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Cystic Fibrosis Transmembrane Conductance Regulator: Temperature-Dependent Cysteine Reactivity Suggests Different Stable Conformers of the Conduction Pathway

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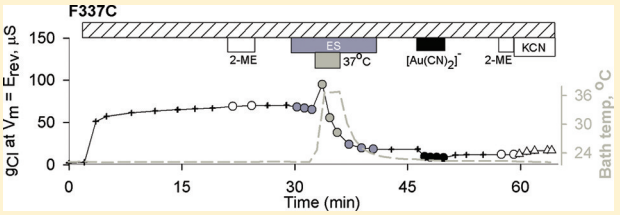
ABSTRACT: Cysteine scanning has been widely used to identify pore-lining residues in mammalian ion channels, including the cystic fibrosis transmembrane conductance regulator (CFTR). These studies, however, have been typically conducted at room temperature rather than human body temperature. Reports of substantial effects of temperature on gating and anion conduction in CFTR channels as well as an unexpected pattern of cysteine reactivity in the sixth transmembrane segment (TM6) prompted us to investigate the effect of temperature on the reactivity of cysteines engineered into TM6 of CFTR. We compared reaction rates at temperatures ranging from 22 to 37 °C for cysteines placed on either side of an apparent size-selective accessibility barrier previously defined by comparing reactivity toward channel-permeant and channel-impermeant, thiol-directed reagents. The results indicate that the reactivity of cysteines at three positions extracellular to the position of the accessibility barrier, 334, 336, and 337, is highly temperature-dependent. At 37 °C, cysteines at these positions were highly reactive toward $MTSES^-$, whereas at 22 °C, the reaction rates were 2−6-fold slower to undetectable. An activation energy of 157 kJ/mol for the reaction at position 337 is consistent with the hypothesis that, at physiological temperature, the extracellular portion of the CFTR pore can adopt conformations that differ significantly from those that can be accessed at room temperature. However, the position of the accessibility barrier defined empirically by applying channel-permeant and channel-impermeant reagents to the extracellular aspect of the pore is not altered. The results illuminate previous scanning results and indicate that the assay temperature is a critical variable in studies designed to use chemical modification to test structural models for the CFTR anion conduction pathway.



Cysteine scanning using both channel-permeant and channel-impermeant, thiol-directed reagents has been employed by several laboratories in investigating the structure of the anion conduction pathway of the CFTR chloride channel.[1−9] A comparison of the reactivity of cysteines engineered into TM6 toward channel-permeant probes {$[Au(CN)_2]^-$ and $[Ag(CN)_2]^-$} and channel-impermeant probes ($MTSET^+$ and $MTSES^-$), assayed at room temperature, suggested that pore geometry imposed a size-selective accessibility cutoff that prevented reaction of channel-impermeant reagents with engineered cysteines that were to the cytoplasmic side of position 338.[10] One exception to the pattern, however, was position 337. When reagents were applied from the extracellular side, a cysteine substituted at position 337 reacted readily with channel-permeant probes, but in our hands, reaction with channel-impermeant probes ($MTSET^+$ and $MTSES^-$) was not detectable. A second concern was that previous studies of cysteine reactions at position 337, also assayed at room temperature, had produced contradictory results. Cheung and Akabas[1,2] reported reactivity of F337C CFTR toward $MTSEA^+$, $MTSET^+$, and $MTSES^-$. Fatehi et al.[5] reported reactivity of F337C CFTR toward $MTSES^-$ but not $MTSET^+$, while Beck et al.[6] reported no reactivity toward $MTSEA^+$ at this locus.

Cysteine scanning studies are routinely conducted at room temperature, rather than at 37 °C, the temperature at which human CFTR would normally function. To probe for possible temperature-induced changes in the accessibility of different protein conformations, we investigated the temperature dependence of cysteine reactivity in TM6. The results indicate that reactivity toward a channel-impermeant reagent, $MTSES^-$, for at least three positions, 334, 336, and 337, is highly temperature-dependent. In particular, the reactivity of a cysteine substituted at position 337 was readily detectable at temperatures above 27 °C, whereas modification by extracellular $MTSES^-$ could not be detected at 22 °C. The apparent activation energy for the reaction exceeded that expected for diffusion or thiol−disulfide exchange in solution as well as CFTR gating, suggesting that the change from low to physiological temperatures is associated with a change in conformation of the outer vestibule of the pore. The position of the size-selective accessibility cutoff for externally applied thiol-reactive probes, however, was unaffected by temperature.

