Journal of Bioenergetics and Biomembranes, Vol. 25, No. 1, 1993

Effects of the ΔF508 Mutation on the Structure, Function, and Folding of the First Nucleotide-Binding Domain of CFTR†

Philip J. Thomas[1] and Peter L. Pedersen[1,2]

Received August 26, 1992; accepted September 15, 1992

The fatal autosomal recessive disease cystic fibrosis (CF) is caused by mutations in the gene which encodes the cystic fibrosis transmembrane conductance regulator (CFTR). Many of these disease-causing mutations, including the deletion of F508 (ΔF508) which accounts for approximately 70% of the disease alleles, occur in one of the two consensus nucleotide binding sequences. Peptide studies have directly demonstrated that the N-terminal nucleotide binding sequences bind adenine nucleotides. Structurally, circular dichroism spectropolarimetry indicates that this region of CFTR assumes a β-stranded structure in solution. The ΔF508 mutation causes a diminution in the amount of β-stranded structure and a concomitant increase in the amount of random coil structure present, indicating that either the mutant peptide has a different native structure or that the conformational equilibrium is shifted toward a more disordered form. Furthermore, the mutant peptide is more sensitive to denaturation, indicating that ΔF508 is a stability, or protein-folding mutant. Here we review these results and discuss their implications for interpreting the behavior of ΔF508 *in situ* and for the rational design of new CF drugs.

KEY WORDS: Cystic fibrosis; cystic fibrosis transmembrane conductance regulator; protein folding; peptides; secondary structure; nucleotide binding; mutant; genetic disease.

INTRODUCTION

Cystic fibrosis (CF) is a common autosomal recessive disease caused by a variety of mutations within the gene encoding the cystic fibrosis transmembrane conductance regulator protein (CFTR) (Riordan *et al.*, 1989; Cutting *et al.*, 1990; Kerem *et al.*, 1990). Nonaffected epithelial cells exhibit a *c*AMP-dependent chloride conductance which is absent in cells isolated from CF patients (Schoumacher *et al.*, 1987; Li *et al.*, 1988; Hwang *et al.*, 1989), thereby accounting for mucus accumulation in the lungs and, perhaps, the pancreatic and sweat abnormalities that are phenotypic of the disease. Expression of the wild-type CFTR in heterologous cells produces a chloride conductance responsive to *c*AMP (Kartner *et al.*, 1991), while in some cases expression of CFTR containing the most common CF-causing mutation, deletion of phenylalanine 508 (ΔF508), did not (Anderson *et al.*, 1991a). Furthermore, expression of the wild-type, but not of the mutant, CFTR corrected the defective chloride conductance in CF cells, indicating that the ΔF508 mutation is causing CF (Rich *et al.*, 1990; Drumm *et al.*, 1990). Moreover, site-directed mutagenesis of putative membrane-embedded residues alters the ion selectivity of the *c*AMP regulated conductance, providing direct evidence that the CFTR protein itself catalyzes the chloride movement (Anderson *et al.*, 1991b).

The *c*DNA sequence of CFTR indicates that it is a protein of 1480 amino acids that is composed of five functional domains (Riordan *et al.*, 1989). A regulatory domain contains serine residues that are required

[1]Department of Biological Chemistry, The Johns Hopkins University School of Medicine, 725 North Wolfe Street, Baltimore, Maryland 21205.

[2]To whom correspondence should be addressed.

0145-479X/93/0100-0011$07.00/0

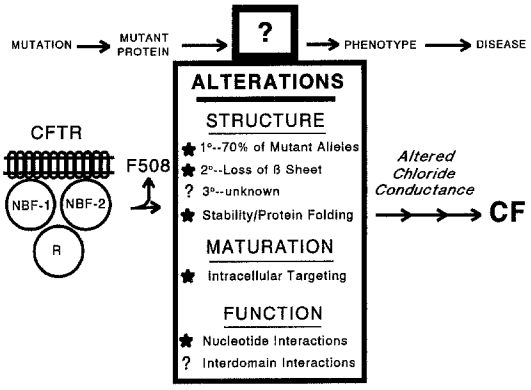

Fig. 1. Overview of the basis of CF: Looking into the black box at a cellular and a molecular level. It is now known that CF is caused by mutations in the gene that codes for the CFTR protein (Riordan *et al.*, 1989; Cutting *et al.*, 1990), and, furthermore, that this protein is responsible for catalyzing a *c*AMP-dependent chloride efflux that is deficient in CF cells. However, the cellular and molecular basis for the effect of CFTR nucleotide binding fold (NBF) mutations on the phenotype are more obscure (black box). In this review we survey work from our own laboratory and others on the effects of the ΔF508 mutation on the structure, function, and maturation-folding of CFTR. Stars indicate areas where there is experimental evidence for mutation-induced alterations, while question marks indicate areas where little or no experimental information exists.

