Characterization of the Proto-oncogenic and Mutant Forms of the Transmembrane Region of Neu in Micelles

We have investigated peptides corresponding to the complete transmembrane region of both proto-oncogenic (Val664) and mutant (Glu664) forms of the receptor Neu in detergent micelles by NMR and CD spectroscopy. Both forms of the peptide appear to adopt similar levels of helicity and dimeric interactions based on the analysis of CD spectra and nuclear Overhauser effect connectivity profiles. There are considerable differences in the chemical shifts of amide and, to a lesser extent, CHα resonances between the two forms of the peptides, and these differences are most pronounced in residues upstream of the mutation site and close to the N terminus of the transmembrane domain. Similarly, there are substantial differences in the amide hydrogen-deuterium exchange rates for residues close to and upstream of the mutation site; amide protons in this region of the proto-oncogenic peptide are much more resistant to exchange than those in the mutant form. In both molecules, residues downstream of the mutation site exhibit slow exchange. We therefore demonstrate that, although transmembrane Neu peptides exhibit similar levels of secondary structure when dispersed in detergent, there are detectable differences in their adopted micellar states that may provide insight into the dimer-promoting ability of the polar transforming mutation.

The catalytic activation of members of the receptor tyrosine kinase (RTK) family is generally initiated by ligand binding to the extracellular domain of a monovalent receptor that promotes dimerization, a process mediated by residues in both the extracellular and transmembrane (TM) domains. Receptor dimerization enables the trans-phosphorylation of the intracellular domain of the partner receptor and the recruitment of signaling proteins, leading to the appropriate downstream cellular response. In Neu, the rat homologue of ErbB2 (a member of the epidermal growth factor receptor subfamily), a single oncogenic mutation (Val664 → Glu) within the TM domain enhances receptor dimerization, leading to constitutive activation (1–3). Substitution of the mutant TM domain from Neu into other RTKs increases catalytic activity and promotes cellular transformation (4–6), demonstrating that there is a common role played by this segment in the activation process. The TM domain of Neu, along with its associated transforming mutation, has become a useful paradigm with which to clarify the role played by the single helical domain in the activation of RTKs.

Recent studies have demonstrated that the presence of polar residues within TM domains can, in principle, promote helix-helix association by providing an additional interhelical stabilizing force acting as both an H-bond donor and acceptor (7–9). The transforming mutation in the receptor Neu occurs within a five-residue dimerization motif that has been identified in the majority of RTKs (10). The local structure, dictated by the primary sequence in the motif, is critical for proper receptor dimerization and activation; replacing residues adjacent to the transforming mutation site with glutamate does not elicit the same behavior (2, 11). Furthermore, upon activation there appears to be important coupling between the kinase domain in the intracellular region of the receptor and the contact point of the helices within the dimer structure (12, 13). Thus, the harmful transforming property of the mutation arises not only because it promotes helix-helix association but because it occurs within the context of a dimerization motif.

Using peptides corresponding to the TM region of mutant Neu reconstituted in lipid bilayers, Smith and colleagues (14) have characterized the dimeric structure at residues close to the mutation site. They determined that the side chain carboxyl group of Glu664 is likely protonated and is a participant in an interhelical hydrogen bond (14). Solid state NMR measurements indicated that there is close association between the side chain carboxyl group of Glu664 and the α-carbon atom of Gly665 of the partner helix (15). These measurements led to a proposed dimer model that was symmetric and possessed a right-handed crossing angle.