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MATERIALS AND METHODS

Mutagenesis and in Vitro Transcription. The methods used for mutagenesis and in vitro transcription were similar to those reported previously.[3,11,12] Briefly, CFTR mutants were generated using site-directed mutagenesis polymerase chain reaction. The Ambion mMessage mMachine T7 Ultra transcription kit (Ambion) was used to generate the CFTR cRNAs for *Xenopus* oocyte injection. The sequences in the region of the mutation were confirmed by direct DNA sequencing.

Preparation and Microinjection of Oocytes. Preparation and microinjection of *Xenopus laevis* oocytes were conducted as previously described in detail.[3,11] The follicular membranes were removed by mechanical agitation (1−2 h) in a Ca^{2+}-free solution containing 82.5 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, 5 mM HEPES (pH 7.5), and 0.2 Wünsch units/mL Liberase Blendzyme 3 (Roche Molecular Biochemicals, Indianapolis, IN). Defolliculated oocytes were washed and maintained in a modified Barth's solution containing 88 mM NaCl, 1 mM KCl, 0.82 mM $MgSO_4$, 0.33 mM $Ca(NO_3)_2$, 0.41 mM $CaCl_2$, 2.4 mM $NaHCO_3$, 10 mM HEPES (hemi-Na), and 250 mg/L Amikacin with 150 mg/L Gentamicin (pH 7.5). Stage V−VI oocytes were treated with 50 nL of CFTR cRNA and cRNA encoding the human β_2-adrenergic receptor per oocyte. The CFTR RNA concentration was adjusted so that the maximum steady state stimulated conductance is less than 200 μS (~12.5 ng/oocyte).

Whole Cell Recordings. Individual oocytes were placed in a 200 μL recording chamber (RC-1Z, Warner) and continuously perfused with Frog Ringer's solution (4 mL/min). The Ringer's solution contained 98 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, 1.8 mM $CaCl_2$, and 5 mM HEPES (hemi-Na) (pH 7.4). CFTR channels were activated using 10 μM isoproterenol (a β-adrenergic agonist) and 1 mM IBMX (a phosphodiesterase inhibitor) as the stimulating cocktail (Isop+IBMX). The Oocyte 725 amplifier (Warner) and the pClamp 8 data acquisition program (Axon Instruments, Inc.) were used for whole cell recordings. Oocytes were maintained in the open circuit condition, and the membrane potential was periodically ramped from −120 to 60 mV over 1.8 s to construct the whole cell $I-V$ plots.

Temperature Control. A Dual Automatic Temperature Controller (CL-200) and an in-line solution heater/cooler (SC-20) (Warner Instruments, Hamden, CT) were used to apply acute temperature changes under a constant flow. The bath temperature was monitored. The temperature signal was digitized and recorded using a USB Data Acquisition Device (DI-158, DATAQ Instruments, Inc., Akron, OH).

Determination of the Apparent Activation Energy. The activation energy was determined using the Arrhenius equation. The equation was rearranged so that the log of the rate constant (k) is a function of the inverse of the temperature (T) (eq 1)

$$\ln(k) = \frac{-E_a}{R}\frac{1}{T} + \ln(A) \quad (1)$$

where A is the pre-exponential factor, R is the gas constant, and E_a is the activation energy determined from the slope of the $\ln(k)$ versus $1/T$ plot. We refer to E_a as the apparent activation energy that may be determined by the nature of the thiol−probe reaction, diffusional access of thiol-directed reagents to the cysteine thiolate, and protein conformational change.

Reagents. Isobutylmethyl xanthine (IBMX), isoproterenol (Isop), 2-mercaptoethanol (2-ME), diethyl dithiocarbamate (DDTC), dithioerythritol (DTE), and $KAu(CN)_2$ were purchased from Sigma (St. Louis, MO). Sodium (2-sulfonatoethyl)methanethiosulfonate ($MTSES^-$) was purchased from Toronto Research Chemicals (Toronto, ON). Potassium cyanide (KCN) was obtained from Fisher Chemicals (Fairlawn, NJ). R. Bridges (Rosalind Franklin University, Chicago, IL) and the Cystic Fibrosis Foundation (CFF) kindly provided $CFTR_{inh}$-172 {4-[4-oxo-2-thioxo-3-(3-trifluoromethylphenyl)thiazolidin-5-ylidenemethyl]benzoic acid, termed CF172 here}.