for the *c*AMP-dependent activation of the protein (Cheng *et al.*, 1991). Two membrane domains, each containing six predicted transmembrane helices, contain residues involved in specifying the ion selectivity of CFTR (Anderson *et al.*, 1991b). Finally, the protein contains two nucleotide-binding consensus sequences (Walker *et al.*, 1982). The ability of the N-terminal nucleotide-binding domain to interact with adenine nucleotides has been directly demonstrated (Thomas *et al.*, 1991, 1992a; Hartman *et al.*, 1992). Despite these advances there are many deficiencies in our understanding of the CFTR protein. For example, how these domains interact to perform their cellular function and whether there are other, as yet not described, solutes for CFTR remain unknown. Studies directed at elucidating the effects of mutations in the CFTR protein on its structure, and subsequently its function, are of particular interest as the cascade of events leading from mutation to structural change to functional alteration to the disease phenotype constitute the molecular basis of CF (Fig. 1). The nucleotide-binding domains are especially interesting in this regard as many prevalent CF-causing mutations are clustered within these domains, including ΔF508. We have focused our attention on the N-terminal nucleotide-binding domain with the goal of answering four fundamental questions: (1) what is the final native structure of the domain, (2) what is the function that it performs in the context of CFTR, (3) how is the final structure attained, that is how does the protein domain fold into an intricate three-dimensional shape, and (4) what are the effects of disease-causing mutations on the structure, function, and folding of the domain. The answers to these questions are both necessary and fundamental to understanding the disease at a molecular level, and, thus, for the rational design of new pharmaceuticals. This review is intended to summarize briefly the progress that has been made toward answering these questions and to identify likely areas of future advance.

STRUCTURAL ASPECTS

The original proposal that CFTR contained nucleotide-binding sites (Riordan, 1989) was based upon the presence of A and B type consensus sequences, which are found in many other nucleotide-binding proteins (Walker *et al.*, 1982). CFTR contains two sets of A and B consensus sequences, one set in the N-terminal half and one in the C-terminal half, indicating that the protein binds at least two moles of nucleotide. Typically, proteins that contain the A and B domains do cellular work at the expense of ATP hydrolysis or catalyze phosphate transfer reactions. High-resolution structures derived by X-ray crystallography exist for several of these enzymes and the related GTP binding proteins (for example, see Story and Steitz, 1992; La Cour *et al.*, 1985; Schultz, 1987; Tong *et al.*, 1991; Jurnak, 1985). Structurally, this class of nucleotide-binding proteins are in a Rossman fold (Rossman *et al.*, 1975) conformation in the crystal state. Although the folding pattern is not necessarily conserved, the general fold consists of a five- to eight-stranded, predominantly parallel β-sheet sandwiched between several α-helices lying across the face of the β-sheet (Story and Steitz, 1992). The A consensus sequence, Gx_4GKT, forms a loop between a β-strand and an α-helix in these structures in which the lysine residue interacts with the pyrophosphate moiety of the nucleotide (Thomas *et al.*, 1992b).

In addition to the A and B consensus regions, there are three other regions of sequence homology observed when the N-terminal nucleotide-binding domain of CFTR is compared to the nucleotide-binding sequences of the ATP synthase α and β subunits and the human P-glycoprotein or mdr (Thomas *et al.*,

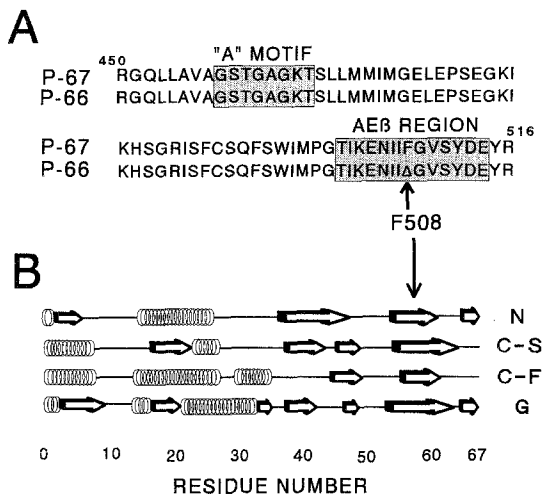

Fig. 2. Structure of the synthetic peptides. (A) Primary structure of the wild-type (P-67) and ΔF508 (P-66) synthetic peptides. The A Motif (Walker *et al.*, 1982) and the predicted β-strand sequence that is terminated by an acidic residue at each end, here called AEβ, are shaded. (B) Secondary structure of P-67 predicted by four independent algorithms. The secondary structure was predicted from the primary sequence by the methods of Novotny and Auffray (1984), N; Chandrasegaran and Smith (1990), C-S; Chou and Fasman (1978), C-F; and Garnier *et al.* (1978), G. Panel B was redrawn from *Science* (Thomas *et al.*, 1991) with permission [Copyright 1991 AAAS].

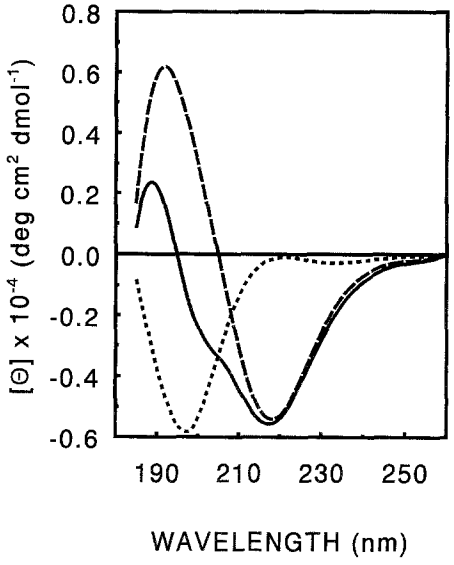

Fig. 3. β-Stranded structure of the synthetic peptides. CD spectra were collected as previously described (Thomas *et al.*, 1992a). The spectra for P-67 (– – –) and P-66 (——) are reprinted here with permission from the *J. Biol. Chem.* The difference spectrum (– – –) was derived by subtraction.