The elucidation of the structure of membrane proteins is a significant challenge because of the technical difficulties associated with their study using techniques such as NMR spectroscopy and x-ray crystallography. It is for this reason that a complete structural picture of the TM domain of Neu in a lipid environment remains elusive. Several lines of evidence suggest that structural details not necessarily confined to the mutation site in the TM domain may offer insight into the process of receptor activation. Molecular dynamics studies of ErbB2/Neu TM peptides, performed in vacuo, have suggested the possibility that portions of this domain may readily adopt α-helix turns capable of reorienting residues found at the dimer interface (16–18). Solvent structures of both proto-oncogenic and mutant forms of the TM domain have displayed helical distortions downstream of the mutation site (19, 20). Therefore, dimer models constructed using canonical α-helices may be overly...
A Study of Transmembrane Neu Peptides in Micelles

EXPERIMENTAL PROCEDURES

Synthesis of Peptides—A hydrophobicity profile of Neu suggests that its TM domain comprises 26 amino acids. The membrane flanking regions contain a proline residue at the N terminus and several basic residues at the C terminus. 36-residue peptides incorporating the entire putative TM domain and several residues from the flanking juxtamembrane regions of both proto-oncogenic and mutant peptides corresponding to the complete TM domain of Neu dispersed in detergent micelles. We have characterized the micelle-adopted states of the peptides using CD and NMR spectroscopy, and we present the banding pattern observed upon running them on SDS-polyacrylamide gels. We demonstrate that both forms of the peptide appear to form helical structures throughout the length of the putative TM region and that they adopt similar levels of interhelical interaction within the micelle environment. However, the transforming mutation causes an appreciable difference in the amide proton exchange rates in residues close to the mutation site and near the N-terminal juxtamembrane region.

Amide Exchange Experiments—Hexa-[15N]-labeled proto-oncogenic and mutant peptides (~1.5 mM) were dispersed in 100 mM DPC at pH 4. The peptide/micelle solution was lyophilized and redissolved in D2O. Amide exchange rates were determined from the rate of decay in successive 1H-15N HSQC spectra acquired continuously over a 24-h time period at 40 °C. Roughly 20 min elapsed between redissolution in D2O and the acquisition of the first spectrum. Each time point took ~ 60 and ~ 75 min for the proto-oncogenic and mutant peptides, respectively. The decay constants were determined by fitting the peaks volumes and fitting the data to a single exponential decay. The curve fitting was performed using the program Origin (version 6.1, Origin Lab Corporation).

RESULTS AND DISCUSSION

There have been three reported NMR studies on peptides corresponding to the TM domain of Neu in all in TFE (19, 20, 32). This is a popular membrane mimetic solvent used to characterize the secondary structure of TM protein fragments; however, because tertiary interactions are generally disrupted, this solvent could not be used to investigate, for instance, the dimeric structure of the peptides. The 1H-NMR-derived monomeric structures of proto-oncogenic and mutant forms of the Neu peptides were found to be very similar in TFE (20, 32). This contradicts an early theoretical investigation, which predicted that the favored conformation of the monomeric form of the proto-oncogenic receptor would be kinked at the mutation site, whereas the mutant form would adopt a canonical α-helix structure (33, 34). In some cases where the protein/micelle complex remains relatively small, standard solution state NMR techniques can be applied to investigate both protein structure and dynamics. Several
investigations of small membrane-associated proteins and TM fragments from larger proteins have been reported in micelles (reviewed in Ref. 36). The effectiveness of micellar systems for NMR studies of membrane proteins is often hindered, however, by poor resolution, spectra that are not generally reproducible, and peptide aggregation (reviewed in Ref. 37).

CD Spectroscopy—The CD spectra of proto-oncogenic and mutant Neu peptides dispersed in SDS and DPC micelles are presented in Fig. 1; for comparative purposes, spectra obtained in the solvent TFE are included in the figure as well. In all three solution conditions, both forms of the peptide are predominantly α-helical, as was predicted by using both a deconvolution algorithm (24) and the value of [θ]222 (25). The predicted α-helical percentages are higher when a deconvolution algorithm was used than when the predictions were based on [θ]222, which produces a different [θ]222/[θ]208 ratio in the two environments. The ratio is very close to 0.8 for both peptides in TFE, which is consistent with the notion that they exist predominantly as monomers in this solvent. In micelles, the [θ]222/[θ]208 ratio ranges between 0.85 and 0.9 for both forms of the peptide. This finding indicates that, within this environment, there is some level of association between the peptides. A comparison of the ratios obtained in micelles for the two peptides does not indicate that one form of the TM domain is more predisposed to dimeric interactions than the other.

