Polar Residues in Membrane Domains of Proteins: Molecular Basis for Helix−Helix Association in a Mutant CFTR Transmembrane Segment†

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ABSTRACT: Polar side chains constitute over 20% of residues in the transmembrane (TM) helices of membrane proteins, where they may serve as hydrogen bond interaction sites for phenotypic polar mutations that arise in membrane protein-related diseases. To systematically explore the structural consequences of H-bonds between TM helices, we focused on TM4 of the cystic fibrosis conductance regulator (CFTR) and its cystic fibrosis- (CF-) phenotypic mutation, V232D, as a model system. Synthetic peptides corresponding to wild-type (TM4-wt) (residues 219−242: LQASAFCGLGFLIVLALFQAGLGR) and mutant (TM4-V232D) sequences both adopt helical structures in SDS micelles and display dimer bands on SDS−PAGE arising from disulfide bond formation via wild-type residue Cys-225. However, the TM4-V232D peptide additionally forms a ladder of noncovalent oligomers, including tetramers, hexamers, and octamers, mediated by a hydrogen bond network involving Asp-Gln side chain−side chain interactions. Ala-scanning mutagenesis of the TM4 sequence indicated that ladder formation minimally required the simultaneous presence of the Cys-225, Asp-232, and Gln-237 residues. As random hydrophobic sequences containing these three residues at TM4 equivalent positions did not oligomerize, specific van der Waals packing interactions between helix side chains were also shown to play a crucial role. Overall, the results suggest that polar mutations in membrane domains, in conjunction with critically positioned polar partner residues, potentially constitute a source of aberrant helix interactions that could contribute to loss of function when they arise in protein transmembrane domains.

The folding of multispanning α-helical transmembrane (TM)[1] domains of membrane proteins into unique three-dimensional structures is directed by the specific packing of the individual TM helices. Because these helices are comprised predominantly of nonpolar residues and are situated in a lipid milieu, the required specificity for folding arises primarily through van der Waals forces in a "knob into holes" context (*1*). Recent reports have shown, however, that side chain−side chain hydrogen bonding also can be a powerful force in the association of TM helices (*2*−*5*). As wild-type polar side chains constitute over 20% of residues in most TM helices, they may serve as a source of interaction sites for phenotypic polar mutations that arise in membrane protein-related diseases (*6*). In the present work, we sought to investigate systematically the range of structural consequences of introducing an additional polar side chain in a TM peptide. For this purpose, we focused on a CF-phenotypic mutation in TM4 from the cystic fibrosis transmembrane conductance regulator (CFTR).

Cystic fibrosis (CF) is the most prevalent genetic disease in Canada and the United States, occurring in ∼1 in 2000 people (*7*). The disease arises through mutations within CFTR, a membrane protein that serves as a chloride ion channel on the apical membrane of epithelial cells (*8*). The most prevalent CF-phenotypic mutation is the ΔF508 mutation that occurs in ∼70% of CF alleles (*9*); this mutation, located in the NBD1 domain, manifests in the disease by causing protein misfolding and subsequent degradation (*10*, *11*). In addition to the ΔF508 mutation, there are hundreds of other mutations that are known to result in CF disease of varying severity; approximately 100 of these are found within the CFTR TM domains (http://www.genet.sickkids.on.ca/cftr-cgi-bin/MutationTable). This latter class of mutations likely produces the disease state by altering the structure/function of the mature protein. Specifically, for mutations involving a change to a polar residue within 1 of the 12 predicted TM segments of CFTR, one may hypothesize that non-wild-type H-bonds disrupt normal protein function by altering helix−helix packing, either within a single CFTR molecule or between molecules.

To investigate the roles of polar side chains in TM helices, we synthesized peptides corresponding to TM4 from CFTR either with or without the CF V232D mutation. We found that whereas the wild-type TM4 peptide failed to form any noncovalent helical associations, the TM4 peptide containing the V232D mutation generates a well-defined oligomeric ladder of TM4 helices mediated through a network of interhelical side chain−side chain H-bonds between D232

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[1] Abbreviations: CFTR, cystic fibrosis transmembrane conductance regulator; TM, transmembrane; SDS−PAGE, sodium dodecyl sulfate−polyacrylamide gel electrophoresis; CD, circular dichroism.

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and Q237, the latter a wild-type residue indigenous to TM4. Results from this in vitro investigation provide insights into the spectrum of structural consequences of membrane-embedded non-wild-type H-bonds and provide guidelines for assessing possible pathogenic mechanisms of disease-inducing mutations.