1991). The homology region nearest to the A consensus region is particularly interesting as it contains F508. Furthermore, this region contains the dicyclohexylcarbodiimide reactive acidic residues that are responsible for the inhibitory effects of this compound on the activity of the ATP synthase (Vignais and Lunardi, 1985) (Fig. 2A). Four different secondary structure prediction algorithms place this region in a β-strand (Thomas *et al.*, 1991) (Fig. 2B), and, thus, it has been designated here as the acidic-ended β-strand or AEβ region. Although these residues have been proposed to be essential to the catalytic mechanism of the ATP synthase, their role in CFTR is unknown.

Two peptides corresponding to the A and the AEβ region of CFTR and the ΔF508 mutant were chemically synthesized and purified for structural and functional characterization (Thomas *et al.*, 1991, 1992a). The sequence of the two peptides is shown in Fig. 2A and the predicted secondary structure for the wild-type peptide is shown in Fig. 2B. The wild-type peptide (P-67) consists of 67 amino acids extending from R450 to R516 in the CFTR sequence. The ΔF508 peptide (P-66) is identical except for the lack of a phenylalanine residue at position 508. Significantly, in agreement with the predicted secondary structure, the wild-type P-67 peptide assumed a β-stranded like conformation in solution according to circular dichroism (CD) spectropolarimetry (Thomas *et al.*, 1991) (Fig. 3). Although the CD spectrum of the mutant P-66 peptide also indicates some β-stranded structure, the appearance of a shoulder near 200 nm and the blue shift in the maximum indicates an increase in the random coil structure in the mutant. In fact, the difference spectrum between the mutant and the wild-type peptide approximates the shape of a random coil spectrum (Fig. 3). These results demonstrate that there is more random coil structure present in the mutant peptide sample, possibly due to a shift in a conformational equilibrium toward a less ordered form and/or to a disruption of the mutant native structure (Thomas *et al.*, 1992a).

In agreement with these results, a peptide fragment of CFTR overexpressed in bacteria containing the A and B consensus sequences also is in a β-stranded conformation (Hartman *et al.*, 1992). However, in this case no significant difference in the structure of the ΔF508 mutant peptide was observed. These seemingly conflicting results can be best explained by differences in the stability of the two peptide systems (see Peptide Stability–Protein Folding below). In

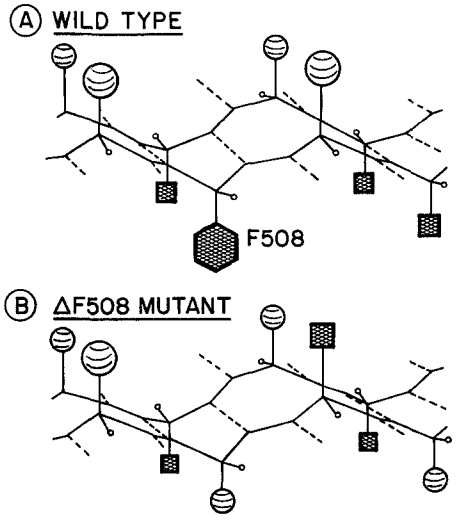

Fig. 4. Effect of a deletion mutation upon a β-sheet structure. One possible effect of the ΔF508 mutation on the β-sheet is illustrated; other possibilities are discussed in the text. (A) Schematic representation of a two stranded β-sheet, drawn after Rossman *et al.* (1975). Spheres and squares represent amino acid side chains. (B) When F508 (hexagon) is removed from the structure, in order to maintain a true β-sheet structure, amino acid side chains (square and sphere on right end of the near β-strand) must be reoriented from one face of the sheet to the other.

brief, whereas the chemically synthesized mutant peptide may be close to the transition between the native and denatured states under the conditions used, the bacterially expressed ΔF508 peptide was perhaps under conditions where the conformational equilibrium favors the native state.

Previously, it was pointed out that the ΔF508 mutation might be expected to have large effects on the structure and/or stability of CFTR because it may reside in a crucial β-strand (Thomas *et al.*, 1991, 1992a). This proposal is supported by genetic evidence that the F508C mutation is benign (Kobayashi *et al.*, 1990) and that the ΔI506/7 mutation causes CF (Kerem *et al.*, 1990). Examination of a model of a two-stranded β-sheet indicates why deletion mutations may be particularly disruptive in regions of repetitive secondary structure (Fig. 4). Removal of an amino acid residue can be accommodated in a β-sheet structure by the formation of a β-bulge which distorts the β-sheet, a register shift in which residues are reoriented from one face of the β-sheet to the other (Fig. 4B), or by a destablization of the region precluding formation of the native secondary structure under physiological conditions. Thus, conservative model building of this type predicts that the ΔF508 mutation would effect the β-sheet structure of the P-67 peptide (Thomas *et al.*, 1991), a prediction that has been experimentally verified (Fig. 3) (Thomas *et al.*, 1992a).