NMR Spectroscopy—Our analysis of the CD profiles suggests that both Neu peptides display similar levels of helicity and dimeric interactions in SDS and DPC micelles. We initially attempted to assign resonances to the Neu peptides dispersed in SDS micelles and, for this purpose, acquired spectra over a range of temperatures and solution pHs. Unfortunately, we were unable to make many assignments in this detergent, in particular, for residues close to the N terminus of the peptides where, in some samples, there appeared to be a lack of detectable signals for several resonances. For this reason, we attempted to characterize the peptides in DPC micelles. In this detergent we were able to obtain better spectral resolution with no apparent loss of signal from residues in the N-terminal region of the molecules. Spectra with the best resolution were acquired at relatively high temperatures and at low solution pHs. This has also been the case for studies of other membrane-bound proteins in detergent micelles (37). Even with the improved spectra obtained in DPC, there was significant overlap of peaks in the crucial amide fingerprint region, and we were not able to fully assign all of the backbone 1H resonances in either form of the peptide using standard two-dimensional homonuclear correlation spectra. To help in this process, we synthesized peptides with 15N amide labels at Val-6, Ala-11, Gly-15, Phe-19, Leu-22, and Gly-27 and used the proton assignments obtained from 15N-resolved spectra as points of departure from which to proceed with the sequential assignment of the other resonances in the peptide. In the final analysis, we were not able to unambiguously assign several resonances at the ends of the peptides. However, these residues are not believed to be found within the helical portion of the TM domain in the native protein. In general, the spectra acquired in DPC were fairly reproducible, although there was some variability in the chemical shift of amide resonances (up to 0.1 ppm), even when an effort was made to prepare samples in a consistent manner. Once dispersed in DPC, the peptides were fairly stable, with little loss of signal detected in samples that were up to a month old.

With respect to the chemical shift of amide resonances, spectra obtained for the proto-oncogenic form of the peptide in DPC were noticeably different from those obtained with the mutant form. This is illustrated in 1H-15N HSQC spectra of the hexa-(15N)-labeled peptides presented in Fig. 2. Once again, for comparative purposes we have presented spectra acquired in the solvent TFE as well, where the peptides adopt very similar structures and display similar chemical shifts in this homogeneous environment (20). This trend is also observed in comparing the HSQC spectra of the peptides in TFE, where there are only small differences in the position of the cross-peaks in
either the proton or the nitrogen dimension (Fig. 2, A and B). In contrast, there is a significant variation in the position of the cross-peaks in the HSQC spectra for the two peptides dispersed in DPC micelles (Fig. 2, C and D). This difference is most pronounced for residues close to the N-terminal region of the peptide. In Fig. 3, the NH-CH$_2$/H$_9251$ region from NOESY spectra of Neu peptides is presented, with arrows drawn to show the sequential connectivities between the NH and CH$_2$/H$_9251$ resonances in the peptides. As noted above, there is considerable overlap of cross-peaks in these spectra, which led to difficulty in making several resonance assignments. Although there are differences in the chemical shifts of amide protons throughout the entire length of the molecules, a noticeable trend is observed in comparing the spectra of proto-oncogenic and mutant Neu peptides in DPC; the amide peak dispersion pattern is similar for residues between Phe-19 and Ile-30 but is significantly different between Val-6 and Thr-12. Because of the change in primary sequence, the position of amide resonances close to the mutation site would be expected to exhibit variability. The chemical shift of an amide proton is very sensitive to its chemical environment (39) and is dictated by both the structural element it is found in and its relative solvent exposure. Our spectra clearly demonstrate that the structural environment of peptides within the micelle is different, and this difference is most pronounced in the N-terminal region of the peptide.