EXPERIMENTAL PROCEDURES

Materials. Fmoc amino acids were obtained from Nova Biochem (San Diego, CA), and PAL-PEG-PS resin was purchased from Applied Biosystems (Foster City, CA). Reagents for peptide synthesis included *N*,*N*-dimethylformamide (Caledon, Ontario, Canada), piperidine (Sigma), *N*,*N*-diisopropylethylamine (DIEA) (Aldrich), methanol (Caledon, Ontario, Canada), and *N*-[(dimethylamino)-1*H*-1,2,3-triazolo-[4,5-*b*]pyridin-1-ylmethylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide (HATU) (PerSeptive Biosystems, Boston, MA). Reagents used in peptide cleavage from solid support were trifluoroacetic acid (TFA) (PerSeptive Biosystems), triisopropylsilane (TIPS) (Aldrich), phenol (Gibco, New York), and ultrapure water. Diethyl ether (Caledon, Ontario, Canada) was used for peptide precipitation. Acetonitrile (MeCN) for HPLC purification was from Caledon (Georgetown, Ontario, Canada). SDS detergent (lauryl sulfate) was from Sigma. The Novex brand of precast gels and buffers (San Diego, CA) was used for SDS−PAGE. Reagents for the Micro BCA assay were from Pierce (Rockford, IL).

Peptide Synthesis. Peptides were synthesized using standard Fmoc chemistry on a PerSeptive Biosystems Pioneer peptide synthesizer. Synthesis employed the use of the Pioneer's standard (45 min) cycle. The HATU/DIEA activator pair was used with a 4-fold excess of amino acid. A low-load (0.18−0.22 mmol/g) PAL-PEG-PS resin was used to produce an amidated C-terminus. Peptides were cleaved with a cocktail of 88% TFA/5% phenol/5% ultrapure water/2% TIPS. Cleaved peptides were precipitated with ice-cold diethyl ether. Centrifuged pellets were dried in a desiccator, redissolved in ultrapure water, and lyophilized.

Peptide Purification. For each peptide, crude peptide powder was dissolved in water, and 10 mg of peptide was loaded onto a C4 preparative RP-HPLC column and eluted with a water/MeCN gradient (30−55% MeCN over 1 h). The major peak generally eluted at approximately 50% MeCN and was collected and lyophilized. Recovery of pure peptide from crude peptide was generally between 20% and 30%. Mass spectrometry was used to confirm the molecular weight of the purified peptide, and the Micro BCA assay was used to determine peptide concentration. Analytical HPLC chromatography confirmed that the final peptide purity was over 95%.

Circular Dichroism Spectroscopy. CD spectra were collected using a Jasco J-720 circular dichroism spectropolarimeter. Samples were measured at peptide concentrations between 20 and 50 μM and were dissolved in a buffer containing 50 mM SDS/50 mM Tris/10 mM NaCl at pH 7.5. Measurements were taken using a quartz cuvette with a 0.1 mm path length. Spectral scans were performed from 250 to 190 nm with a step resolution of 0.2 nm, a speed of 20 nm/min, and a bandwidth of 1.0 nm.

Table 1: Sequences of Peptides Derived from the CFTR TM Segment 4 (Residues L219−R242)[a]

Peptide	Sequence
TM4-wt	KKK-LQASAFCGLGFLIVLALFQAGLGR-MKKK
TM4-VD	KKK-LQASAFCGLGFLIDLALFQAGLGR-MKKK
TM4-VD(L219A)	KKK-AQASAFCGLGFLIDLALFQAGLGR-MKKK
TM4-VD(Q220A)	KKK-LAASAFCGLGFLIDLALFQAGLGR-MKKK
TM4-VD(S222A)	KKK-LQAASAFCGLGFLIDLALFQAGLGR-MKKK
TM4-VD(F224A)	KKK-LQASAACGLGFLIDLALFQAGLGR-MKKK
TM4-VD(C225A)	KKK-LQASAFAGLGFLIDLALFQAGLGR-MKKK
TM4-VD(C225S)	KKK-LQASAFSGLGFLIDLALFQAGLGR-MKKK
TM4-VD(G226A)	KKK-LQASAFCALGFLIDLALFQAGLGR-MKKK
TM4-VD(L227A)	KKK-LQASAFCGAGFLIDLALFQAGLGR-MKKK
TM4-VD(G228A)	KKK-LQASAFCGLAFLIDLALFQAGLGR-MKKK
TM4-VD(F229A)	KKK-LQASAFCGLGALIDLALFQAGLGR-MKKK
TM4-VD(L230A)	KKK-LQASAFCGLGFAIDLALFQAGLGR-MKKK
TM4-VD(I231A)	KKK-LQASAFCGLGFLADLALFQAGLGR-MKKK
TM4-VD(L233A)	KKK-LQASAFCGLGFLIDAALFQAGLGR-MKKK
TM4-VD(L235A)	KKK-LQASAFCGLGFLIDLAAFQAGLGR-MKKK
TM4-VD(F236A)	KKK-LQASAFCGLGFLIDLALAQAGLGR-MKKK
TM4-VD(Q237A)	KKK-LQASAFCGLGFLIDLALFAAGLGR-MKKK
TM4-VD(C-term(A6))	KKK-LQASAFCGLGFLIDLALFQAAAAA-AKKK
TM4-VD(CDQL)	KKK-LAAAAACALAALADLALAQAAAAA-AKKK
TM4-VD(scramble)	KKK-AALSAGCLMLAGADALFLQGFGIL-FKKK