Other, more ambitious, attempts have been made in the past to build molecular models of the entire nucleotide-binding fold (Hyde *et al.*, 1990; Mimura *et al.*, 1991; Shyamala *et al.*, 1991). These models are based upon secondary structure predictions, known high-resolution crystal structures of related proteins, and the dubious assumption that CFTR folds in the same pattern as AdK, an assumption that does not hold true for Rossmann-fold proteins of known crystalline structure (Schultz, 1987; Story and Steitz, 1992). Neither of these hypothetical models place F508 within the Rossman fold. Until a high-resolution structure is solved, both molecular models should be viewed with extreme caution.

FUNCTIONAL ASPECTS

One function of the nucleotide-binding domain has been directly demonstrated with CFTR peptides (Thomas *et al.*, 1991, 1992a; Hartman *et al.*, 1992). Nucleotide binding to the synthetic peptides P-67 and P-66 is shown in Fig. 5. Measurement of the binding constants for TNP-ATP, TNP-ADP, and TNP-AMP were determined from the fluorescence enhancement observed when these analogues interact with the peptide. The respective K_ds for the nucleotides for P-67 and P-66 were 410 and 490 nM for TNP-ATP, 320 and 580 nM for TNP-ADP, and 1260 and 830 nM for TNP-AMP. The apparent K_ds for ATP corrected for competition with TNP-ATP were 590 μM for P-67 and 830 μM for the P-66 mutant peptide. All of the dissociation constants were within approximately one standard deviation for the two peptides, indicating that under the conditions employed the ΔF508 mutation did not effect nucleotide binding, and thus, F508 is not directly involved in the nucleotide-binding function (Thomas *et al.*, 1992a). However, a small but reproducible reduction in the fluorescence maximum of the mutant peptide may reflect a shift in the conformational equilibrium to a less ordered nonfunctional form (Fig. 5).

These affinities are in good agreement with the half-maximal ATP concentration required for *c*AMP-activated chloride conductance (Anderson *et al.*, 1991c). Experiments with the N-terminal nucleotide-binding peptide overexpressed in bacteria and nucleotide affinity probes also show that the ΔF508 mutant

