A Mixture of α-Helical and $3_{10}$-Helical Conformations for Involucrin in the Human Epidermal Corneocyte Envelope Provides a Scaffold for the Attachment of Both Lipids and Proteins*

(Received for publication, April 28, 1999, and in revised form, July 12, 1999)

N. D. Lazo‡ and Donald T. Downing‡

From the Department of Dermatology, University of Iowa, Iowa City, Iowa 52242

Involucrin plays an important role in the lipid and protein compound envelopes of mammalian epidermal corneocytes. In the present study, model peptides containing the consensus repeating units PEQQEGQLEL and LEQQEGQLEH, found in the central region of human involucrin, were studied by circular dichroism spectroscopy, molecular modeling, and energy minimization. These peptides have intrinsic α-helix-forming properties as indicated by their circular dichroic spectra obtained in the presence of 2,2,2-trifluoroethanol. Peptide (LEQQEGQLEH)$_3$ had an α-helix content of 100% in 100% 2,2,2-trifluoroethanol at 0 °C. The energy-minimized α-helix showed that only 50% of the glutamate side chains may be available for the attachment of lipids. However, when a $3_{10}$-helix was assumed for the GQL or GQLE residues in LEQQEGQLEH, all of the glutamate side chains were arrayed on one face of the helix, and all of the glutamine side chains were arrayed on the opposite face. A similar result was obtained when the nonhelical part of PEQQEGQLEL was assumed to contain a β-turn III, which is equivalent to a short portion of $3_{10}$-helix. The results of this study suggest that when the central segment of human involucrin is predominantly α-helical, accompanied by short $3_{10}$-helical segments, the protein can function as a scaffold for the attachment of both lipids and proteins.

Corneocytes are the thin, flat cells that form the outer surface of mammalian skin, and they play a vital role in the epidermal permeability barrier. Each corneocyte possesses a protein envelope, approximately 15 nm in thickness, which is composed of several structural proteins including involucrin (1–4), cystatin α (5, 6), loricrin (7–9), elafin (10), small proline-rich proteins (11, 12), keratin intermediate filaments (13), trichohyalin (14), and filaggrin (15). These proteins are crosslinked together by $N^\gamma$-(γ-glutamyl)lysine isodipeptide bonds formed by the action of transglutaminases and also by disulfide bonds. Studies of the structure and assembly of the protein envelope indicate that involucrin is one of the proteins that is deposited first and that it forms a scaffold for the subsequent deposition of the other proteins (3, 16–18).

Covalently attached to the exterior of the corneocyte protein envelope is a lipid envelope composed mainly of ω-hydroxysteroids (19, 20). Chemical evidence indicates that these lipid molecules are attached to proteins through ester bonds, since they are easily released by treatment with mild alkali (19, 20). The availability of sufficient ester bonds requires a protein that is rich in free carboxyl side chains (21). Thus, involucrin, which has a high glutamate content (~20% of the amino acids), has been proposed to act as a scaffold for the attachment of the lipid layer to the protein envelope (22). This idea was confirmed by biochemical and mass spectrometric characterization of lipopeptide fragments obtained from human corneocyte envelopes by proteolysis, which indicated that approximately 35% of the peptides were derived from involucrin (23). Thus, from studies of protein and lipid envelopes, it is generally accepted that involucrin is covalently attached to both proteins and lipids.

Biophysical characterization of human involucrin using secondary structure prediction, CD1 and electron microscopy indicated that involucrin is a rod-shaped molecule that possesses 50–75% α-helical content (4). It was suggested that the glutamine residues are circumferentially distributed along the length of the α-helix and that this arrangement maximizes intermolecular cross-linking between involucrin and other proteins (4). The distribution of glutamate residues and its role in the attachment of lipids were not addressed in that study. Initially, we predicted that human involucrin might adopt a β-sheet structure, since this could provide the required surface density of glutamate residues for the attachment of lipids (22). This prediction seemed to be supported by solid-state nuclear magnetic resonance studies of corneocyte envelopes isolated from pig epidermis, which suggested that the cross-linked proteins are predominantly in the β-sheet conformation (24). X-ray diffraction studies of murine corneocyte envelopes also indicated the dominant presence of β-sheets (25). However, human involucrin is a distinctly different protein than that of lower mammals (26), and therefore, care must be taken in extrapolating results with lower mammals to humans.