[a] Alanine residues used in scanning mutagenesis are underlined.

SDS−PAGE. Peptide samples were subjected to SDS−polyacrylamide gel electrophoresis using Novex precast 10−20% Tricine gels and buffers (Novex, San Diego, CA).

Molecular Models. Three-dimensional models were generated using Swiss-PdbViewer (*12*) and Xfit (*13*) software programs.

RESULTS

Design of CFTR TM4 Peptides. For the wild-type TM4 peptide (TM4-wt), residues 219−243 from the full-length protein were included, along with a non-native C-terminal methionine (for prospective radiolabeling purposes) (*14*) and non-native N- and C-terminal lysine residues. The latter so-called "Lys tags" were appended to confer water solubility to the peptide, which facilitates peptide purification, handling, and characterization (*15*). Of significant advantage, Lys-tagged TM peptides retain the ability to insert into membrane-mimetic environments such as SDS micelles, where they adopt helical secondary structure(s) with native-like tertiary contacts (*15*). The TM4 peptide was designed identically to TM4-wt except that it contained the V232D mutation. The sequences of the synthesized peptides are given in Table 1.

Effect of the V232D Mutation on Helical Packing. SDS−PAGE was employed to assess helix−helix interactions of each of the initial two peptides. This method represents a well-established tool for the analysis of the oligomeric state(s) of TM helices (*2*−*5*, *15*−*26*). Figure 1 shows the contrasting migration patterns of the two peptides: the TM4-wt peptide migrated to mobilities consistent with those of a monomer (3.3 kDa) and a dimer (6.6 kDa). However, the TM4 peptide containing the V232D mutation displays additional bands consistent with those of a tetramer, hexamer, and octamer. Additional higher order species (decamer, dodecamer, and larger) become observable for the TM4-VD peptide if it is overloaded on a gel (data not shown).

Scanning Mutagenesis of the TM4-VD Peptide. To identify specific residues both responsible and essential for the self-

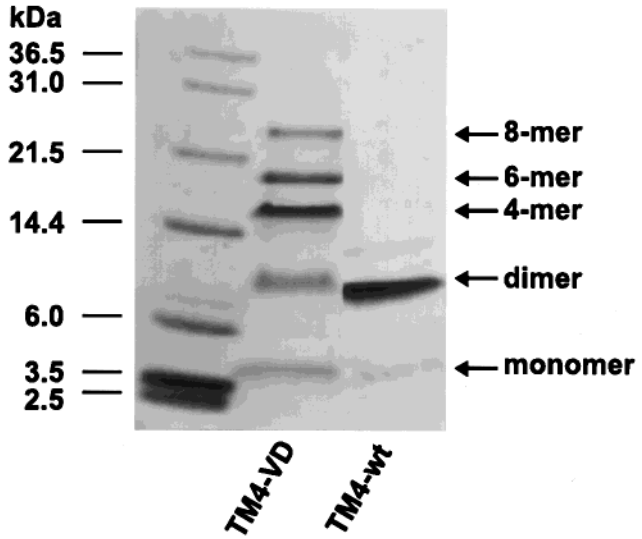

FIGURE 1: SDS−PAGE analysis of peptides derived from CFTR transmembrane helix 4. Samples were loaded onto a 10−20% Tricine gel and run at 125 V (constant voltage) for 90 min. Molecular weight markers, peptide lanes, and band oligomerization states are indicated on the diagram.

