequence, Glu-Thr-Asp-Thr-(Leu_3-Trp-Val)$_2$-Pro-Gly. (An amino-terminal Met has been omitted because it is assumed to function only as a chain initiator in protein synthesis.)

The use of conformational energy calculations has proven to be highly successful (7–10) in predicting the three-dimensional structures of a number of polypeptides, including smaller peptides, gramicidin S, and collagen models (7–10). The agreement between theory and experiment in these cases was excellent. The strategies employed in these papers and in recent calculations on the conformations of polar tetrapeptides (11) involve judicious combinations of the local minima for simple peptides to construct longer peptide chains. Recently, a one-dimensional Ising model for the folding of basic pancreatic trypsin inhibitor that accounts for all long-range peptide interactions has been proposed (12).

In this paper, we present a simple method for combining the local energy minima for the component di- and tripeptides of the leader sequence that allows for construction of long peptide chains. This method involves combinations of only nondegenerate conformations of component peptides—i.e., only conformations of each peptide with different backbone conformations are used in the combination of peptide minima. This method eliminates many redundant minima and greatly reduces the number of energy minimizations required in the construction of the polypeptide chain while all representative conformations of the component peptides are considered.

These calculations are well suited to the study of leader sequences because they take into account all interatomic interactions—i.e., short-, medium-, and long-range interactions in the peptide chain. Furthermore, because leader sequences exist inside highly nonpolar environments within the membrane, the effects of solvent—e.g., water—are not important in determining the structure of the molecule.

METHODS

The conformational energy of a polypeptide chain may be expressed as the sum of the electrostatic, nonbonded, hydrogen bonding, and torsional energies (7). The potentials employed are those used in the ECEPP (Empirical Conformational Energy for Polypeptides and Proteins) Program (7). In these calculations all backbone and side-chain dihedral angles are allowed to vary while bond lengths and bond angles are held fixed (7).

The problem in calculating the structure of a long polypeptide chain is the large number of local energy minima that exist in the conformation space of the molecule. Because all leader sequences contain long stretches of hydrophobic amino acid residues, we began with the repeating hydrophobic decapeptide, (Leu_3-Trp-Val)$_2$. We assumed that the hydrophobic core is crucial in determining the overall structure of the polypeptide. Thus, we studied the conformational preferences for the pentapeptide unit, Leu_3-Trp-Val. This was further subdivided into the two sections, Leu_3 and Trp-Val. To obtain all of the allowed (low energy) conformations for the former sequence, all of the allowed conformations for the blocked dipeptide Ac-Leu_2-$NHCH_3$ were determined by combining all of the allowed conformations for the single residue minima (13, 14) for Ac-Leu-$NHCH_3$. All of these conformations were then sub-

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jected to energy minimization by using the method of Powell (15). All of the resulting low energy minima that were within 3 kcal/mol of the global (calculated lowest energy) minimum were then combined with the single residue minima for the terminally blocked Leu and were then subjected to energy minimization (15).

The 3-kcal/mol cutoff has been selected because it has been shown that for all terminally blocked single residues and for a large number of blocked di- and tripeptides, all structures that contribute significantly to the partition function for the isolated molecules have energies that lie within 3 kcal/mol of the global minimum (7, 14). In our calculations for the smaller peptide units, the number of structures that exist within this cutoff is sufficiently large so that many different types of structures—e.g., α-helices, chain reversals, bends, etc.—are represented in the combination of states employed in building the polypeptide chain.

An identical procedure to that used for Leu_3 was applied to the sequence Trp-Val. A total of 364 structures for Ac-Leu_3-$NHCH_3$ and 66 structures for Ac-Trp-Val-$NHCH_3$ resulted. The problem then became how to combine the minima for these two sequences in a way that would sufficiently sample the conformation space and yet not involve the excessive number of energy minimizations that would result from combining 364 × 66 minima.