The optical CD data not only indicate that the two forms of TM Neu peptides adopt similar levels of helicity in detergent micelles, but that these levels are similar to those observed in TFE, where it was determined previously that the adopted $\alpha$-helical structure incorporates residues from Val-6 through to Ile-30 (20). Even though there are significant differences in the chemical shifts of amide protons in DPC, the NMR data are consistent with the formation of a helical structure in residues 6 through 30 in both forms of the peptide. An examination of the CH$_2$/H$_9251$ chemical shifts for these residues indicates they are uniformly upfield of random coil values, which is a strong indication of $\alpha$-helix formation (39). The CH$_2$/H$_9251$ chemical shift deviations from random coil values (CSDs) for residues 6 through 30 are presented as a histogram in Fig. 4A for both peptides. Once again, it can be seen in this figure that, for the residues in the C-terminal region of the peptide, the CH$_2$/H$_9251$ CSDs are almost identical. There are observable differences in the histogram, however, for residues close to the N terminus (e.g. Thr-9, Ala-11, and Thr-12), and, where differences exist, the CH$_2$/H$_9251$ CSDs are more negative for residues found in the proto-oncogenic peptide. This is potentially indicative of a more rigid helical structure in this region for the form of the molecule. It should be noted that, among the residues that we were able to assign at the ends of the peptides, the CH$_2$/H$_9251$ shifts are very close to random coil values (data not shown).

The NOE connectivity profile is presented for both peptides in Fig. 4B. Even in light of the considerable overlap of peaks in
the NOESY spectra, which prevented the assignment of several peaks, the pattern for both peptides is typical of an α-helix, with several Hα-NH$_{i+3}$, Hα-NH$_{i+4}$, and Hβ-NH$_{i+1}$ contacts for residues between 6 and 30. Curiously, we observed no NH$_{i}$-NH$_{i+1}$ contacts in the mutant form of the peptide in the N-terminal region, whereas several were observed in the proto-oncogenic peptide. As was noted earlier concerning differences in the CHα CSDs for residues in this region, this provides evidence that, although both the mutant and proto-oncogenic peptides are helical, the structure formed by the mutant peptide may exhibit more flexibility than that adopted by the proto-oncogenic peptide.

Amide Exchange Experiments—While initially attempting to obtain resonance assignments of Neu peptides in SDS micelles, we solubilized the peptide/micelle complex in D$_2$O. This procedure helps the assignment process by simplifying the spectra in the amide fingerprint region, because the signal from amide protons close to the ends of the molecule will disappear rapidly since they are generally unstructured and/or solvent exposed.

We noticed that, among the residues that we were able to assign in this detergent, those located at the N terminus of the peptide appeared to exchange very rapidly, whereas residues situated at the C terminus exhibited almost no loss of proton signal over several hours (data not shown).

To further investigate the exchange properties of the peptides in micelles, we dissolved the hexa-(15N)-labeled forms of both the proto-oncogenic and mutant Neu peptides dispersed in DPC micelles in D$_2$O (pH 4, 40 °C) and monitored the loss of amide signal intensity in successive 1H-NH HSQC spectra over a 24 h time period. Representative spectra for both peptides acquired at various time points following the initiation of exchange are presented in Fig. 5, and representative decay curves are presented in Fig. 6. Confirming what was seen in SDS, probes situated in the C-terminal half of both peptides (i.e. Phe-19, Leu-22, and Gly-27) display almost no visible exchange over the course of the experiment. After 24 h in D$_2$O, we performed a two-dimensional 1H-NOESY spectrum on the mutant peptide to verify whether the exchange trend observed with these three probes in the C-terminal half of the peptides was consistent for all of the residues in this region. As was observed at the labeled sites, we saw strong amide signals for all of the residues between Phe-19 and Ile-30, whereas no signals could be detected for any of the other residues in the peptide.

There is a considerable difference in the amide exchange behavior between proto-oncogenic and mutant forms of the peptide in two of the three probes (Ala-11 and Gly-15) in the N-terminal half; Val-6 exchanged within the dead-time of the experiment in both cases (i.e. rates of <20 min). In the proto-oncogenic peptide, the amide signal from Ala-11 exhibits a decay constant of ~840 min, whereas Gly-15 exhibits almost no signal decay over the duration of the experiment and exchanges as slowly as probes in the C-terminal half of the peptide (Fig. 6A). In the mutant form of the peptide, the amide signals at Ala-11 and Gly-15 decay much faster than in the proto-oncogenic form, with decay constants of ~66 min and ~830 min, respectively (Fig. 6B).