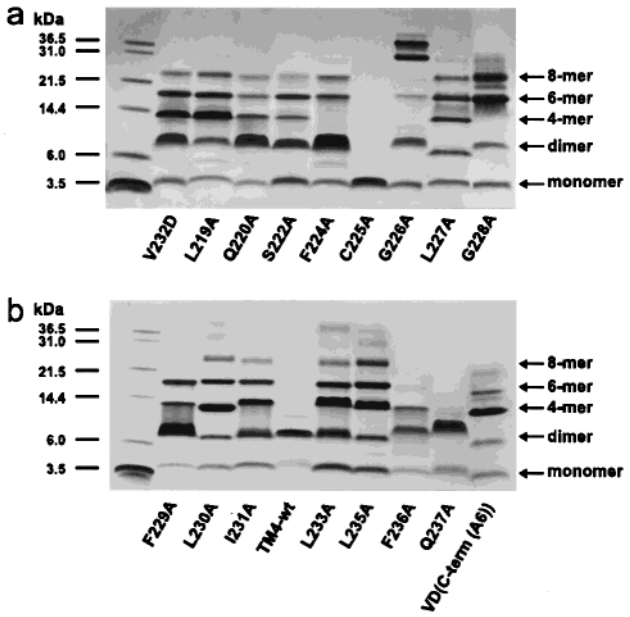

FIGURE 2: SDS−PAGE analysis of Ala-scanning CFTR TM4 peptides (see Table 1). Analysis was performed as described in Figure 1. Molecular weight markers, peptide lanes, and band oligomerization states are indicated on the diagram. Panel a: Ala scanning of peptide positions 219−228. Panel b: Ala scanning of peptide positions 229−237. Positions 238−242 plus the non-native Met residue were all converted to Ala residues in the VD(C-term-(A6)) peptide.

association of the TM4-VD peptide into a ladder of oligomers, we performed Ala-scanning mutagenesis. In these experiments, each residue in the CFTR TM4 segment was replaced individually from residues 219 through 237 inclusive with an alanine residue; residues 221, 223, and 234 are already Ala in the native sequence. In addition, residues 238 through 243 (and the non-native Met residue) inclusive were probed for a possible role in peptide oligomerization by simultaneously replacing this full segment with a polyalanine sequence. All peptides used in this study are given in Table 1. Upon analysis by SDS−PAGE, we observed that ladder formation is highly tolerant to substitution at most positions in the sequence (Figure 2); i.e., only three residues, C225, D232, and Q237, appeared to constitute an absolute requirement for the formation of helical oligomers beyond that of a dimer. These results led to the proposition that oligomer formation is mediated by specific side chain−side chain hydrogen bonds between D232 and Q237.

Further analysis revealed that substitution at a few positions does have discernible effects on peptide oligomerization. For example, the point mutations Q220A, S222A, F224A, and F229A each resulted in species patterns with a more heavily populated dimer band versus the higher order oligomer bands. In contrast, the G226A mutation stabilized the oligomer ladder, as evidenced by the presence of additional higher order oligomeric bands corresponding to decamers and dodecamers.

Analysis of the Peptide Secondary Structure by Circular Dichroism Spectroscopy. To gain further insights into the nature of the TM4 oligomerization, the TM4 peptides were examined by CD spectroscopy. Figure 3 shows that the peptides are helical in SDS micelles. Using the [θ] value at 222 nm, these peptides were calculated to be approximately 50% helical (*27*). Considering that only ca. 18−22 of the 31 residues of each peptide are expected to be embedded within the SDS micelle interior, these values correspond to a high degree of helicity for the actual transmembrane residues. Additionally, we noted that peptide oligomerization did not significantly alter secondary structure of the individual helical components, as both TM4-VD and TM4-VD-(CA) had superimposable spectra. All other TM4 peptides were similarly helical in SDS micelles. CD spectra were concentration-independent over an order of magnitude of peptide concentration.