In the present study, we used model peptides to show that the α-helical character that has been demonstrated for the involucrin molecule as a whole (4) can be adopted by the specific regions that are rich in glutamate residues. Also, using computer modeling, we investigated whether an α-helical conformation would array the glutamate residues of these regions appropriately for attachment of the lipid envelope. Our results suggest that a mixture of α-helical and $3_{10}$-helical conformations in the central segment of involucrin provides sufficient surface density of glutamate side chains for the attachment of lipids.
EXPERIMENTAL PROCEDURES

Peptide Synthesis and Characterization—Peptides were synthesized in the Protein Structure Facility of the University of Iowa using an Applied Biosystems 431A peptide synthesizer. A stepwise solid-phase procedure using rink amide MBHA resin and fluorenylmethoxycarbonyl-protected amino acids was employed. The crude peptides were purified by preparative C18 reverse-phase HPLC. The purity of all peptides used in this study was confirmed to be >95% by analytical C18 reverse-phase HPLC. Peptide identity was confirmed by electrospray-ionization mass spectrometry and by amino acid analysis.

Circular Dichroism—CD spectra were acquired using an Aviv Associate model 62DS spectrometer. Stopped optical cells with a path length of 1 mm were used in all acquisitions. Spectra were obtained by taking readings every 0.5 nm with an averaging time of 4 s and a bandwidth of 1 nm. Ellipticity measurements were expressed as mean residue ellipticity, \( \theta \), in units of deg cm\(^2\) dmol\(^{-1}\) and calculated from the equation,

\[
\theta = \theta_{\text{obs}} (\text{MRW}/10l[C]) \quad \text{(Eq. 1)}
\]

in which \( \theta \) is the observed ellipticity in degrees, MRW is the mean residue weight of the peptide (molecular weight divided by the number of residues), \([C]\) is the peptide concentration in g/liter, and \( l \) is the optical path length in centimeters. Peptide concentrations were determined by quantitative amino acid amino acid analysis. The helix content of each peptide was determined from the values of their mean residue ellipticities at 222 nm (\( \theta_{222} \)). Based on CD studies of model peptides that mimic the \( \alpha \)-helical regions of intermediate-filament proteins, the \( \theta_{222} \) for a 100% helix was taken as –43,000 deg cm\(^2\) dmol\(^{-1}\) (27).

Computer Modeling—Energy minimization of possible structures for (PEQQEGQLEL)\(_1\)–3 and (LEQQEGQLEH)\(_1\)–3 was performed on a Silicon Graphics Indigo workstation in the Image Analysis Facility of the University of Iowa using Sybyl 6.4 software. The conformational energies were minimized using the Kollman all-atom force field and Kollman charges using 1,000 iterations or until convergence was reached. Energies for the 10-residue repeating units were calculated by subtracting the energy of the 20-residue peptide from that of the 30-residue peptide. These are reported here in units of kcal/mol-residue.

RESULTS

An important feature of human involucrin is the presence of a central segment of high homology whose consensus repeat sequence is (P/L)EQQQEGQLE/K/H (2). From CD, molecular modeling, and electron microscopy, it has been shown that the central segment contains a large proportion of \( \alpha \)-helices (4). Our initial molecular modeling of this segment, using a graphical method developed for the expression of local spatial relationships between amino acid residues (28), is shown in Fig. 1. A predominantly \( \alpha \)-helical conformation for the central segment is indicated, with most of the glutamate side chains arrayed on one face of the \( \alpha \)-helices, and most of the glutamine side chains arrayed on the opposite face. To check the validity of this model, peptides containing the consensus 10-residue repeating units LEQQEGQLEH and PEQQEGQLEL were synthesized and investigated by CD spectroscopy, revealing random coil conformation in water (Fig. 2) but \( \alpha \)-helical structures in TFE (Fig. 3). In addition, (PEQQEGQLEL)\(_1\)–3 and (LEQQEGQLEH)\(_1\)–3 sequences were subjected to energy minimization by computer, confirming their predominantly \( \alpha \)-helix-forming properties in conformations that would maximize the attachment of both lipids and proteins.