To resolve this problem, we made use of the fact that many of the minima for each peptide are degenerate—i.e., involve similar backbone conformations but different side chain conformations. Thus, of all the low energy conformations—i.e., those within 3 kcal/mol of the global minimum for each peptide segment—only the nondegenerate minima were combined. This approach is especially well suited to the hydrophobic amino acid residues whose side chains are incapable of hydrogen bonding and have small partial atomic charges, thereby minimizing their contribution to the overall structure due to electrostatic and hydrogen-bonding interactions. Aside from aqueous solvation effects that are not involved in the apolar membrane environment, only three considerations are important in determining their conformations: minimization of unfavorable steric interactions, maximization of packing interactions (i.e., attractive dispersion interactions), and local torsional potentials that determine rotational isomeric states for the side chains. If two side chains in local minima interact unfavorably, they can—under energy minimization—cross the low torsional barriers and adopt conformations in which the bad contacts are removed and in which, at the same time, the attractive dispersion interactions are optimized. Thus, because side chain–side chain interactions are nonspecific, it is not necessary to combine all possible rotational isomeric minima for the hydrophobic amino acid residues to obtain lowest energy structures.

Application of this method resulted in 324 different conformations for the pentapeptide that were subjected to energy minimization. Only 6 of the resulting structures had energies within 3 kcal/mol of the global minimum. The low energy conformations for the decapeptide $(Leu_3\text{-Trp-Val})_2$ were computed by combining the 6 best structures of the two identical pentapeptides with each other. Again only 6 low energy conformations resulted.

These structures were then extended by the addition of Pro-Gly, the terminal dipeptide. The numerous low energy conformations for this dipeptide have been determined (16). However, only four low energy conformers for Pro exist (14). (Only the backbone dihedral angle, ψ, assumes different values; the dihedral angle, ϕ, is fixed at $-75°$.) For each such Pro conformation, the best backbone conformation for Gly was selected. In this way four Pro-Gly conformations were combined with the six lowest energy $(Leu_3\text{-Trp-Val})_2$ conformations determined above. Energy minimization was then performed on each of these initial conformations in which only the terminal Trp-Val-Pro-Gly residues were allowed to move. The remainder of the dodecapeptide was held fixed in its lowest energy conformation—namely, an α-helix. Of the resulting structures, three low energy conformations were found.

The conformational preferences for the amino terminal peptide, Glu-Thr-Asp-Thr, were then explored. The same method used for combining the minima described above was used for this tetrapeptide. The low energy nondegenerate dipeptide minima for Glu-Thr and for Asp-Thr were determined and then combined to yield the possible low energy nondegenerate conformations for the tetrapeptide. In this case, all minimizations were performed with a carboxyl-terminal Leu residue held fixed in its α-helical conformation as determined from the previously minimized hydrophobic decapeptide, Leu_3-Trp-Val-Leu_3. This procedure was employed because it was assumed that the hydrophobic segment is the nucleation site (as discussed above). This assumption will be further justified in the *Results and Discussion*. The lowest energy structures for all peptide segments were then combined and the energy of the entire hexadecapeptide was then minimized allowing all variables to change.

RESULTS AND DISCUSSION

Low Energy Minima for Ac-Leu_3-$NHCH_3$ and Ac-Trp-Val-$NHCH_3$. The lowest energy conformers for Leu_3 are given in Table 1. The global minimum is a compact structure with a central C_7 NH—O=C hydrogen bond. It may be noted that the conformational energy of the α-helical conformation (conformer 2 of Table 1) is close to that of the global minimum, the former being only 0.2 kcal/mol higher in energy. Of the 364 energy minimizations for Leu_3, about 50 structures had energies that were within 3 kcal/mol of the global minimum, but only 21 had distinctly different backbone conformations. The fully extended

Table 1. Four energy minima for Ac-Leu_3-$NHCH_3$*

Conformer	ϕ_1	ψ_1	χ_1	χ_2	χ_2^1	χ_2^2	ϕ_2	ψ_2	χ_1	χ_2	χ_2^1	χ_2^2	ϕ_3	ψ_3	χ_1	χ_2	χ_2^1	χ_2^2	E_{tot},† kcal/mol
1‡	−63	−54	177	65	54	60	−96	58	−56	174	61	69	161	−57	177	64	54	59	0.0
2§	−73	−41	179	64	54	59	−67	−37	180	64	53	59	−69	−50	178	63	54	59	0.2
3	−80	96	180	63	52	59	−67	−45	179	64	54	60	−84	79	−174	69	55	60	0.4
4¶	−150	125	180	66	54	60	−150	132	−178	68	55	60	−154	158	60	136	59	58	5.8

* All dihedral angles are in degrees. The dihedral angle, ω, for rotation around the C′-N peptide bond has been omitted because it is always close to 180°.