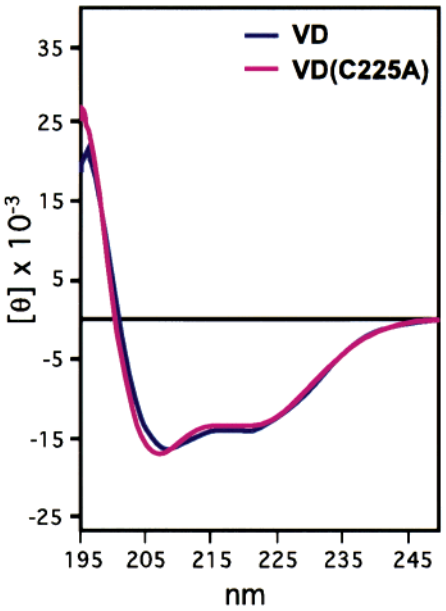

FIGURE 3: Secondary structure determination of TM4 peptides using circular dichroism spectroscopy. Samples were prepared using 20−50 μM peptide, 50 mM SDS/Tricine, and 10 mM NaCl, at pH 7.5. Spectra are TM4-VD and TM4-VD(C225A).

Oxidation State of C225. The TM4 peptides contain a single wild-type Cys residue at position 225. Mass spectrometry indicated these peptides were purified as monomers (data not shown). However, using the TM4-VD peptide as an example, we showed that, during sample handling under

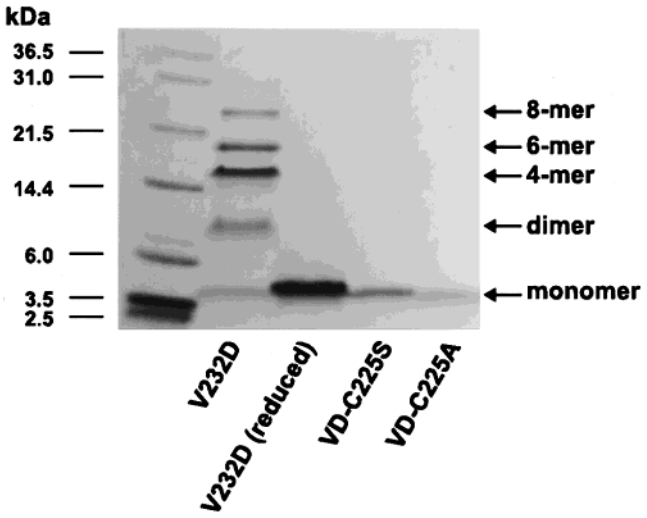

FIGURE 4: SDS−PAGE analysis of CFTR TM4 peptides (see Table 1). Analysis was performed as described in Figure 1. Molecular weight markers, peptide lanes, and band oligomerization states are indicated on the diagram. The V232D peptide was reduced in the lane indicated by incubating the peptide with TCEP in aqueous solution for 5 min prior to addition of Tricine sample buffer.

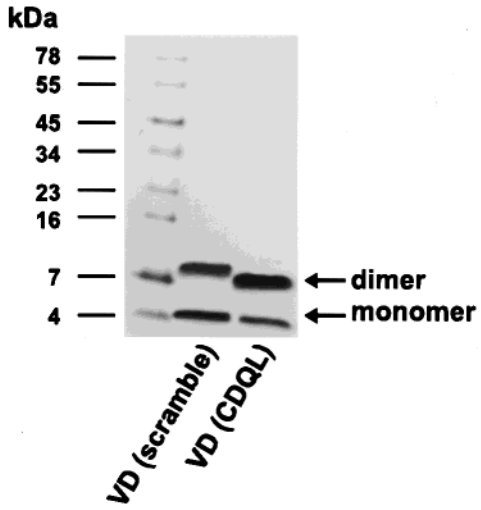

FIGURE 5: SDS−PAGE analysis of CFTR TM4-VD(scramble) and TM4-VD(CDQL) peptides (see Table 1). Analysis was performed as described in Figure 1. Molecular weight markers, peptide lanes, and band oligomerization states are indicated on the diagram.

ambient conditions, Cys-225 becomes largely involved in an interhelical disulfide bond (Figure 4). Treatment of the TM4-VD peptide with the reducing agent TCEP (*28*) prior to electrophoresis resulted in a single monomeric band for the TM4-VD peptide (and all other TM4 peptides). This result established that a disulfide-linked dimer is the "building block" for the higher order oligomers. This concept is reinforced by the observation that TM4-VD peptides with an Ala or Ser at position 225 are also completely monomeric on SDS−PAGE. Furthermore, HPLC-isolated disulfide-linked dimers displayed an identical oligomeric ladder on SDS−PAGE (without a monomer band), confirming the disulfide dimer as the building block and negating the possibility of odd-numbered oligomers in the ladder (data not shown).