RESULTS

The representative experiments compiled in Figure 1 illustrate the dramatic effect of increased temperature on the rate of reaction of $MTSES^-$ with F337C CFTR. $MTSES^-$ was chosen for this comparison for two reasons. First, the thiol−disulfide

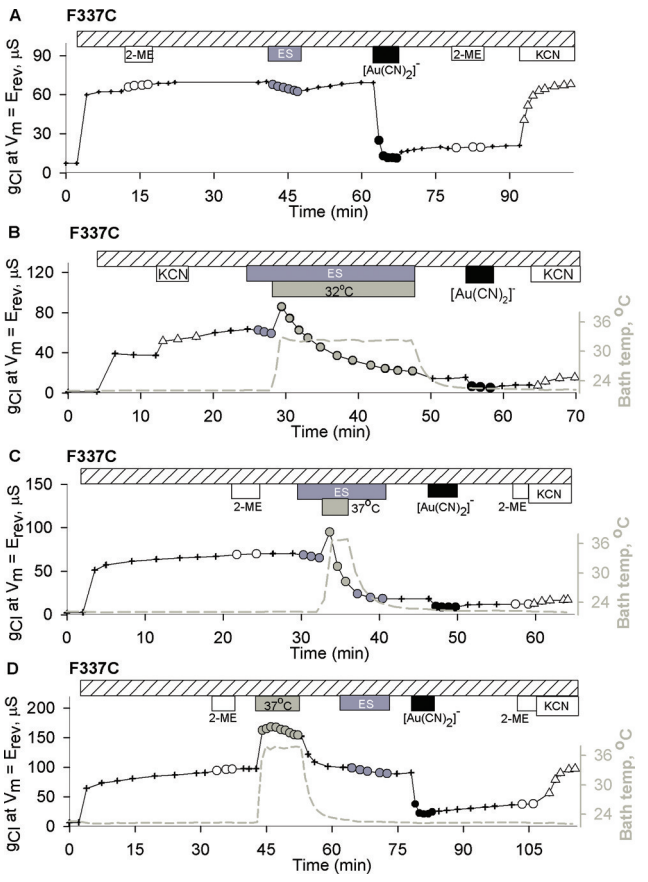

Figure 1. Increased temperature dramatically increased the reactivity of F337C CFTR toward $MTSES^-$. Oocytes expressing F337C CFTR were activated using a stimulatory cocktail (10 μM Isop and 1 mM IBMX, hatched bar and cross hairs) and then exposed to 1 mM 2-ME to reverse any spurious reactions of the substituted cysteine.[11] (A) Exposure to 1 mM $MTSES^-$ (dark gray bar and circles) produced no reaction, but 1 mM $[Au(CN)_2]^-$ (black bar and circles) produced profound inhibition that was not reversed by 1 mM 2-ME but was reversed by 1 mM KCN (white bar and triangles). (B) Increasing the temperature to 32 °C in the presence of 1 mM $MTSES^-$ produced a modest reaction rate. (C) Increasing the temperature to 37 °C in the presence of 1 mM $MTSES^-$ produced a rapid reaction. (D) After the oocyte had been heated to 37 °C and cooled to 22 °C, exposure to 1 mM $MTSES^-$ produced no evidence of a reaction.

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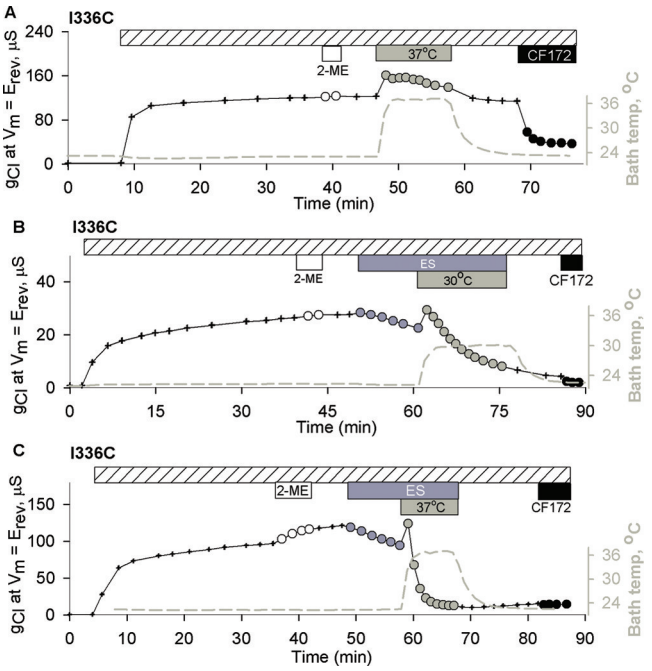