† Total conformational energy as defined in *Methods*. All energies are expressed relative to the energy of conformer 1, whose energy = −1.24 kcal/mol.

‡ A chain reversal conformation.

§ The lowest energy α-helical conformation.

¶ The lowest energy extended conformation.

Table 2. Representative energy minima for Ac-Trp-Val-$NHCH_3$*

Conformer	ϕ_1	ψ_1	χ_1	χ_2	ϕ_2	ψ_2	χ_1	χ_2^1	χ_2^2	E_{tot},† kcal/mol
1‡	−82	108	179	79	−89	94	179	56	66	0.0
2§	154	151	180	91	149	143	67	65	54	0.3
3	153	143	176	77	−80	121	175	54	64	0.3
4¶	−73	−36	−176	88	−74	−45	173	53	63	2.9

* All dihedral angles are in degrees.
† All conformational energies are expressed relative to the energy of conformer 1, whose energy = −4.62 kcal/mol.
‡ Double C_7 conformation.
§ Fully extended conformation.
¶ α-Helical conformation.

conformation for Leu_3 (conformer 4 in Table 1) had an energy >5 kcal/mol higher than that of the global minimum, indicating that in the absence of other longer range stabilizing interactions, an extended conformation is unlikely for this peptide.

The combination of the single residue minima for Ac-Trp-Val-$NHCH_3$ gave a total of 66 final structures, 21 of which had energies within 3 kcal/mol of the global minimum and 14 of which had unique backbone conformations. The lowest energy conformers for this blocked peptide are given in Table 2. The global minimum is a double C_7 structure. Interestingly, the α-helical conformation for this molecule is of relatively high energy when compared with that of the global minimum (compare E_{TOT} of conformer 4 with that of conformer 1 of Table 2).

Low Energy Minima for Ac-Leu_3-Trp-Val-$NHCH_3$ and Ac-(Leu_3-Trp-Val)$_2$-$NHCH_3$. When the 21 lowest energy distinct Leu_3 structures were combined with the similarly selected 14 Trp-Val minima (a total of 324 conformers in all), all of the 6 lowest energy structures contained Leu_3 in an α-helix, whereas the terminal Trp-Val assumed a variety of conformations (using the nomenclature of ref. 14, AA, CC, EC, CA, EA, EC), the most stable of which was an α-helix. Thus, the global minimum was an α-helix even though this structure was not the lowest energy structure for either Leu_3 or Trp-Val and was, in fact, of relatively high energy in the latter case. Because the number of low energy conformers for Ac-Leu_3-Trp-Val-$NHCH_3$ was quite limited and the Trp-Val residues could exist in a number of different conformations (including reverse turns), it was of great interest to investigate the low energy structures for the repeating decapeptide, Ac-(Leu_3-Trp-Val)$_2$-$NHCH_3$. When the 6 minima found for the blocked hexapeptides were combined—i.e., when the 36 possible starting conformations were subjected to energy minimization—again only 6 low energy structures were obtained, in all of which the first eight residues were α-helical. The carboxyl-terminal Trp-Val existed in the same 6 conformations as obtained for the blocked hexapeptide. It is most notable that the central Trp-Val sequence in this peptide in all 6 lowest energy structures became α-helical and could no longer exist in the alternate conformations that it adopted in the blocked hexapeptide.

It was noted above that the fully extended conformation for Ac-Leu_3-$NHCH_3$ (conformer 4 of Table 1) was of relatively high energy when compared with that of the global minimum. However, it is possible that if two extended sequences of Leu_3 were separated by a hairpin loop conformation for Trp-Val so that they would be involved in a β-pleated sheet, the conformational energy of such a structure might be quite low. Thus, two Leu_3 sequences were placed in the lowest energy extended conformations for this tripeptide and separated by Trp-Val in all of its low energy nonhelical conformations, most of which are chain-reversal structures. No structure was obtained that was <25 kcal/mol higher in energy than the lowest energy α-helical conformation for this sequence. Thus, β-pleated sheet and fully extended conformations are highly unlikely for the central hydrophobic residues.