Oligomeric State of Nonspecific Sequences. To assess whether residues C225, D232, and Q237 are sufficient to drive the association of TM4 helices, two additional peptides were designed and synthesized: TM4-VD(scramble) and TM4-VD(CDQL) (Table 1). Both peptides retain the Lys tags and contain residues C, D, and Q at positions equivalent to those in the TM4-VD peptide. However, the TM4-VD-(scramble) peptide contains the remaining residues randomly scrambled, while in the TM4-VD(CDQL) peptide, all remaining positions are replaced by Ala with the exception of the native Leu residues that were retained to ensure the peptide remains sufficiently hydrophobic to insert into SDS micelles (*29*). When these two peptides were examined on SDS−PAGE, both ran as monomer and disulfide dimer, but neither was able to form noncovalent oligomers (Figure 5). Thus, the C, D, and Q residues are necessary, but not sufficient, to drive the noncovalent association of TM4-VD peptides. These results provide strong evidence that the structures of TM4-VD higher order oligomers are sequence-specific and that their formation requires additional determinants that are not immediately identified by Ala-scanning mutagenesis.

DISCUSSION

Hydrogen bonding within the membrane domains of proteins can result in strong TM helix−helix association (*2*−*5*). In the present work, we sought to obtain systematic knowledge concerning the detailed prerequisites under which these interactions can take place. In this context, we simulated H-bond formation in membrane-interactive peptides using segments featuring the CF-phenotypic mutation V232D from TM4 of the CFTR transmembrane domain. We found that TM4 peptide−peptide association devolved from three basic structural elements in confluence: (i) dimers linked through preformed disulfide bonds; (ii) H-bonds between D232 and neighboring Q237 in a partner helix; and (iii) specific van der Waals packing of wild-type TM4 residues.

Role of a Disulfide Link. Formation of a disulfide link via C225 residues plays an essential role in the SDS-resistant association of TM4-VD. The capacity of the S−S linked dimer, but not the reduced monomer, to promote oligomerization likely emanates from the spatial orienting effect the disulfide bond imparts to the downstream Asp and Gln residues, arising from the facts that (i) the S−S dimer creates a parallel, in-register situation (*30*) for two TM helices and (ii) the S−S cross-link impedes free rotation around the major axes of the two helices with respect to one another. Thus, when compared to the monomeric TM4-VD helix, the S−S link creates an arrangement where two Asp-Gln H-bonds are formed per molecule rather than one. Similarly, the associating S−S dimers would have more favorable intermolecular van der Waals contacts per molecule than two interacting TM4 monomers. Thus, the increased number of stabilizing contacts may be the reason disulfide-linked dimers form SDS-resistant noncovalent oligomers while TM4-VD monomers do not. Finally, while monomeric TM4 peptides could not associate in SDS micelles, less disruptive environments such as the native lipid bilayer may promote such interactions.

Role of Wild-Type "Polar Partner" Residues. The observation that Q237, a wild-type polar residue, is critical for ladder-type interhelical association is consistent with previous findings in this laboratory that a conceptually analogous Asp-

Gln side chain−side chain H-bond forms in a helix−loop−helix construct derived from CFTR helices 3 and 4 (*6*). Gln and Asp are known to preferentially form stable H-bonds in helical folds of soluble proteins (*31*). In addition, the strength of apolar hydrogen bonds (i.e., those in a membrane environment, free from competing water molecules) is illustrated by the fact that their calculated Gibbs free energy of formation is predicted to be ca. −5 kcal/mol, whereas this value may be reduced to ca. −0.5 kcal/mol in an aqueous environment (*32*). This notion is also in agreement with the observation that certain polar side chains (Asn, Gln, Asp, or Glu) can drive the association of model TM segments to form SDS-resistant noncovalent oligomers (*2*−*5*).

Sequence-Specific Packing Is a Requirement for Ladder Formation. Given the apparent strength of H-bonds in apolar environments, one might postulate that the presence of a strongly polar residue (such as Asp, Gln, Asn, or Glu) would cause the association of any membrane-embedded helix. From this work, we discovered that this is not always the case, as nonspecific TM model peptides retaining only the correct spatial arrangement of the critical C, D, and Q residues [TM4-VD(scramble) and TM4-VD(CDQL)] could not form an oligomeric ladder. These latter observations demonstrate that the proposed H-bonding network is necessary, but not itself sufficient, to drive the higher order association of TM helices. Therefore, there must be additional sequence-specific interhelical contacts that stabilize ladder formation, a result that implies the inherent capacity of the CFTR TM4-VD helices to self-associate via a complementary interfacial packing motif. While this work does not address the specific residues comprising this motif, disruption of such interactions, which would typically include van der Waals packing, aromatic ring stacking, and some additional but not strictly required H-bonds, may explain why peptides with Ala substitutions for wild-type residues Q220, S222, F224, or F229 are found to destabilize the noncovalent oligomers (Figure 2).