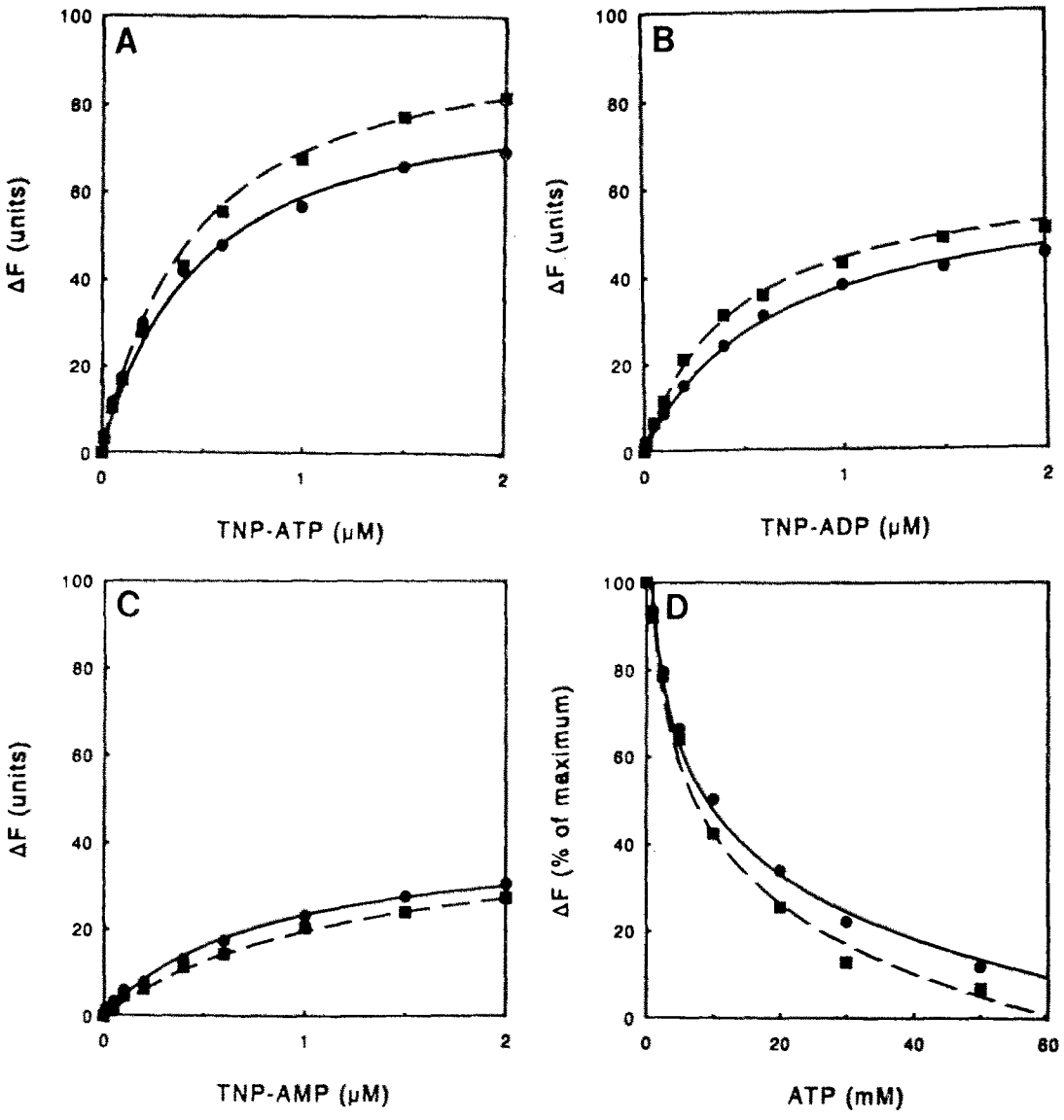

Fig. 5. Nucleotide-binding function of P-67 and P-66. Adenine nucleotide binding as reflected in the enhancement by 5 μM P-67 (- - - -) or P-66 (——) of (A) TNP-ATP, (B) TNP-ADP, and (C) TNP-AMP fluorescence in 50 mM Tris-Cl, pH 7.4. Fluorescence enhancement was measured by exciting at 410 nm and measuring the emission at 540 nm (Thomas *et al.*, 1992a). (D) ATP competition with 5 μM TNP-ATP for the binding site on P-67 (dashed line) or P-66 (solid line). Panels A and D were reprinted with permission from *J. Biol. Chem* (Thomas *et al.*, 1992a).

interacts with nucleotides in a manner similar to the wild-type, although in these studies the reported affinity for ATP is reduced (Hartman *et al.*, 1992). This preparation has also been reported to form anion channels when reconstituted into planar lipid bilayers (Arispe *et al.*, 1992). Thus, although it is clear that the region is capable of binding adenine nucleotides, this domain of CFTR may have other functions as well. However, it is not clear if these activities are mutually exclusive. Unfortunately, nucleotide-binding experiments were not reported for the full-length CFTR recently purified from expressing Sf9 cells (Bear *et al.*, 1992a). Hydrolysis was not detected in either the wild-type or mutant synthetic peptides (Thomas *et al.*, 1991, 1992a) and was not reported for the bacterially expressed domain (Hartman *et al.*, 1992; Arispe *et al.*, 1992) or the purified CFTR from Sf9 cells (Bear *et al.*, 1992a).

Examination of other CF-causing mutations indicate that some are not only in regions of homology but are changes of conserved residues. These mutations may be altering the function of CFTR in the absence of large conformational changes since these residues, unlike F508, may be directly interacting with the ligand. For example, the G458V (Cuppens *et al.*, 1990) and G1244E (Devoto *et al.*, 1991) mutations which change the first glycine residue in the Gx_4GKT sequence of the A homology region and the R560T (Kerem *et al.*, 1990) mutation in the $Rx_{6\text{-}8}h_4D$ sequence of the B homology region are known to cause CF. Moreover, if the importance of the AEβ region proposed here holds true, along with the known deletion mutations, it might be predicted that mutation of the conserved acidic residues E504, D513, and E514 (Thomas *et al.*, 1991) may cause CF as well.

PEPTIDE STABILITY–PROTEIN FOLDING

The most dramatic altertion of any characteristic of the CFTR polypeptide by the ΔF508 mutation directly observed is the reduction in the stability of the P-66 peptide when contrasted with the P-67 peptide (Fig. 6) (Thomas *et al.*, 1992a,c). When the denaturant urea was used to unfold the peptides, the mutant peptide reached the midpoint of the transition between the folded native and unfolded denatured states at 2.7 M (Fig. 6A). Significantly, the wild-type peptide required nearly a 30% increase in the urea concentration to reach the same point. The difference in stability of the two peptides may account for the observation that P-66 precipitates from solution at high concentration as the pH approaches 7 and the wild-type peptide P-67 does not (Thomas *et al.*, 1992a). The decreased resistance to denaturation was also reflected in the nucleotide-binding function of the ΔF508 peptide (Fig.6B). At 2.5 M urea, P-66 retained only one-third of its ability to bind TNP-ATP, while the wild-type P-67 peptide retained one-half of its binding capacity. At 4 M urea P-66 no longer bound the nucleotide, while P-67 retained a small but significant binding capacity. This reduced structural stability, and consequently function, may indicate that defective protein folding is the basis of most cases of CF.

When considered in the context of protein folding, several disparate experimental observations can be readily understood. First, defective protein folding due to the ΔF508 mutation could certainly be the basis of the defective trafficking of this mutant observed in several heterologous expression systems (Cheng *et al.*, 1990; Gregory *et al.*, 1991). Second, altered CFTR stability can explain the observations that ΔF508 CFTR is not functional when expressed in some cell types (Rich *et al.*, 1990), has reduced but wild type-like functional activity in other cell types (Dahlmanns *et al.*, 1991; Drumm *et al.*, 1991), and is wild-type both quantitatively and qualitatively in other cells (Bear *et al.*, 1992b). Wild-type activity and levels of CFTR are observed when Sf9 cells express the ΔF508 mutant (Bear *et al.*, 1992b). It is important to note that these cells are grown at 27°C and low temperature is one