Model Peptides of Involucrin Possess Intrinsic Helix-forming Properties—CD spectra of the 20- and 30-residue peptides containing the repeating units PEQQEGQLEL and LEQQEGQLEH in phosphate-buffered saline at pH 7 show a strong minimum at approximately 198 nm, corresponding to the \( \pi-\pi^* \) transition of the random coil (Fig. 2). Similar spectra were obtained after the addition of 2 molal sodium sulfate, a salt that is known to promote structure formation in peptides that contain hydrophobic residues.\(^2\)

\(^2\) N. D. Lazo and D. T. Downing, unpublished observations.

![Fig. 1. Molecular modeling of the central segment of human involucrin using a graphical method developed in this laboratory (28). Circles represent the side chains of the amino acid residues. The central spiral represents the peptide backbone in helical regions. Most of the glutamate side chains are arrayed on one face of the \( \alpha \)-helices, and most of the glutamines are arrayed on the opposite face.](image-url)

![Fig. 2. CD spectra of the 20- and 30-residue model peptides of the central segment of human involucrin in phosphate-buffered saline at pH 7. solid line, (PEQQEGQLEL)\(_1\)–3; dashed line, (PEQQEGQLEL)\(_2\)–3; dotted line, (LEQQEGQLEH)\(_1\)–3; and dotted line, (LEQQEGQLEH)\(_2\)–3. At pH 2.7, below the \( pK_a \) of the glutamate side chains, the peptides remained predominantly random coil. \( \alpha \)-Helices were formed by all of the synthetic peptides in the presence of TFE, a nonpolar solvent that has been shown to promote native-like \( \alpha \)-helical structures in peptides with intrinsic \( \alpha \)-helix forming properties (29–32). TFE has a dielectric...](image-url)
constant that closely approximates that of the interior of proteins and has recently been shown to promote structure formation by minimizing the exposure of the peptide backbone to water (30, 32). Thus, solvents with high concentrations of TFE may mimic the hydrophobic environment of involucrin found in the corneocyte envelope. Fig. 3 presents CD spectra of (PEQQEGQLEL)1–3 and (LEQQEGQLEH)1–3. Peptide (PEQQEGQLEL)2 was insoluble in 100% TFE but was soluble in 25–75% TFE. The spectra of (LEQQEGQLEH)2 and (LEQQEGQLEH)3 in 75–100% TFE were characteristic of the peptides in 100% TFE at 0 °C. Peptides (PEQQEGQLEL)2 and (LEQQEGQLEH)2 in 25–75% TFE. The spectra of (LEQQEGQLEH)2 and (LEQQEGQLEH)3 (Fig. 3, a–c) had minima at approximately 220 nm and at 202–203 nm, indicating a lower proportion of α-helix.

Table I shows the θ222 and corresponding α-helix contents of the peptides in 100% TFE at 0 °C. Peptides (PEQQEGQLEL)2 and (PEQQEGQLEL)3 had similar helix contents, which suggests that α-helix formation in these peptides is independent of chain length. In 25–75% TFE, the series of (PEQQEGQLEL)1–3 peptides exhibited similar spectra, indicating similar helix contents (Fig. 3, a–c), perhaps because the α-helix formed by the PEQQEGQLEL repeating unit is interrupted by the proline of the next repeating unit. Proline lacks a peptide NH group that can participate in backbone hydrogen bonding, and because of its cyclic ring structure, it is energetically unfavorable for the preceding residue to form an α-helix (34). On the other hand, the helix content of peptides containing the LEQQEGQLEH repeating unit was dependent on the length of the chain (Fig. 3, d–f; Table I); peptide (LEQQEGQLEH)2 had the highest helicity, with a θ222 of -41,900 deg cm2 dmol-1, corresponding to an α-helix content of almost 100%.