There are two interesting features regarding the amide exchange behavior of the peptides in detergent micelles. The first is the extremely slow rate of exchange exhibited by residues in the C-terminal region of both peptides. This is indicative of the formation of a very stable protein-micellar complex in this region of the peptide. The second interesting feature concerns the effect of the polar mutation on the relative exchange rates seen in both peptides. Clearly, the exchange rate at Gly-15, which is directly adjacent to the mutation site, is faster in the mutant form of the peptide. What is most intriguing is that, even though Phe-19 and Ala-11 are both close to the mutation site, the mutation has an effect only on the rate of exchange at Ala-11; Phe-19 exchanges very slowly in both forms of the peptide (Fig. 6).

Amide exchange measurements have been used to investigate the structure and dynamics of water-soluble proteins for several years (reviewed in Refs. 40 and 41). This technique has been used more recently to characterize the dynamics of several membrane-associated proteins in detergents, primarily to deduce which residues are likely to rest near the membrane surface versus those that will penetrate the hydrophobic core (42–47). An exchange at a given amide in soluble proteins is inhibited due to hydrogen bonding and/or its burial within the protein hydrophobic core. Similarly, within a micelle environment the protection from exchange for an amide proton found in a helix could be the result of its participation in a backbone hydrogen bond and/or because of its burial within the hydrophobic micellar core. It is impossible to clearly distinguish whether the difference in exchange rates at Gly-15 and Ala-11 is a result of helical destabilization or because of a difference in solvent exposure. Based on studies of other peptides in solution, the protection from exchange afforded by participation in a backbone hydro-
gen bond is on the order of minutes to hours, whereas residues buried deep within the hydrophobic core of a protein can be protected from exchange for up to weeks at a time. Because our NMR and CD data indicate that both peptides are helical in the N-terminal region, this tends to suggest that there is a difference in the level of solvent exposure at these sites. It is possible that, in the mutant form of the peptide, this portion of the helix is drawn close to the surface of the micelle in order to solvate the polar glutamate side chain. This possibility remains an interesting area open to further investigation.

The Use of Micelles to Investigate the Impact of Polar Residues within a TM Domain—Because of its ability to promote cellular transformation, the identification of the Val664Glu mutation within the transmembrane region of Neu has generated tremendous interest in the mechanism by which the presence of this residue promotes receptor dimerization. Polar mutations within the TM region of the cystic fibrosis transmembrane conductance regulator (CFTR) have also been implicated in some forms of cystic fibrosis (48). Micellar systems are frequently used to investigate membrane protein structure and dynamics, and it is important to establish whether these systems offer a reliable method with which to mimic the effect exerted by polar residues within a bilayer environment.

Many investigators have used SDS-PAGE analysis to quantify the level of interaction in TM domains (49–51), and this method was used in part to demonstrate the ability of polar mutations to drive helix-helix association in a small membrane-soluble peptide (7). However, through extensive work with the protein glycophorin A, Engelman and co-workers have argued that the effects of polar mutations, specifically their ability to promote interhelical association, may not be properly reflected in micellar systems (49, 50, 52, 53). They have theo-
neric forms of both Neu peptides have a molecular mass of ~4 kDa.

The dimerization-promoting ability of the Val664 → Glu transformation is not reflected in the migration pattern of TM Neu peptides on SDS-polyacrylamide gels (Fig. 7). The major band for the proto-oncogenic peptide runs at a position corresponding to ~1.5× its molecular mass, whereas the mutant form migrates as a major band at a position corresponding to ~1.0× its mass. (We have noted previously that concentrating the proto-oncogenic peptide in TFE prior to incubation in loading buffer seems to promote aggregation (20)). This banding pattern could be rationalized as a rapidly migrating dimer species for proto-oncogenic Neu versus a monomeric species for the mutant peptide; however, the subtle difference in the migration pattern may also be because the overall shape of the polar group and the energetic cost associated with water penetration into the bilayer.