The fact that the whole hydrophobic decapeptide exists in only six lowest energy conformations—in all of which the first eight residues are in an α-helix—indicates that this sequence is very likely a tight structural unit around which adjacent res-

Table 3. Lowest energy conformer for the hexadecapeptide Ac-Glu-Thr-Asp-Thr-(Leu_3-Trp-Val)$_2$-Pro-Gly-$NHCH_3$*

Residue	Dihedral angle†					
1 Glu	−76 (ϕ),	103 (ψ),	−176 (χ_1),	175 (χ_2)	−99 (χ_3),	−179 (χ_3^1)
2 Thr	−78 (ϕ),	−61 (ψ),	45 (χ_1),	168 (χ_2^1),	64 (χ_2^2)	
3 Asp	−66 (ϕ),	91 (ψ),	173 (χ_1),	92 (χ_2),	16 (χ_2^1)	
4 Thr	−95 (ϕ),	87 (ψ),	−53 (χ_1),	72 (χ_2^1),	65 (χ_2^2)	
5 Leu	−65 (ϕ),	−38 (ψ),	179 (χ_1),	63 (χ_2),	54 (χ_2^1),	59 (χ_2^2)
6 Leu	−62 (ϕ),	−40 (ψ),	178 (χ_1),	63 (χ_2),	53 (χ_2^1),	59 (χ_2^2)
7 Leu	−70 (ϕ),	−37 (ψ),	179 (χ_1),	63 (χ_2),	53 (χ_2^1),	59 (χ_2^2)
8 Trp	−68 (ϕ),	−43 (ψ),	−177 (χ_1),	83 (χ_2)		
9 Val	−65 (ϕ),	−43 (ψ),	165 (χ_1),	51 (χ_2^1),	58 (χ_2^2)	
10 Leu	−63 (ϕ),	−43 (ψ),	177 (χ_1),	64 (χ_2),	55 (χ_2^1),	60 (χ_2^2)
11 Leu	−68 (ϕ),	−41 (ψ),	179 (χ_1),	64 (χ_2),	54 (χ_2^1),	60 (χ_2^2)
12 Leu	−64 (ϕ),	−46 (ψ),	178 (χ_1),	65 (χ_2),	54 (χ_2^1),	59 (χ_2^2)
13 Trp	−152 (ϕ),	129 (ψ),	171 (χ_1),	83 (χ_2)		
14 Val	−91 (ϕ),	128 (ψ),	178 (χ_1),	58 (χ_2^1),	63 (χ_2^2)	
15 Pro	79 (ψ)					
16 Gly	81 (ϕ),	−80 (ψ)				

* The final conformational energy = −50.8 kcal/mol.
† In degrees; the dihedral angle, ω, is always close to 180°.

idues adopt conformations that are compatible with this unit. Thus, the carboxyl-terminal dipeptide, Pro-Gly, and the amino-terminal tetrapeptide, Glu-Thr-Asp-Thr, were joined to the lowest energy decapeptide structures, assuming that the α-helix would be preserved for the first eight residues.

Structures of the Hexadecapeptide. When Pro-Gly was added to the hydrophobic decapeptide, the carboxyl-terminal sequence Trp-Val-Pro-Gly was allowed to move under energy minimization while the first eight residues were held fixed. Three lowest energy conformers were obtained within 3 kcal/mol of the global minimum. In all of these conformers, the carboxyl-terminal Trp residue assumed a fully extended conformation while the succeeding valine formed a C_7 structure. Three of the four allowed conformations for Pro ($\psi = 75°$, 135°,