An isodichroic point at 201.5 nm was observed for the (LEQQEGQLEH)1–3 peptides (Fig. 3, d–f), which suggests that, under all conditions of TFE concentration and temperature used, each amino acid residue in the peptides existed as either random coil or α-helix (35). On the other hand, no isodichroic point was observed in the spectra of the (PEQQEGQLEL)2 and (PEQQEGQLEL)3 (Fig. 3, b–c), indicating the presence of more than two conformations.

Energy-minimized Structures Containing α-Helices with or without 3₁₀-Helices—All residues of (LEQQEGQLEH)1 were assigned backbone torsion angles corresponding to an α-helix (δ = -57°, ϕ = -47°), and then the minimum conformational energy of the peptide was computed. Fig. 4a shows the α-helix after energy minimization. The conformational energy of the LEQQEGQLEH repeating unit was -16.3 kcal/mol-residue, which is lower than the energy of a corresponding β-strand (-14.0 kcal/mol-residue). However, in the pure α-helix, as shown in Fig. 4a, only 50% of the glutamate side chains may be available for the attachment of lipid. Therefore, possible modifications of the α-helical conformation of (LEQQEGQLEH)3 that would maximize its potential for the attachment of both lipids and proteins were investigated. These modifications began with the glycine residue, which, primarily because of the conformational flexibility of its backbone (36), is a known α-helix breaker. It is also known that facile transitions between α-helix and 3₁₀-helix occur in proteins and that these transitions play an important role in protein recognition and function (37–39). Most 3₁₀-helices in proteins are short (3–4 residues long) compared with α-helices, which have a mean length of 10 residues (40). Fig. 4b shows the energy-minimized structure of (LEQQEGQLEH)3 in which the segment GQL was modeled as a 3₁₀-helix, i.e. the backbone torsion angles of GQL were initially set to θ = -50° and ϕ = -28°. In the resulting conformation, all of the glutamate side chains were arrayed on one face of the helix, and all of the glutamine side chains were arrayed on the opposite face. The computed conformational energy of the structure in Fig. 4b was -15.3 kcal/mol-residue.

| Peptide          | θ222 (10^3 deg cm^2 dmol^-1) | % α-helix |
|------------------|------------------------------|----------|
| (PEQQEGQLEL)_2   | 25.2                         | 58.6     |
| (PEQQEGQLEL)_3   | 24.8                         | 57.7     |
| (LEQQEGQLEH)_1   | 25.3                         | 58.8     |
| (LEQQEGQLEH)_2   | 36.5                         | 84.9     |
| (LEQQEGQLEH)_3   | 41.9                         | 97.4     |
conformations that fulfill the putative function of the protein, presenting all of the glutamate residues in a surface density adequate for attachment of the corneocyte lipid envelope.

The model peptides that we have investigated in this study represent two parts of the central segment of human involucrin. Residues 176–225 of the protein are made up of five repeats of PEQQEQLLEL. Our studies of the peptides containing PEQQEQLLEL indicate that this segment of the protein may contain five α-helical regions separated from each other by four LPEQ segments. Energy calculations indicate that the LPEQ segments may form a β-turn III, equivalent to a segment of a 3_10-helix (43), resulting in a linear structure in which all of the glutamate side chains are sequestered on one face, and all of the glutamine side chains are on the opposite face. The model peptides that contain the consensus repeat sequence LEQQEQLLEQ possess intrinsic α-helix-forming properties that are dependent on chain length. This suggests that the sections that join the repeating units form part of the α-helix.

Previous molecular modeling of α-helices from the repeating unit LEQQEQLLEQ by Yaffe and co-workers (4) show that when an extra amino acid is inserted between the consensus repeating units, a clustering of the glutamine residues on one face of the helix results. Here, we have shown that when an α-helix and a 3_10-helix are present in the 10-residue repeating units, helical structures are obtained in which all of the glutamate side chains are sequestered on one face of the helix, and all of the glutamine side chains are clustered on the opposite face. The CD spectrum of human involucrin in phosphate buffer at neutral pH indicated a θ_222 of −18,000 deg cm^2 dmol^{-1} (4). Since a 3_10-helix possesses an α-helix-like CD spectrum (41) and 3_10-helices have been proposed as intermediates along the α-helix unfolding pathway (37, 42), it has been proposed that a value between −15,000 and −30,000 deg cm^2 dmol^{-1} for the θ_222 of helical peptides or proteins suggests the presence of a mixture of nascent helix, 3_10-helix, and α-helix (37).