Acknowledgments.—We are very grateful for Marc Genest's work in synthesizing the Neu peptides at the Canadian Protein Engineering Network of Centres of Excellence (PENCE). Thanks also to Richard and Raquel Épand for their help with the CD experiments, Christophe Furie and Valerie Robertson for helpful suggestions, and Joseph Chu for technical assistance.

REFERENCES

1. Bergmann, C. K., Hung, M. C., and Weinberg, R. A. (1986) Cell 57, 657–665
2. Cao, H., Bangalore, L., Bormann, B. J., and Stern, D. F. (1992) EMBO J. 11, 923–932
3. Sajot, N., and Genest, M. (2000) Eur. J. Biochem. 267, 603–608
4. Zhou, F. X., Merianos, H. J., Brunger, A. T., and Engelman, D. M. (2001) Nat. Struct. Biol. 8, 163–164
5. Zhou, F. X., Merianos, H. J., Brunger, A. T., and Engelman, D. M. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 2245–2250
6. Sternberg, M. J., and Gulick, W. J. (1990) Protein Eng. 3, 245–248
7. Cao, H., Bangkok, L., Bormann, B. J., and Stern, D. F. (2002) EMBO J. 21, 923–932
8. Bell, C. A., Tynan, J. A., Hart, K. C., Meyer, A. N., Robertson, S. C., and Donoghue, D. J. (2000) Mol. Biochem. Cell 11, 3589–3599
9. Zhou, F. X., Merianos, H. J., Brunger, A. T., and Engelman, D. M. (2001) Eur. J. Biochem. 267, 603–608
10. Sternberg, M. J., and Gulick, W. J. (1990) Protein Eng. 3, 154–160
11. Cao, H., Bangalore, L., Bormann, B. J., and Stern, D. F. (2002) EMBO J. 21, 923–932
12. Bell, C. A., Tynan, J. A., Hart, K. C., Meyer, A. N., Robertson, S. C., and Donoghue, D. J. (2000) Mol. Biochem. Cell 11, 3589–3599
13. Chen, L. I., Webster, M. K., Meyer, A. N., and Donoghue, D. J. (1997) J. Cell Biol. 137, 619–631
14. Smith, S. O., Smith, C. S., and Bormann, B. J. (1996) Nat. Struct. Biol. 3, 252–258
15. Smith, S. O., Smith, C. S., Shoker, S., Peerssen, O., Zlizn, M., and Amito, S. (2000) Biochemistry 39, 1870–1875
16. Duseau, J. P., Garnier, N., and Genest, M. (1997) J. Biomol. Struct. Dyn. 15, 555–572
17. Sajot, N., and Genest, M. (2000) Eur. J. Biochem. 293, 648–662
18. Sajot, N., and Genest, M. (2001) J. Biomol. Struct. Dyn. 19, 15–31
19. Goetz, M., Carlotti, C., Bontems, F., and Dufourc, E. J. (2001) J. Magn. Reson. 132, 133–143
20. Houliston, R. S., Hodges, R. S., Sharom, F. J., and Davis, J. H. (2003) FEBS Lett. 535, 39–43
21. Jones, D. H., Ball, E. H., Sharpe, S., Barber, K. R., and Grant, C. W. (2000) J. Magn. Reson. 143, 271–285
22. Duseau, J. P., Garnier, N., and Genest, M. (1997) J. Biomol. Struct. Dyn. 15, 555–572
23. Fleishman, S. J., Schlessinger, J., and Ben Tal, N. (2002) Proc. Natl. Acad. Sci. U. S. A. 99, 15597–15602
24. Boman, G., Murer, R., and Jermick, R. (1992) Protein Eng. 5, 191–195
25. Chang, C. T., Wu, C. S., and Yang, J. T. (1978) Anal. Biochem. 91, 13–31