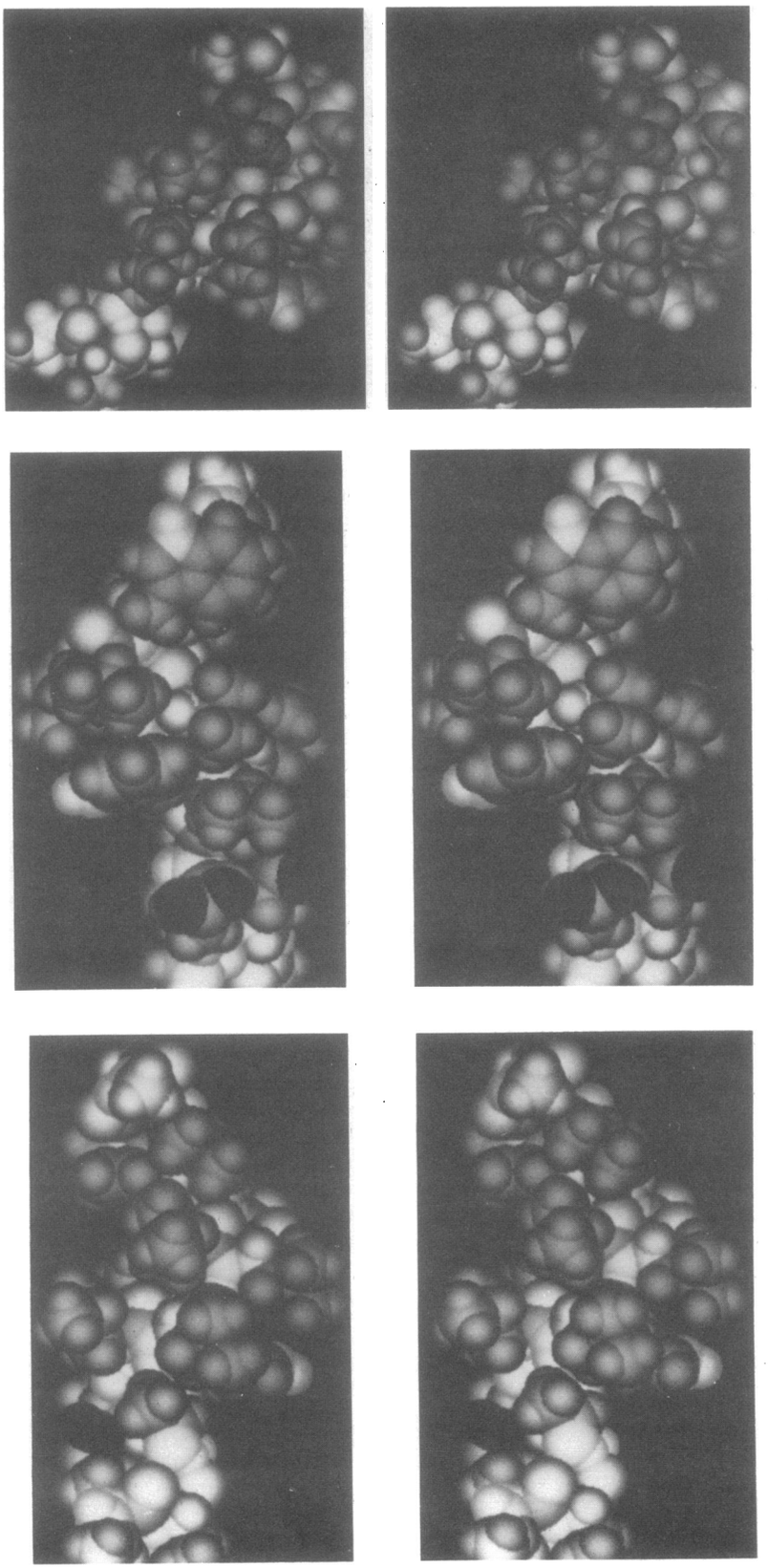

FIG. 1. Space-filling stereo views of the calculated lowest energy conformer for murine pre-κ light chain leader sequence (Table 3). The color scheme is: gray, nonpolar side chain atoms; dark black, carboxyl oxygens of Asp and Glu. All other atoms are white, including the NH atoms of the Trp indole ring. (*Top*) An overall view of the molecule, the polar section being in the lowermost part of the figure. (*Middle*) The chain reversal at the carboxyl end of the molecule (uppermost part of the figure), the side chain packing, and the orientation of the two Trp rings. (*Bottom*) The course of the central α-helix with the chain reversal pointing toward the viewer at the top of the figure.

and −55°) occur in these low energy conformations whereas Gly could exist in a number of low energy minima. In the lowest energy minimum for the hexadecapeptide (Table 3), the carboxyl-terminal sequence, Trp-Val-Pro-Gly, forms a chain reversal.