Our energy calculations on the helical structures that contain the repeating unit LEQQEQLLEQ indicate a slightly higher energy for the structures that contain 3_10-helices than for the pure α-helix. The 3_10-helix is more tightly wound and is less stable than the α-helix due to several close contacts and less optimal hydrogen bond geometry (44). However, there is no disallowed region between the α-helical and the 3_10-helical conformations in the Ramachandran plot, and therefore α-helix to 3_10-helix transformations can easily occur (44). Furthermore, calculations indicate that the low dielectric of proteins and the presence of lipid membranes could provide conditions that may stabilize the 3_10-helix (45). In the corneocyte envelopes, the orienting effect of the bound lipids on one side of involucrin and the cross-linked proteins on the other side could also stabilize the 3_10-helices. It also may be significant that esterification of the envelope lipids to the involucrin glutamates would remove the carboxylate electrostatic charges that might oppose α-helix formation. Furthermore, lateral hydrophobic interactions would tightly pack the attached envelope lipids, stabilizing and condensing the involucrin conformation.

There are two important reasons for having most if not all of the glutamate side chains sequestered on one face of involucrin in the human epidermal corneocyte envelope. First, it provides the high density of glutamate side chains required for attachment of the lipids in the interface between the protein and lipid compound envelopes of the corneocyte (19–23). For this, every molecule in a tightly packed monolayer of hydroxyacylamides is required to be attached through ester bonds to an underlying planar surface of protein (20–22). Second, isolating the glutamate side chains on one face of involucrin may increase the


Structure-Function Correlation in Human Involucrin

activity of transglutaminase for the formation of isodipeptide cross links, since it has been shown that in addition to being exposed on the surface of the protein, there should be no charged residues on either side of the target glutamine (46). The results presented here indicate that involucrin existing predominantly as $\alpha$-helices and $3_{10}$-helices can function as a scaffold not only for the attachment of proteins but also for the attachment of lipids.