Models for the Oligomeric Peptide Ladder Formed by CFTR TM4(VD). Figure 6a shows a possible molecular model of the disulfide-linked TM4-VD helical dimer consistent with the above results, highlighting the three residues observed to be essential for the formation of the noncovalent oligomers. The disulfide link is modeled with a bond length of ∼2 Å and a bond angle of ∼90° between the two Cys side chains, values typically observed in soluble proteins (*33*, *34*). D232 and the Q237 residues are calculated to be 140° apart, situating them on opposing faces of the helix, thereby allowing two H-bonds to form between a pair of dimers (Figure 6b). Figure 6c illustrates the tetrameric complex in Figure 6b in a view that allows observation of the proposed Asp-Gln H-bonds. Here, the Asp carboxylate oxygen atom and the Gln carboxamide nitrogen atom are ∼2.9 Å apart, sufficiently close for strong H-bond formation. For simplicity, the tetrameric complex is modeled without a crossing angle; however, the average crossing angle of TM helices has been reported to be +20° (*35*), an arrangement that would bring both pairs of D and Q residues closer, further facilitating the formation of the H-bonds. Figure 7 is a highly schematized diagram that depicts how a chain of disulfide-linked dimers could then be formed progressively through the proposed H-bonding network. According to this model, the assembly of the ladder satisfies the H-bonding potential of the Asp and Gln residues at the helix−helix interfaces. However, in this linear arrangement, the open ends of the ladder have Asp and Gln residues whose H-bonding potential remains unmet. As the ladder gets longer, the ratio of satisfied/unsatisfied polar residues (i.e., enthalpic stabilization) increases; apparently, the concomitant decrease in entropy mitigates against indefinite peptide oligomerization.

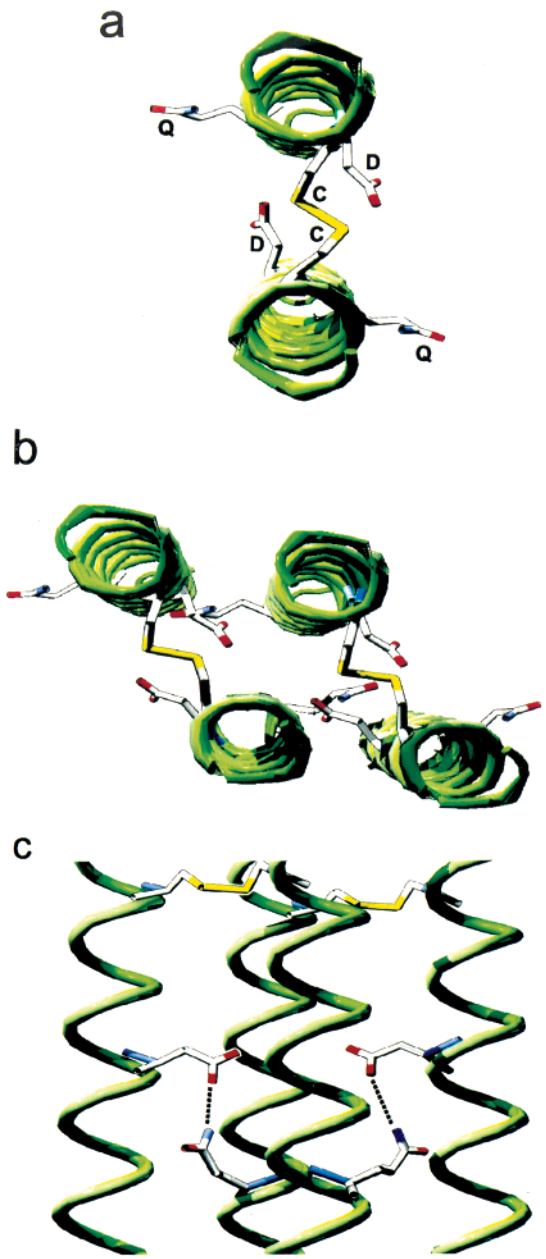

FIGURE 6: Molecular models proposed for the oligomerization of the CFTR transmembrane helix 4 containing the V232D mutation (TM4-VD). Panel a: Disulfide-linked dimer viewed down the helical long axis from the N-terminus. Residues implicated in mediating oligomerization are labeled (C = Cys-225; D = Asp-232; Q = Gln-237). The yellow bar represents the S−S bond. Panel b: Proposed scheme for noncovalent oligomerization of a pair of disulfide-linked dimers via Asp-Gln side chain−side chain H-bonding. The tetrameric complex is viewed down the helical long axis from the N-terminus. Panel c: Tetrameric complex in panel b viewed perpendicular to the long axis. Hydrogen bonds are represented with dotted lines. For clarity, only two pairs of Asp-Gln residues are shown.