26. Shaka, A. J., Lee, C. S., and Pines, A. (1988) J. Magn. Reson. 77, 274–293
27. Pottier, A. J., Bociek, V., and Skinner, E. F. (1996) J. Biomol. NMR 2, 661–665
28. Kay, L. E., Keifer, P., and Saarinen, T. (1992) J. Am. Chem. Soc. 114, 10663–10665
29. Oting, G., Senn, H., Wagner, G., and Wuthrich, K. (2004) J. Magn. Reson. 70, 500–505
30. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
31. Schagger, H. (1994) in A Practical Guide to Membrane Protein Purification (Schagger, H., and Von Jagow, G., eds) Academic Press, San Diego
32. Guillich, W. J., Bottomley, A. C., Lofts, P. J., Doak, D. G., Mulvey, D., Newman, R., Crumpston, M. J., Sternberg, M. J., and Campbell, I. D. (1992) EMBO J. 11, 43–48
33. Brandt-Rauf, P. F., Pincus, M. R., and Chen, J. M. (1989) J. Protein Chem. 8, 775–786
34. Brandt-Rauf, P. F., Backowsky, S., and Pincus, M. R. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 5660–5664
35. MacKenzie, K. R., Prestegard, J. H., and Engelman, D. M. (1997) Science 276, 131–133
36. Damberger, P., Jarvet, J., and Graslund, A. (2001) Methods Enzymol. 339, 271–285
37. Opeka, S. J., Ma, C., and Marassi, F. M. (2001) Methods Enzymol. 339, 285–313
38. Lau, S. Y., Taneja, A. K., and Hodges, R. S. (1984) J. Biol. Chem. 259, 13253–13261
39. Williamson, M. P. (1999) Biopolymers 47, 1433–1443
40. Englander, S. W., and Kallenbach, N. R. (1983) Q. Rev. Biophys. 16, 521–565
41. Englander, S. W. (2000) Ann. Rev. Biochem. Biomol. Struct. 69, 213–238
42. Almeida, F. C., and Opeka, S. J. (1997) J. Mol. Biol. 270, 481–495
43. Bader, R., Rytz, G., Lorch, M., Beck-Sickinger, A. G., and Zerbe, O. (2002) Biochemistry 41, 8031–8042
44. Nielson, J. D., and Sykes, B. D. (1988) Biochemistry 27, 2753–2762
45. Shao, H., Jiao, S. A., and Zalogis, M. G. (1999) J. Mol. Biol. 285, 755–773
46. Williams, K. A., Farrow, N. A., Deber, C. M., and Kay, L. E. (1996) Biochemistry 35, 5145–5157
47. Yee, A., Symczyna, B., and O'Neil, J. D. (1999) Biochemistry 38, 6489–6498
48. Therien, A. G., Grant, F. E., and Deber, C. M. (2001) Nat. Struct. Biol. 8, 2043–2052
49.红色字体表示文献引用。
597–601
49. Arkin, I. T., Adams, P. D., MacKenzie, K. R., Lemmon, M. A., Brunger, A. T.,
and Engelman, D. M. (1994) EMBO J. 13, 4757–4764
50. Lemmon, M. A., Flanagan, J. M., Treutlein, H. R., Zhang, J., and Engelman,
D. M. (1992) Biochemistry 31, 12719–12725
51. Simmerman, H. K., Kobayashi, Y. M., Autry, J. M., and Jones, L. R. (1996)

52. Engelman, D. M., Adair, B. D., Brunger, A., Flanagan, J. M., Hunt, J. F.,
Lemmon, M. A., Treutlein, H., and Zhang, J. (1993) Soc. Gen. Physiol. Ser.
48, 11–21
53. Russ, W. P., and Engelman, D. M. (1999) Proc. Natl. Acad. Sci. U. S. A. 96,
863–868

A Study of Transmembrane Neu Peptides in Micelles

J. Biol. Chem. 271, 5941–5946