REFERENCES

1. Rice, R. H., and Green, H. (1977) Cell 11, 417–422
2. Eckert, R. L., and Green, H. (1986) Cell 46, 583–589
3. Eckert, R. L., Yaffe, M. B., Crish, J. F., Murthy, S., Ronke, E. A., and Welter, J. F. (1993) J. Invest. Dermatol. 100, 613–617
4. Yaffe, M. B., Beegen, H., and Eckert, R. L. (1992) J. Biol. Chem. 267, 12233–12238
5. Kartasova, T., Cornelissen, B. J. C., and van der Putte, P. (1987) Nucleic Acids Res. 15, 5945–5962
6. Takahashi, M., Teruha, T., and Katanuma, K. (1992) FEBS Lett. 308, 79–82
7. Mehril, T., Hohl, D., Rothnagel, J. A., Longley, M. A., Bundman, D., Cheng, C., Lichti, U., Bisher, M. E., Steven, A. C., Steiner, P. M., Yosua, S. H., and Roop, D. R. (1990) Cell 61, 1103–1112
8. Hohl, D., Mehril, T., Lichti, U., Turner, M. L., Roop, D. R., and Steiner, P. M. (1998) J. Biol. Chem. 266, 6626–6636
9. Yoneda, K., Hohl, D., McBride, O. W., Wang, M., Cehrs, K. U., Idler, W. W., Roop, D. R., and Steiner, P. M. (1992) J. Biol. Chem. 267, 18960–18966
10. Nonomura, K., Yaminishi, K., Yasuno, H., Nara, K., and Hirose, S. (1994) J. Invest. Dermatol. 103, 88–91
11. Kartasova, T., and van der Putte, P. (1988) Mol. Cell. Biol. 8, 2195–2203
12. Gree, M. A., Lorand, L., Lane, W. S., Barden, H. P., Pararneswaran, K. N. P., and Kveddar, J. V. (1995) J. Invest. Dermatol. 104, 204–210
13. Steiner, P. M., and Marekov, L. N. (1995) J. Biol. Chem. 270, 17792–17711
14. Lee, S.-C., Kim, I.-G., Marekov, L. N., O’Reege, E. J., Parry, D. A. D., and Steiner, P. M. (1993) J. Biol. Chem. 268, 12164–12176
15. Steven, A. C., and Steiner, P. M. (1994) J. Cell Sci. 107, 693–700
16. Steiner, P. M. (1995) Cell Death Differ. 2, 33–40
17. Robinson, N. A., LaCelle, P. T, and Eckert, R. L. (1996) J. Invest. Dermatol. 107, 101–107
18. Steiner, P. M., and Marekov, L. N. (1997) J. Biol. Chem. 272, 2021–2030
19. Wertz, P. W., and Downing, D. T. (1987) Biochim. Biophys. Acta 917, 108–111
20. Swartzendruber, D. C., Wertz, P. W., Madison, K. C., and Downing, D. T. (1988) J. Invest. Dermatol. 86, 709–713
21. Wertz, P. W., Madison, K. C., and Downing, D. T. (1989) J. Invest. Dermatol. 92, 109–112
22. Downing, D. T. (1992) J. Lipid Res. 33, 301–313
23. Marekov, L. N., and Steiner, P. M. (1998) J. Biol. Chem. 273, 17763–17770
24. Lazo, N. D., Meine, J. G., and Downing, D. T. (1995) J. Investig. Dermatol. 105, 296–300
25. White, S. H., Mirejovsky, D., and King, G. I. (1992) Biochemistry 27, 3725–3732
26. Tseng, H., and Green, H. (1990) Mol. Biol. Evol. 7, 293–302
27. Lazo, N. D., and Downing, D. T. (1997) Biochemistry 36, 2559–2565
28. Downing, D. T. (1995) Proteins Struct. Funct. Genet. 23, 204–217
29. Sönnschen, F. D., Van Eyk, J. E., Hodges, R. S., and Sykes, B. D. (1992) Biochemistry 31, 8790–8798
30. Cammer-Goodwin, A., Allen, T. J., Oslick, S. L., McClure, K. F., Lee, J. H., and Kemp, D. S. (1996) J. Am. Chem. Soc. 118, 3082–3096
31. Luo, P., and Baldwin, R. L. (1997) Biochemistry 36, 8415–8421
32. Kentsis, A., and Sosnick, T. R. (1998) Biochemistry 37, 14613–14622
33. Holzwarth, G., and Doty, P. (1965) J. Am. Chem. Soc. 87, 218–228
34. Pielu, L., Nemethy, G., and Scheraga, H. A. (1987) Biopolymers 26, 1587–1600
35. Scholtz, J. M., Qian, H., York, E. J., Stewart, J. M., and Baldwin, R. L. (1991) Biopolymers 31, 1463–1470
36. Blaber, M., Zhang, X.-J., and Matthews, B. W. (1993) Science 260, 1637–1640
37. Timmers, G. L. (1995) Biochemistry 34, 3873–3877
38. Gerstein, M., and Chothia, C. (1992) J. Mol. Biol. 210, 133–149
39. McPhalen, C. A., Vincent, M. G., Picot, D., Jansons, J. N., Lesk, A. M., and Chothia, C. (1992) J. Mol. Biol. 227, 197–213
40. Barlow, D. J., and Thornton, J. M. (1988) J. Mol. Biol. 201, 601–619
41. Sudha, T. S., Vijayakumar, E. K. S., and Balaram, P. (1983) Int. J. Pept. Protein Res. 22, 464–468
42. Soman, K. V., Karim, A., and Case, D. A. (1991) Biopolymers 31, 1351–1361
43. Creighton, T. E. (1993) Proteins: Structures and Molecular Properties, 2nd Ed., W. H. Freeman and Co., NY
44. Tonolo, C., and Benedetti, E. (1991) Trends Biochem. Sci. 16, 350–353
45. Smythe, M., Huston, S. E., and Marshall, G. R. (1993) J. Am. Chem. Soc. 115, 11984–11985
46. Greenberg, C. S., Bircbkichler, P. J., and Rice, R. H. (1991) FASEB J. 5, 3071–3077