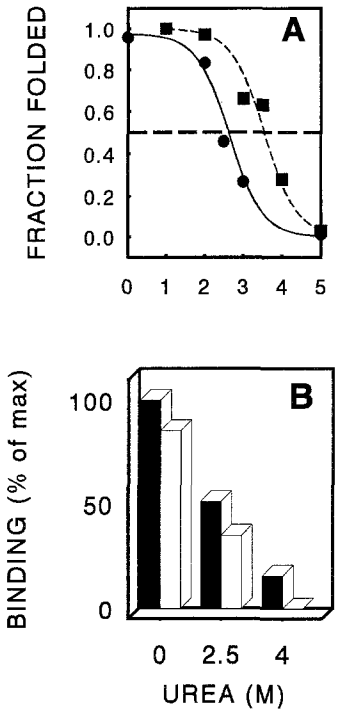

Fig. 6. Stability of the peptides. Peptide folding was assessed as the stability of the mutant and wild-type peptides in the denaturant urea. (A) Stability curves for P-67 (– – –) and P-66 (——) were derived by determining the secondary structure of the peptides in the CD spectropolarimeter as a function of denaturant concentration. The amount of β-stranded structure present in each peptide was determined at several different concentrations of urea by monitoring the mean residue molar ellipticity at 218 nm as previously described (Thomas *et al.*, 1992a). An increase in the ellipticity at this wavelength toward a less negative value indicates a transition from a β-stranded structure to a random coil. The fraction folded was taken to be one under nondenaturing conditions and zero where no further change was observed at high urea concentrations. (B) Stability was also determined by measuring the relative binding function of the peptides in urea. Nucleotide binding of P-67 (closed bars) and P-66 (open bars) was determined as previously described (see Fig. 5). The fluorescence maximum under each condition was calculated as a percentage of the P-67 maximum in the absence of urea.

obvious way to stabilize a protein. The cell types that express ΔF508 CFTR at reduced or nondetectable levels are all grown at higher temperature. In these cases the differences between functional expression and lack of activity are small and may be explained by slight differences in the intracellular milieu in these cells, some being more stabilizing than others. Finally, defective protein folding may account for the differences in the CD spectra for the synthetic and bacterially expressed peptides, as discussed above.

PERSPECTIVES AND CONCLUSIONS

In this review we have attempted to consider the structure, function, and maturation of CFTR within the context of the biochemical and biophysical data from studies of peptides corresponding to the N-terminal nucleotide-binding domain. The work of several laboratories indicates that CFTR binds nucleotides, is responsible for *c*AMP-dependent chloride conductance, and may have other functions and solutes not presently known. For example, in the case of CFTR, whereas the chloride conductance is mediated by a channel-like activity, it is not clear why ATP hydrolisis would be required. However, such dependence upon ATP hydrolysis has been demonstrated (Anderson *et al.*, 1991c). Furthermore, the familial resemblance to other traffic ATPases, which by definition require ATP hydrolysis, is strong, perhaps arguing for active transport of a second solute. Other possibilities are that the nucleotide-binding domain and/or the AEβ region are directly involved in the maturation of CFTR.

The peptide results discussed here in detail provide clear evidence that the ΔF508 mutant is a protein-folding mutant. Thus, if under intracellular conditions the ΔF508 mutant is near the transition from denatured to native (Fig. 7A), simple equilibrium arguments can explain the altered maturation of mutant CFTR observed in different cell types (Rich *et al.*, 1990; Cheng *et al.*, 1990; Drumm *et al.*, 1991; Dahlmanns *et al.*, 1991; Bear *et al.*, 1992b). However, it should also be considered that during the process of CFTR maturation the protein most likely passes through several intermediate states. These states share characteristics of both the native and denatured states, such as considerable secondary structure and exposed hydrophobic surfaces. Hydrophobic interactions between the intermediates could lead to aggregation, thereby kinetically trapping the immature protein in

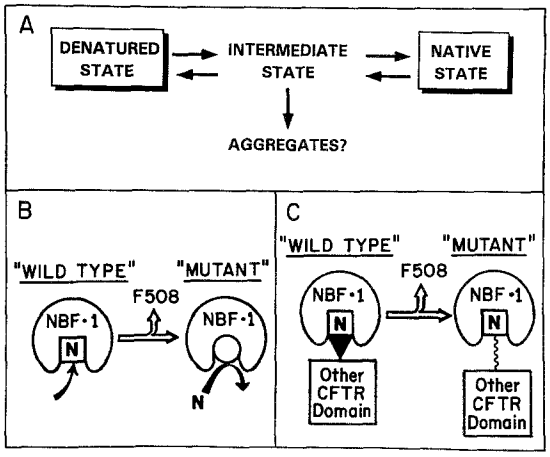

Fig. 7. Perspectives on the effects of the ΔF508 mutation on CFTR maturation and function. (A) Defective folding of CFTR leading to protein aggregation and/or proteolysis. It is clear from peptide studies that the ΔF508 mutation destabilizes this region of CFTR. The simplest interpretation of this finding is that the molecular basis of CF is an inability of the mutation protein to reach its final native state. The mutant folding pathway may involve intermediates that are particularly susceptible to aggregation and/or proteolysis, thereby accounting for the reduced levels of ΔF508 CFTR observed in the membranes of many expression systems. (B) Alteration of nucleotide interactions with CFTR. Although under conditions that favor the native state, no difference is observed in the nucleotide-binding ability of the ΔF508 mutant peptide (Thomas *et al.*, 1992a; Hartman *et al.*, 1992), the possibility remains that the nucleotide is not bound in a functional manner; for instance, the putative hydrolytic activity may be defective, accounting for qualitative differences observed in ΔF508 CFTR activity (Dahlmanns *et al.*, 1991; Drumm *et al.*, 1991). It is also possible that the binding domain itself plays some role in the proper maturation of CFTR. (C) Alteration of interdomain interactions in CFTR. Once again, these interactions may play a role in CFTR maturation and function. It is important to note that domain destabilization could compromise any of these processes.

an off pathway form or increasing its sensitivity to intracellular proteolysis. Typically, chaperonins prevent these interactions, allowing the nascent protein to proceed to the functional native state (Flynn *et al.*, 1991). If altered stability of a folding intermediate is indeed involved, treatments that reduce aggregate formation and limit proteolysis may prove useful.