Figure 2. Increased temperature altered the rate of modification of I336C CFTR by $MTSES^-$. Oocytes expressing I336C CFTR were activated using a stimulatory cocktail. (A) Increased temperature reversibly increased CFTR conductance (gray bar and circles). After the oocyte had been cooled to 22 °C, it was exposed to 10 μM CF172, a CFTR inhibitor.[32] (B) Increasing the superfusate temperature to 30 °C in the presence of 1 mM $MTSES^-$ resulted in an increased rate of reaction. After the ooctye had been cooled to 22 °C, it was exposed to 10 μM CF172. (C) Increasing the superfusate temperature to 37 °C in the presence of 1 mM $MTSES^-$ further increased the rate of reaction with $MTSES^-$. After the oocyte had been cooled to 22 °C, it was exposed to 10 μM CF172.

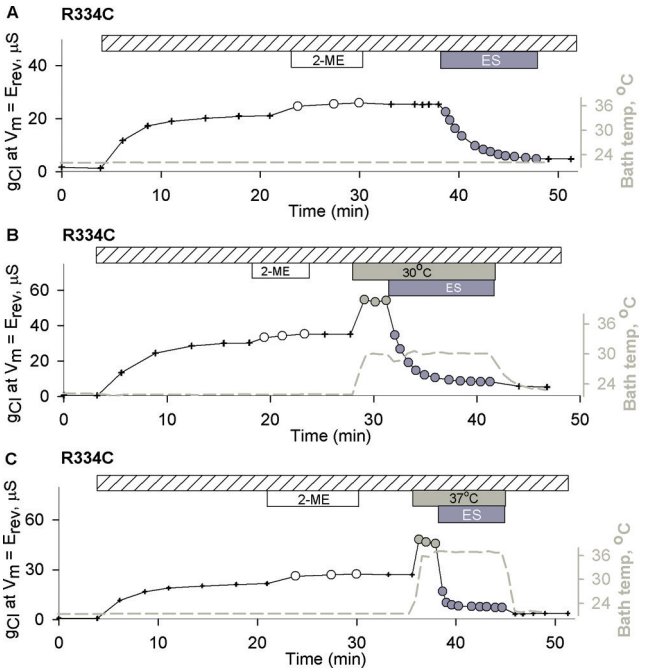

Figure 3. Increased temperature altered the rate of modification of R334C CFTR by $MTSES^-$. An oocyte expressing R334C CFTR was activated using a stimulatory cocktail and then (A) exposed to 1 mM 2-ME (white bar and circles) and 3 μM $MTSES^-$ (dark gray bar and circles) at room temperature. (B) Exposure to 3 μM $MTSES^-$ at 30 °C (gray bar and circles) resulted in an increased rate of reaction. (C) Exposure to 3 μM $MTSES^-$ at 37 °C (gray bar and circles) further increased the rate of reaction.

