Cystic Fibrosis Transmembrane Conductance Regulator-associated ATP and Adenosine 3’-Phosphate 5’-Phosphosulfate Channels in Endoplasmic Reticulum and Plasma Membranes

(Received for publication, January 14, 1997)

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Cystic fibrosis (CF) is characterized by abnormal regulation of epithelial ion and fluid transport due to mutations in the CF transmembrane conductance regulator (CFTR), an apical membrane-localized Cl– channel, that usually prevent it from exiting the endoplasmic reticulum. Defective or absent CFTR in the epithelium is believed to disrupt fluid balance in human airways and thereby contribute to chronic respiratory inflammation. Patch-clamp of the plasma membrane and outer membrane of the nuclear envelope of nuclei isolated from CFTR-expressing Chinese hamster ovary cells revealed that CFTR is associated with a regulated ATP channel in both membrane compartments. CFTR expression was also shown to be associated with permeability to another adenosine nucleotide, adenosine 3’-phosphate 5’-phosphosulfate, the universal sulfate donor in cells. These results may provide a link between the ion channel function of CFTR and abnormal glycoprotein processing observed in CF.

Cystic fibrosis (CF) is a common genetic disease characterized by abnormal regulation of epithelial ion and fluid transport due to mutations in CFTR, an apical membrane-localized cAMP-regulated Cl– channel (1–8). Hundreds of different mutations (1) can affect CFTR Cl– channel function by several mechanisms (8). However, most CF is caused by lack of CFTR in the plasma membrane due to mutations, including the most common one (ΔF508-CFTR) (8), that affect the ability of fully translated CFTR to exit from the endoplasmic reticulum (ER), where it is synthesized (9–12). Defective or absent CFTR in the epithelium is believed to disrupt fluid balance in human airways and thereby compromise mucociliary clearance of inflammatory particles, including bacteria (13). The resulting chronic inflammation and tissue damage in the lung are the cause of most mortality in CF (14). Nevertheless, it remains unclear if the lack of plasma membrane Cl– channel activity can fully account for airway pathology in CF (13, 15). Human airways are Na+-absorbing tissues (16, 17), and the role of CFTR in transepithelial Cl– transport is still undefined because Cl– seems to be at electrochemical equilibrium across the apical membrane (18), and NaCl absorption is enhanced in CF (17, 19, 20). Furthermore, other transport and biochemical abnormalities in the lung in CF may not be readily explained by the lack of plasma membrane CFTR Cl– channel activity, including elevated Na+ permeability through amiloride-sensitive channels (13, 17, 19, 21) and abnormal regulation by cAMP of another airway Cl– channel, the outward rectifier (22–25). In addition, altered glycoprotein processing is observed in respiratory cells from CF patients (26–29). CF airway epithelial cells express higher levels of the receptor for Pseudomonas aeruginosa, which may be a consequence of its diminished sialylation (14, 28), and secreted and cell-surface glycoconjugates are hypersulfated in the lung in vivo and in primary cultures of airway cells from CF patients (27, 30–32). The evidence suggesting that abnormal protein sulfation is a fundamental biochemical defect in CF (31, 32) seems paradoxical in light of the identification of CFTR as a plasma membrane Cl– channel.

A recent study suggested that abnormal regulation of the outward rectifier in CF involves defective plasma membrane ATP permeability (33) and results from two studies by independent groups indicated that CFTR may either conduct ATP or be closely associated with a separate ATP conductance (33, 34). However, these results are currently rather controversial because they have not been observed by all investigators (35–37). The possibility that CFTR is associated with ATP permeability has prompted us to consider whether other transport and protein-processing abnormalities in CF are related to this conductance. Specifically, in the present study we have considered whether protein processing alterations in CF might be related to an association of CFTR with ATP permeability in intracellular membranes. We recently demonstrated that CFTR as well as ΔF508 CFTR function as Cl– channels when they are localized in the ER membrane (38). That wild-type CFTR functions in the ER as well in plasma membranes suggests that it also likely functions in intermediate compartments, including the Golgi, the site of most protein processing, including sialylation and sulfation. CF in most patients may therefore be associated with lack of Cl– channel activity not only in the plasma membrane but in the Golgi as well. If CFTR is associated with nucleotide conductances, altered nucleotide permeability in intracellular membranes may possibly contribute to cellular dysfunction in CF.