Regardless of whether the altered protein stability is an equilibrium or a kinetic problem *in situ*, knowledge of the structural basis of this defect is crucial to understanding the development of the ΔF508 form of CF. In this regard, identification of second-site mutations which alleviate the CF phenotype of ΔF508 mutants may be particularly instructive. It is also possible that the defective folding of CFTR is not a global process but rather is limited to the local

N-terminal nucleotide-binding domain. Thus, ΔF508-altered stability of the domain may compromise the ability of CFTR to interact with nucleotide in a productive manner (Figs. 6, 7B) or may alter the ability of the nucleotide-binding domain to interact functionally with other domains of CFTR (Fig. 7C).

When considering the molecular basis of human genetic disorders, there are three general classes of mutations: mutation of functional residues, mutation of crucial structural residues that indirectly effect function, and mutation of structural residues that effect ability of the protein to reach the functional native state. Here, we have reviewed data and presented arguments that CF caused by the ΔF508 mutation is an example of the third class of mutation that alters the ability of the protein to reach its final functional structure in the membrane. Furthermore, along with evidence that the mutant protein retains wild type-like function when it reaches the membrane (Dahlmanns *et al.*, 1991; Drumm *et al.*, 1991; Bear *et al.*, 1992b), this finding indicates that conditions and compounds that stabilize ΔF508 CFTR may alter the pathological course of the disease.

ACKNOWLEDGMENTS

This work was supported by National Institutes of Health grant DK43962 to P.L.P. We would like to thank Dr. P. Shenbagamurthi for the peptide synthesis, J. Hullihen for technical help, and Drs. Y. Ko and J. Sondek for helpful comments.

REFERENCES

Anderson, M. P., Rich, D. P., Gregory, R. J., Smith, A. E., and Welsh, M. J. (1991a). *Science* **251**, 679–682.

Anderson, M. P., Gregory, R. J., Thompson, S., Souza, D. W., Paul, S., Mulligan, R. C., Smith, A. E., and Welsh, M. J. (1991b). *Science* **253**, 202–205.

Anderson, M. P., Berger, H. A., Rich, D. P., Gregory, R. J., Smith, A. E., and Welsh, M. J. (1991c). *Cell* **67**, 775–784.

Arispe, N., Rojas, E., Hartman, J., Sorscher, E. J., and Pollard, H. B. (1992). *Proc. Natl. Acad. Sci. USA* **89**, 1539–1543.

Bear, C. E., Li, C., Kartner, N., Bridges, R. J., Jensen, T. J., Ramjeesingh, M., and Riordan, J. R. (1992a). *Cell* **68**, 809–818.

Bear, C. E., Jensen, T. J., and Riordan, J. R. (1992b). *Biophys. J.* **61**, A127.

Chandrasegaran, S., and Smith, H. O. (1990). In *Second Annual New England Biolabs Workshop on Biological DNA Modifications*, New England Biolabs, Berlin, p. 106.

Cheng, S. H., Gregory, R. J., Marshall, J., Paul, S., Souza, D. W., White, G.A., O'Riordan, C., and Smith, A. E. (1990). *Cell* **63**, 827–834.

Cheng, S. H., Rich, D. P., Marshall, J., Gregory, R. J., Welsh, M. J., and Smith, A. E. (1991). *Cell* **66**, 1027–1036.

Chou, Y., and Fasman, G. D. (1978). *Adv. Enzymol.* **47**, 45–148.

Cuppens, H., Marynen, P., De Broeck, C., and Cassiman, J. J. (1990). *Pediatr. Pulmonol. Suppl* **5**, 203.

Cutting, G. R., Kasch, L. M., Rosenstein, B. J., Zielenski, J., Tsui, L.-C., Antonarakis, S. E., and Kazazian, H. H. (1990). *Nature (London)* **346**, 366–369.

Dahlmanns, W., Barbry, P., Champigny, G., Jallat, S., Dott, K., Dreyer, D., Crystal, R. G., Pavirani, A., Lecocq, J.-P., and Lazdunski, M. (1991). *Nature (London)* **354**, 526–528.

Devoto, M., Ronchetto, P., Fanen, P., Telleria Orriols, J. J., Romeo, G., Gossens, M., Ferrari, M., Magnani, C., Seia, M., and Cremonesi, L. (1991). *Am. J. Hum. Genet.* **48**, 1127–1132.

Drumm, M. L., Pope, H. A., Cliff, W. H., Rommens, J. M., Marvin, S. A., Tsui, L.-C., Collins, F. S. Frizzell, R. A., and Wilson, J. M. (1990). *Cell* **62**, 1227–1233.