exchange reaction deposits a negatively charged substituent in the pore that is expected to profoundly inhibit anion conduction,[4,10] rendering the progress of the reaction readily detectable. Second, the anionic character of $MTSES^-$ makes it most comparable to the channel-permeant probe, $[Au(CN)_2]^-$. In a previous study, we reported that whereas the channel-permeant probe, $[Au(CN)_2]^-$, reacted with a cysteine substituted at position 337, the channel-impermeant probe, $MTSES^-$, did not. In many such experiments conducted at room temperature, no change in conductance was detectable upon exposure of an oocyte expressing F337C CFTR to 1 mM $MTSES^-$, even for periods exceeding 10 min. Repeated attempts to detect a reaction at room temperature sometimes gave rise to results like that depicted in Figure 1A, however. In this instance, exposure of the oocyte expressing F337C CFTR to $MTSES^-$ at room temperature evoked what at first appeared to be a very slow rate of reaction, but the decline spontaneously reversed upon removal of the reagent from the superfusate. Subsequent exposure of the oocyte to the channel-permeant probe, $[Au(CN)_2]^-$, produced a profound inhibition as previously reported,[10] confirming that the cysteine thiolate remained unreacted. The inhibition following the reaction of $[Au(CN)_2]^-$ was not reversed by exposure to a competing thiol, 2-ME, but was readily reversed by exposure to the high-affinity metal ligand, CN^-, as expected from previous studies.[10,12] Figure 1B depicts an experiment in which an oocyte expressing F337C CFTR was exposed to 1 mM $MTSES^-$ at 22 °C, and the superfusate temperature was then increased to 32 °C in the presence of the mixed disulfide. Increasing the superfusate temperature in the presence of 1 mM extracellular $MTSES^-$ resulted in a 64% ($\pm$1; n = 3) decrease in conductance. Addition of 1 mM $[Au(CN)_2]^-$ afterward produced a small irreversible decrease in conductance that was reversed by exposure to 1 mM KCN, suggesting that even after a 20 min exposure, a small fraction of the cysteine thiols was not modified by $MTSES^-$ at 32 °C. Similar results were seen at 37 °C (Figure 1C), except that increasing the bath temperature to 37 °C in the presence of the mixed disulfide resulted in a more rapid reduction in conductance, indicative of a robust reaction of the mixed disulfide probe with the substituted cysteine at the elevated temperature. Figure 1D depicts an experiment designed to test the reversibility of the temperature-induced changes in the reactivity of a cysteine at position 337. An oocyte expressing F337C CFTR was heated to 37 °C for 10 min and then cooled to 22 °C. The temperature excursion evoked an increase in conductance that was sustained during the period of elevated temperature but was reversed rapidly when the temperature of the superfusate was returned to 22 °C. This behavior is identical to that reported previously for wild-type (wt) CFTR channels[13,14] and reflects increases in open probability and single-channel conductance evoked by the elevated temperature. Subsequent exposure to $MTSES^-$ at 22 °C produced no evidence of a reaction, but block by 1 mM $[Au(CN)_2]^-$ confirmed the presence of the reactive thiolate. This result indicates that, although reaction of $MTSES^-$ with F337C CFTR was readily detectable at 37 °C, exposure to the elevated temperature did not result in any irreversible change in the channel.

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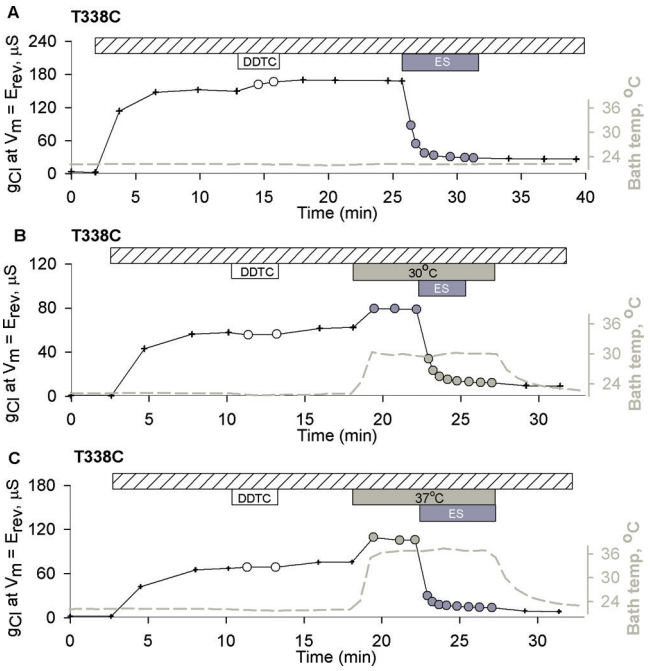

Figure 4. Increased temperature increased the rate of reaction of T338C CFTR with $MTSES^-$. An oocyte expressing T338C CFTR was activated using a stimulatory cocktail and then (A) exposed to 100 μM DDTC to reverse spurious thiol reactions (white bar and circles). Exposure to 5 μM $MTSES^-$ (dark gray bar and circles) profoundly inhibited the T338C CFTR conductance. (B) The bath temperature was increased to 30 °C (gray bar and circles), and the oocyte was exposed to 5 μM $MTSES^-$ at the elevated temperature. (C) The bath temperature was increased to 37 °C (gray bar and circles), and the oocyte was exposed to 5 μM $MTSES^-$ at the elevated temperature. Oocytes were pretreated with 1 mM DTE (>24 h) to reverse any reaction with contaminating trace metals.[11]

Figures 2–4 contain the results of similar experiments conducted with oocytes expressing R334C, I336C, and T338C CFTR channels. In the case of R334 and I336, exposure to $MTSES^-$ (3 μM and 1 mM, respectively) produced reactions at 22 °C that were slow but, unlike that of F337C CFTR, readily discernible at room temperature. Both reaction rates were enhanced at 30 and 37 °C. The reaction of $MTSES^-$ with T338C CFTR was the most rapid at 22 °C and was nevertheless increased at 37 °C.