The models in Figure 6 clearly illustrate that Asp and Gln H-bonds can form between TM4 helices. However, due to

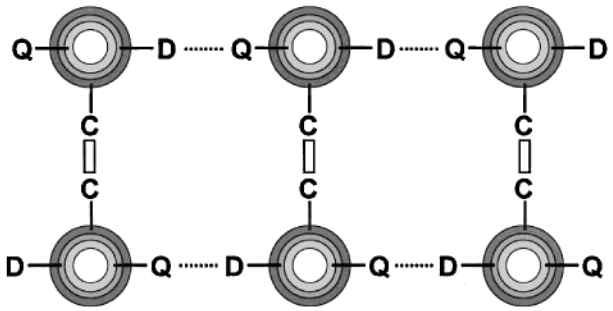

FIGURE 7: Schematic diagram illustrating how disulfide-linked dimers may form higher noncovalent oligomers in a chainlike fashion. See text for further discussion.

the vertical displacement between the two residues along each helix, only certain combinations of side chain χ angles will give rise to an arrangement where the two polar groups are sufficiently proximal to interact. The specifics of this geometry may be the reason polar residues and the surrounding packing interactions are both required for the noncovalent association of TM4 helices. As well, an alternative to this model could involve an antiparallel, arrangement between pairs of S−S linked dimers. Although this may be physically possible, it does not appear to offer any advantage for positioning the polar partners. Furthermore, such an arrangement would eliminate specific stabilizing van der Waals contacts that we expect must occur for parallel TM4 helices.

Implications of Peptide Oligomer Formation for the Pathogenic Mechanism of CF Disease. The V232D mutation in TM4 creates a membrane-buried electrostatic locus, whether it is in the context of a TM peptide or in the full-length protein. The present findings may thus relate to the full-length protein in that the D232 residue could form an interhelical H-bond with a neighboring native polar side chain [Q237 or other proximal polar partner residue] within the TM domain of a single CFTR molecule. Since many of the membrane-based CF-phenotypic mutations involve a hydrophobic-to-polar side chain change (*6*), non-native polar partnering may represent a general phenomenon underlying not only mutant structures of CFTR but also that of other members of the ABC transporter superfamily, a class of proteins currently associated with a number of genetic disorders (*36*).

While intimate association of two identical TM helices obviously cannot arise in the membrane domain of a monomeric multispanning protein, such a phenomenon could also play a role in membrane proteins which dimerize/oligomerize through specific helices, either in normal or aberrant states. For example, where the V232D mutation arises in full-length CFTR, the D232 residue on one TM4 helix could partner with the Q237 residue in the complementary TM4 helix in another CFTR molecule, to produce a functionally impaired H-bonded dimer. Given the evidence for dimers of full-length CFTR and related ABC transporters in vivo (*37*−*43*), the formation of the Asp-Gln hydrogen bond mediating the association of two TM4 helices is plausible.

Conclusions. In the single-spanning TM peptides studied here, the V232D mutation results in a homophilic association of TM4 helices mediated by D232 and Q237 interhelical H-bonding. In a helix−loop−helix construct consisting of CFTR TM segments 3 and 4, this same mutation leads to the formation of an interhelical H-bond between D232 in helix 4 and Q207 in helix 3 (*6*). That the V232D mutation produces a non-wild-type H-bond in both systems suggests that a given mutant polar residue arising in the CFTR membrane domain could have multiple polar partners available in vivo. Indeed, the analysis given by Therien et al. (*6*) suggests that the many wild-type polar amino acids embedded throughout the CFTR membrane domain can readily partner with polar CF-phenotypic mutations. Nevertheless, it should be emphasized that, in the present work, polar residues were necessary but not sufficient to cause the noncovalent association of TM helices, indicating that any disease-inducing effect of polar residues in these segments will ultimately be context-specific.

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