In the case of the amino-terminal sequence, Glu-Thr-Asp-Thr, we found that of the allowed Asp-Thr conformations only one was compatible with the succeeding α-helix—namely, a double C_7 conformation that contains a number of highly favorable side chain-backbone hydrogen bonds. When the low energy minima for Glu-Thr were added to this structure, a number of favorable conformations were found. In the lowest energy form of Glu-Thr, in the amino-terminal sequence, Glu adopts the C_7 conformation while Thr is α-helical. These lowest energy tetrapeptide conformers were then joined to the previously determined lowest energy conformer for the dodecapeptide, $(Leu_3\text{-Trp-Val})_2$-Pro-Gly, and the energy of the hexadecapeptide was minimized, allowing for changes in all the variables.

Space-filling stereo views of the global minimum structure are shown in Fig. 1, and the final dihedral angles are given in Table 3. Even allowing all residues to vary freely failed to alter the very stable α-helical conformation of the central hydrophobic octapeptide. The final structure contains a flattened, polar amino-terminal tetrapeptide followed by a tightly packed α-helix for the next eight residues, followed by a chain reversal at the terminal tetrapeptide sequence.

It is interesting that 8 of 16 residues of our calculated structure exist in an α-helix, consistent with the experimental results of Rosenblatt *et al.* (4). These workers found that about 40% of the signal sequence for preproparathyroid hormone exists in an α-helix in a nonpolar solvent (4).

It has been suggested (5) that some signal sequences may exist in an extended structure in order to span the membrane. The total span length for our calculated structure is about 30 Å so that it can span the hydrophobic core of the lipid bilayer in cell membranes (also about 30 Å thick). Our calculations for this relatively short leader sequence also show that an extended structure is unlikely (as discussed above). Preliminary calculations on two other leader sequences—for influenza hemaglutinin and for the fd major coat protein—indicate that an α-helix is much more stable than a fully extended structure.

Now that we have calculated from basic units the structure of a leader sequence, it is possible to analyze other sequences in the same manner to determine how similar these sequences are in structure. However, we should note several basic assumptions in the methodology we have employed. First, we have assumed that the hydrophobic sequence is the crucial one. This assumption is based on the observation that all leader sequences have such a hydrophobic core with little or no other similarities in sequence. Thus, when we constructed the amino- and carboxyl-terminal sequences, these were added to a fixed preexisting global minimum α-helical octapeptide. It is possible, though, that the tetrapeptide may cause unwinding of the helix from the end by providing competing stabilizing interactions. Such a possibility is unlikely for three reasons: (*i*) the amino-terminal peptide can assume very low energy conformations fully compatible with the succeeding α-helix; (*ii*) the fact that a number of signal peptides do not contain hydrophilic residues at the amino-terminal end but rather begin with the hydrophobic sequence (17); and (*iii*) when the energy of the whole hexadecapeptide was minimized, allowing all variables to change, no structure was found in which any of the eight hydrophobic residues changed conformation from an α-helix. Nonetheless, we are testing this assumption by combining different peptide blocks in the energy minimization procedure.

Second, in our methods we used 3 kcal/mol as the cutoff for including viable structures. This choice of cutoff has been justified in the *Methods*. We now further add that from our calculations, by using this cutoff, quite distinct structural patterns occur and—in the case of the α-helix—no low energy structures for either Leu_3 or Trp-Val that were not α-helical were viable for the penta- and octapeptides. However, it is possible that structures of peptides whose energies are >3 kcal/mol above that of the global minimum can be stabilized by long-range interactions, though this is quite unlikely, especially in view of our calculations on β-sheet conformations of the hydrophobic octapeptide.

Finally, there is our use of the criterion of nondegeneracy. This criterion allowed us to reduce the number of starting conformations for minimization to a reasonable number. As described earlier, the absence of specific interactions between side chains of hydrophobic residues enables us to sample the energy space by limiting our starting conformations, when combining peptide units, to the nondegenerate structures for each of the units. The extension of this method to the polar amino acids is more questionable, and the results obtained in the search for the low energy minima for the amino-terminal tetrapeptide must be regarded as preliminary because not all possible side chain positions were used to obtain a final structure. It should further be noted that the hydrophilic sequence has been assumed to exist in the apolar membrane so that the polar side chains are un-ionized. Whether similar results would be obtained for this peptide with solvated ionized side chains is an open-ended question.

One satisfying aspect of these calculations is that it resulted in a specific and structured conformation for the molecule. Extensive testing of the assumptions of the approach to the calculations described here must be undertaken. Furthermore, a variety of other leader sequences must be analyzed to determine whether there are shared three-dimensional features of these varied sequences.

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