The second-order rate coefficients for the reaction of $MTSES^-$ with CFTR constructs bearing cysteines at positions 334 and 336–338 are listed in Table 1. Shown also is the apparent activation energy calculated on the basis of eq 1 using the slope obtained from fitting the Arrhenius plots shown in Figure 5. At room temperature, the measured rates of reaction

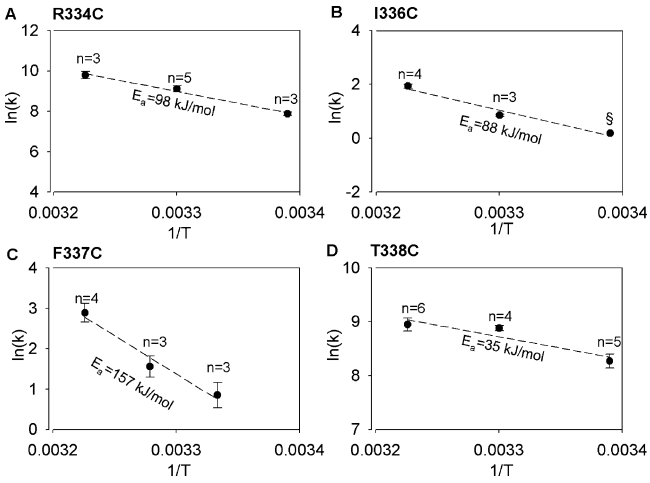

Figure 5. Arrhenius plots for (A) R334C, (B) I336C, (C) F337C, and (D) T338C CFTR. The right-most value in panel B is from ref 10. Values, based on Table 1, represent means ± the standard error of the mean.

varied over 4 orders of magnitude, despite the relative proximity of these four positions in the protein, suggesting that the orientation or electrostatic environment of the engineered cysteine side chains may differ significantly in different locations. Reactions at all four cysteines were enhanced by increasing the temperature to 37 °C, but the effect varied from ~2-fold for a cysteine at position 338 to >7-fold for a cysteine at position 337. As expected, the apparent activation energies varied widely, ranging from being that expected for disulfide exchange[15,16] for T338C CFTR to values in the range of those generally associated with protein conformational change.[17−20]

Figure 6 summarizes the inhibition by $MTSES^-$ of CFTR conductance at 22 °C (27 °C for F337C CFTR) and 37 °C in oocytes expressing CFTR constructs bearing substituted cysteines at positions extracellular to (334 and 336–338) and cytoplasmic to (339–342 and 344) the apparent accessibility cutoff defined by Alexander et al.[10] It is apparent that, despite the dramatic increases in the reaction rates of cysteines extracellular to the cutoff, the position of the cutoff was unchanged at 37 °C. That is, whatever conformational restriction prevents the reaction of extracellularly applied $MTSES^-$ with cysteines cytoplasmic to position 338 remained intact at 37 °C. The ability of cysteines at more cytoplasmic positions to react with channel-permeant, thiol-directed probes is documented by Alexander et al.[10] Recently, Bai et al.[7] and El Hiani and Linsdell[8] used detached membrane patches to demonstrate the reactivity of deeper-lining cysteines to $MTSES^-$ applied from the cytoplasmic side. However, the two studies differ substantially in the position of the apparent

Table 1. Temperature Dependence of $MTSES^-$ Modification

	k_{MTSES} (M^{-1} s^{-1})				
mutant	22 °C	30 °C	32 °C	37 °C	E_a (kJ/mol)
R334C	2648 ± 259 (n = 3)	9411 ± 1210 (n = 5)		18407 ± 3240 (n = 3)	98
I336C	1.2[a]	2.3 ± 0.1 (n = 3)		6.9 ± 0.4 (n = 4)	88
F337C	2.6 ± 0.7 (27 °C)[b] (n = 3)		5.1 ± 1.2 (n = 3)	19.4 ± 4.4 (n = 4)	157
T338C	4067 ± 573 (n = 5)	7192 ± 370 (n = 4)		7972 ± 1019 (n = 6)	35

[a]Value from ref 10. [b]The reaction rate was undetectable at 22 °C, so the value determined at 27 °C was used. Values are means ± the standard error of the mean.