Drumm, M. L. Wilkinson, D. J., Smit, L. S., Worrell, R. T., Strong, T. V., Frizzell, R. A., Dawson, D. C., and Collins, F. S. (1991). *Science* **254**, 1797–1799.

Flynn, G. C., Pohl, J., Flocco, M. T., and Rothman, J. E. (1991). *Nature (London)* **353**, 726–730.

Garnier, J., Osguthorpe, D. J., and Robson, B. (1978). *J. Mol. Biol.* **120**, 97–120.

Gregory, R. J., Rich, D. P., Cheng, S. H., Souza, D. W., Paul, S., Manavalan, P., Anderson, M. P., Welsh, M. J., and Smith, A. E. (1991). *Mol. Cell. Biol.* **11**, 3886–3893.

Hartman, J., Huang, Z., Rado, T. A., Peng, S., Jiling, T., Muccio, D. D., and Sorscher, E. J. (1992). *J. Biol. Chem.* **267**, 6455–6458.

Hwang, T.-C., Lu, L., Zeitlin, P. L., Gruenert, D. C., Huganir, R., and Guggino, W. B. (1989). *Science* **244**, 1351–1353.

Hyde, S. C., Emsley, P., Hartshorn, M., Mimmack, M. M., Gileadi, U., Pearce, S. R., Gallagher, M. P., Gill, D. R., Hubbard, R. E., and Higgins, C. F. (1990). *Nature (London)* **346**, 362–365.

Jurnak, F. (1985) *Science* **230**, 32–36.

Kartner, N., Hanrahan, J. W., Jensen, T. J., Naismith, A. L., Sun, S., Ackerley, C. A., Reyes, E. F., Tsui, L.-C., Rommens, J. M., Bear, C. E., and Riordan, J. R. (1991) *Cell* **64**, 681–691.

Kerem, B.-S., Zielenski, J., Markiewicz, D., Bozon, D., Gazit, E., Yahav, J., Kennedy, D., Riordan, J. R., Collins, F. S., Rommens, J. M., and Tsui, L.-C. (1990). *Proc. Natl. Acad. Sci. USA* **87**, 8447–8451.

Kobayashi, K., Knowles, M. R., Boucher, R. C., O'Brien, W. E., and Beaudet, A. L. (1990). *Am. J. Hum. Genet.* **47**, 611–615.

La Cour, T. F. M., Nyborg, J., Thirup, S., and Clark, B. F. C. (1985). *EMBO J.* **4**, 2385–2388.

Li, M., McCann, J. D., Liedtke, C. M., Nairn, A. C., Greengard, P., and Welsh, M. J. (1988). *Nature (London)* **331**, 358–360.

Mimura, C. S., Holbrook, S. R., and Ames, G. F.-L. (1991). *Proc. Natl. Acad. Sci. USA* **88**, 84–88.

Novotny, J., and Auffray, C. (1984). *Nucleic Acids Res.* **12**, 243–255.

Rich, D. P., Andersen, M. P., Gregory, R. J., Cheng, S. H., Paul, S., Jefferson, D. M., McCann, J. D., Klinger, K. W., Smith, A. E., and Welsh, M. J. (1990). *Nature (London)* **347**, 358–363.

Riordan, J. R., Rommens, J. M., Kerm, B.-S., Alon, N., Rozmahel, R., Grzelczak, Z., Zielenski, J., Lok, S., Plavsic, N., Chou, J.-L., Drumm, M. L., Iannuzzi, M. C., Collins, F. S., and Tsui, L.-C. (1989). *Science* **245**, 1066–1073.

Rossmann, M. G., Lijas, A., Brädén, C. I., and Banaszak, L. J. (1975). In *The Enzymes* (Boyer, P. D., ed.), Vol.11, 3rd ed., Academic Press, New York, pp. 61–102.

Schoumacher, R. A., Shoemaker, R. L., Halm, D. R., Tallant, E. A., Wallace, R. W., and Frizzell, R. A. (1987). *Nature (London)* **330**, 752–754.

Schultz, G. (1987). *Cold Spring Harbor Symp. Quant. Biol.* **LII**, 429–439.

Shyamala, V., Biachwal, V., Beall, E., and Ames, G. F.-L. (1991). *J. Biol. Chem.* **266**, 18714–18719.

Story, R. M., and Steitz, T. A. (1992). *Nature (London)* **355**, 374–376.

Thomas, P. J., Shenbagamurthi, P., Ysern, X., and Pedersen, P. L. (1991). *Science* **251**, 555–557.

Thomas, P. J., Shenbagamurthi, P., Sondek, J., Hullihen, J., and Pedersen, P. L. (1992a). *J. Biol. Chem.* **267**, 5727–5730.

Thomas P. J., Garboczi, D. N., and Pedersen, P. L. (1992b) *Acta Physiol. Scand.* **146**, 23–29.

Thomas, P. J., Sondek, J., Ko, Y. H., and Pedersen, P. L. (1992c). *Biophys. J.* **61**, A62.

Tong, L., deVos, A. M., Milburn, M. V., and Kim, S.-H. (1991). *J. Mol. Biol.* **221**, 751–754.

Vignais, P., and Lunardi, J. (1985). *Ann. Rev. Biochem.* **54**, 977–1014.

Walker, J. E., Saraste, M., Runswick, M. J., and Gay, N. J. (1982). *EMBO J.* **1**, 945–951.