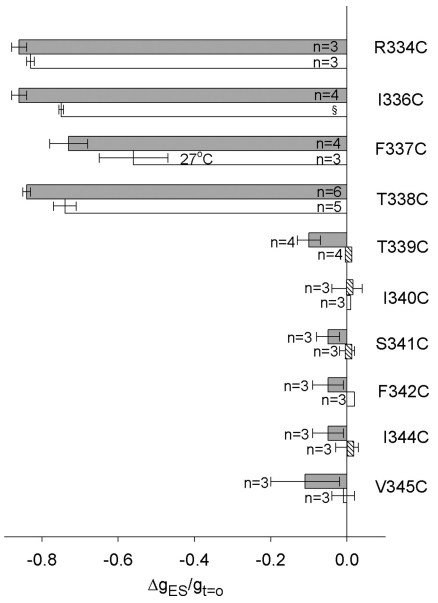

Figure 6. Summary of the effects of temperature on the reactivity of CFTR constructs toward $MTSES^-$. The percent block of CFTR conductance by $MTSES^-$ was defined as the change in conductance induced by $MTSES^-$, Δg_{ES}, divided by the conductance at time zero of exposure, $g_{t=0}$, at 22 °C (27 °C for F337C, white bars) and 37 °C (dark gray bars). The small hatched bars at the base of the *Y*-axis indicate cases where the value was indeterminant or near zero. The values at 37 °C (gray bars) from T339C to V345C reflect small changes in conductance in response to the temperature pulse, rather than reactivity toward $MTSES^-$. The section mark denotes data taken from ref 10. Values represent means ± the standard error of the mean.

size cutoff defined by the reactivity toward cytoplasmically applied reagents.

DISCUSSION

The results presented here provide evidence of a striking temperature dependence of the reactivity of cysteines engineered into the sixth transmembrane domain (TM6) of the CFTR chloride channel toward a channel-impermeant, thiol-directed reagent, $MTSES^-$. To the best of our knowledge, this is the first report comparing substituted cysteine reactivity at different temperatures, in particular, "physiological temperature" (37 °C). The findings have implications for assays routinely used to test molecular models of the CFTR channel as well as for our understanding of what structure of the pore domain is most relevant to the function of the channel in vivo.

Previous room-temperature cysteine scanning studies from several laboratories implicated three residues in TM6, R334, I336, and T338, as being likely to lie within the outer vestibule of the CFTR pore.[3−6,10] Cysteines substituted at these positions reacted with both channel-permeant reagents like $[Au(CN)_2]^-$ and $[Ag(CN)_2]^-$ and larger, channel-impermeant reagents like $MTSES^-$ and $MTSET^+$, as expected if these residues formed part of a wide, extracellular end of the channel that coupled the rate-limiting portion of the pore to the extracellular solution. Increasing the temperature increased the reaction rate for the extracellularly applied, channel-impermeant reagent, $MTSES^-$, at all three positions. At room temperature, the most rapid modification was seen at position 338. The rate at 22 °C, ∼4000 M^{-1} s^{-1}, is ∼1 order of magnitude less than that reported for the reaction of $MTSES^-$ with 2-mercaptoethanol in free solution.[21] The activation energy, 35 kJ/mol, is in the range reported for thiol−disulfide exchange, 30−40 kJ/mol,[15,16] and exceeds that reported for the diffusion of alanine in water, 19 kJ/mol.[22] The room-temperature rate of reaction at position 334 was comparable to that at position 338, but the rate of reaction was more temperature-dependent, which is evident from the higher energy of activation. The activation energy, 98 kJ/mol, lies within the range reported for CFTR channel gating, 70−150 kJ/mol.[14] In addition, the rate at 37 °C actually exceeded that seen at position 338 despite the fact that position 338 is thought to lie somewhat deeper in the pore. These findings point to the increased accessibility of channel conformations that result in deocclusion of the substituted cysteine at position 334 with an increase in temperature.

Modification rates at positions 336 and 337 were dramatically lower than those at positions 334 and 338, by 3 orders of magnitude at 22 °C. Given the proximity of these positions in the protein, the low rates suggest some sort of occlusion of the substituted cysteine or some other environmental feature such as a nearby negative charge that would attenuate the rate of the thiol−disulfide exchange reaction at room temperature.[21,23,24] The activation energy seen for the reaction at position 336 was well above that reported for thiol−disulfide exchange, as if an apparent occlusion of the substituted cysteine is reduced at elevated temperature due to a conformational change.

Cysteine modification at position 337 appears to represent a limiting case in that the reaction rate was undetectable at 22 °C (Figure 1A) and robust at 37 °C, exceeding that seen at position 336 at 37 °C. The estimated energy of activation, 157 kJ/mol, was much larger than at other positions and exceeded that associated with the opening of CFTR channels,[14] suggestive of a conformational change that accompanied the increased temperature.

The observed accessibility of a cysteine at position 337 to the channel-impermeant reagent, $MTSES^-$, at 37 °C was important for two reasons. First, it is clear that at elevated temperature, the channel can access conformations that permit a cysteine at position 337 to react with both channel-permeant and larger, channel-impermeant reagents, thereby bringing the reactivity of a cysteine at position 337 in line with that seen at positions 334, 336, and 338. The observed nonselective reactivity at position 337 places this position in the outer vestibule of the pore as defined by probe accessibility. This finding does not, in and of itself, resolve the discrepancy between our data and those of Cheung and Akabas[1,2] and Fatehi et al.,[5] who reported reactivity of F337C CFTR toward externally applied $MTSES^-$ at room temperature, but the observation of a slow, but discernable reaction rate at 27 °C (Table 1) raises the possibility of some, as yet unidentified, condition of their experiments that allows the CFTR channel to access conformations at temperatures within the 22 °C−27 °C range in which the thiol−disulfide exchange reaction can occur at position 337.

The temperature-induced changes in cysteine reactivity reported here, in particular that of a cysteine at position 337, are similar to the nondenaturing conformational changes reported for enzymes like adenosine deaminase[19] and carbonic anhydrase.[20] These enzymes can apparently access different functional states, some dominant at physiological temperature and others dominant at lower temperatures. Massey et al.[17] had previously reported similar, thermally induced, reversible changes in the structure of D-amino acid oxidase. These thermally induced transitions between relatively stable, functional

states have been likened to a partial unfolding from more compact low-temperature forms to more open, high-temperature forms characterized by increased exposure of nonpolar amino acid side chains to the aqueous environment.[18,19] Similarly, the large changes in cysteine reactivity reported here suggest that the outer portion of the CFTR pore can access more open conformations at 37 °C that render a cysteine substituted at position 337 reactive toward larger, externally applied reagents. Importantly, however, the position of the previously defined cutoff in reactivity toward channel-impermeant reagents appears to be unchanged at physiological temperature, indicating that the barrier to the permeation of $MTSES^-$ remains intact and located to the cytoplasmic side of T338.

The reactivity of cysteines substituted at either of two adjacent locations, positions 337 and 338, differed dramatically. The results suggest that the cysteine side chain at position 337 is occluded in the substates of the channel that predominate at room temperature, and it is less reactive even at 37 °C for reasons that could involve steric as well as electrostatic effects.[23,24] A goal of future modeling and MD simulations will be to understand the striking difference in cysteine reactivity at these two positions in the protein.

There are more than 1800 mutations known in the CFTR gene. The most common, ΔF508, results in a protein characterized by a temperature-sensitive folding defect.[25] The ΔF508 CFTR channel is expressed in the plasma membrane of mammalian cells at 28 °C, but increasing the temperature leads to the disappearance of the surface protein with a half-time of 1−2 h,[26−31] suggesting that exposure to a physiologic temperature produces unfolding that leads to internalization of surface protein and ultimately degradation within the cell. The results presented herein suggest that there may be a general propensity of the CFTR protein toward low-to-physiological-temperature conformational change that is apparent even in the outer vestibule of the pore, well away from site of the most common mutation in the first nucleotide binding domain, NBD1. Temperature-induced partial unfolding can produce, as in this instance, stable conformers, whereas in disease-related mutants like ΔF508, the natural tendency to unfold at physiological temperature may be exacerbated by a defect that drives the conformational change farther along the unfolding pathway toward a state of denaturation that triggers degradative, quality control processes in the cell. Understanding this continuum may offer insights into the temperature-dependent unfolding process seen in mutant CFTRs.

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ABBREVIATIONS

CFTR, cystic fibrosis transmembrane conductance regulator; IBMX, isobutylmethyl xanthine; Isop, isoproterenol; 2-ME, 2-mercaptoethanol; DTE, dithioerythritol; KCN, potassium cyanide; DDTC, diethyl dithiocarbamate; $MTSES^-$, sodium (2-sulfonatoethyl)methanethiosulfonate; CF172 ($CFTR_{inh}$-172), 4-[4-oxo-2-thioxo-3-(3-trifluoromethylphenyl)-thiazolidin-5-ylidenemethyl]benzoic acid.

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