MATERIALS AND METHODS

CHO cells that stably expressed wild-type CFTR and the parental CHO cell line (controls; no CFTR) were used (11, 39, 40). Cells were...
cultured, and nuclei were isolated as described previously (38). Standard patch-clamp techniques were applied: (i) excised inside-out and cell-attached configurations for the plasma membrane, and (ii) excised patches (cytoplasmic side facing into the pipette) for the outer nuclear membrane (38). Stable (15–60 min) seals (10–100 μm) were obtained using heat-polished electrodes (tip <0.5 μm in diameter) with 10–50 megohms resistance. Ag/AgCl electrodes were used without salt bridges. When using low (~5.2 and 0.7 mM) Cl− solutions (below) in the pipette, the electrodes were not backfilled with Cl−-containing solutions. These low Cl− conditions did not affect our ability to record currents, probably because the currents that the electrodes were required to pass were small. Junction potentials were corrected in all experiments by an initial offset applied by the amplifier, with one exception relating to the third part of Fig. 4A, which has not been corrected for the junction potential that arose during the switch from the ATP to the Cl− solutions. The measured offset was ~6 mV, which does not significantly affect the interpretations. All experiments were performed at room temperature as described previously (38). Data were digitally filtered at 300 Hz unless indicated. Channel openings and closings were automatically detected using the Tasc computer program, and amplitudes were determined by computer-assisted manual measurements of detected events. Data analyses were limited to records with low baseline drift. Kinetic analyses were restricted to single-channel patches. Results are expressed as mean ± S.E. The following solutions were used: Solution A, high Cl− solution containing 120 mM N-methyl-D-glucamine chloride, 3 mM MgCl2, 0.1 mM CaCl2, 1.1 mM EGTA, 10 mM HEPES, and 5 mM glucose, pH 7.3 with HCl; Solution B, high ATP solution containing 100 mM Na2ATP, 5 mM KCl, 1 mM MgSO4, 0.1 mM CaCl2, 1.1 mM EGTA, and 10 mM HEPES, pH 7.3 or 7.1 with NaOH for plasma and nuclear membrane patches, respectively; Solution C, high PAPS solution containing 100 mM Li4PAPS, 5 mM KCl, 1 mM MgSO4, 0.1 mM CaCl2, 1.1 mM EGTA, and 10 mM HEPES, pH 7.3 with NaOH. Unless otherwise specified, PKA (180 nM) and MgATP (1 mM) were added to or included in the bath or pipette solutions for excised plasma membrane or nuclear membrane patches, respectively. Thus, MgATP2− concentration was always ~2 mM. In the ATP solutions, the extra 99 mM ATP is mostly ATP4− because it was added as Na2ATP. Therefore, the free Mg2+ concentration was always ~1 μM. Thus, neither Mg2+ nor MgATP2− were significant variables in our experiments. The bath was continuously perfused until PKA was added to it. The perfusion was halted during these periods because of the high cost of PKA and the large quantities required for perfusion. Cell-attached patches were stimulated by a mixture designed to elevate intracellular levels of cAMP containing forskolin (5 μM), 3-isobutyl-1-methylxanthine (0.1 mM), and the cAMP analog chlorophenylthio-cAMP (0.1 mM) applied directly to the bath to achieve the desired final concentrations. DIDS was added to the pipette or bath for plasma membrane or nuclear membrane patches, respectively.

RESULTS AND DISCUSSION

Stably transfected CHO cells were used to examine whether plasma membrane-localized as well as ER-localized CFTR are conductive to ATP. We first reconfirmed (39) the presence of CFTR Cl− channels in the plasma membrane of CFTR-expressing CHO cells. With Cl− solutions in the bath and pipette, elevation of intracellular cAMP levels activated CFTR Cl− channel activities in cell-attached patches (eight of nine patches) with familiar properties (39, 41, 42) (data not shown). In excised inside-out patches with the catalytic subunit of PKA and MgATP in the bath, 6.1 ± 0.7 pS linear Cl− channels were observed (16 of 18 patches) with typical CFTR characteristics (Fig. 1A).

We used the inside-out excised patch configuration to determine whether membranes from these cells exhibited ATP conductances. With 100 mM ATP solutions in the bath and pipette, channel activities were observed with features similar to those of CFTR Cl− channels (Figs. 1 and 2); i) linear current-voltage (IV) relation with a slope conductance of 4.5 ± 0.3 pS (13 of 14 patches; Fig. 1, B and C); ii) PKA-dependence, because in the absence of PKA, they were not observed (0 of 5 patches; Fig. 2A); and iii) insensitivity to DIDS (Fig. 2B), whereas 50 μM glybenclamide, an inhibitor of CFTR Cl− channels (33, 43), reduced channel open probability (Po) (3 of 3 patches; Fig. 2C). A 10-fold ATP gradient shifted the reversal potential from 0 mV to approximately −12 mV, indicating permeability to ATP (Fig. 1C). Of note, this reversal potential is close to that expected for permeation by a quadrivalent anion, i.e. ATP4− (RT/4F in C/C0 = −15 mV), the overwhelmingly dominant ionic species in these experiments. Cl− was eliminated as the current carrier because similar channels (4.6 ± 0.2 pS) were present in solutions containing 0.7 mM NaCl in the bath (2 of 10 of 11 patches; Fig. 3A) in Tris−-ATP and Na2ATP solutions in which the Cl− concentration was reduced from the normal 5.2 mM (Figs. 1B and 2) to only 0.7 mM (Fig. 3). Na+ was also excluded as the current carrier because in inside-out patches under symmetrical conditions, 4.6 ± 0.1 pS channels were present when Na2ATP was replaced with Tris−ATP (eight of nine patches; Fig. 3B), and no channels were observed when

FIG. 1. CFTR channel activities in inside-out patches of plasma membranes of CFTR-expressing CHO cells. A, CFTR Cl− channels recorded from inside-out patch in symmetrical Cl− solution (Solution A) with PKA (180 nM) in the bath. Membrane potentials (V) were as indicated. Dashed line represents the closed state. This figure is representative of 16 patches. Kinetic analysis for three patches that contained single channels (data not shown) revealed Po = 0.6, τo = 16 ms (77% of openings), τc1 = 1150 ms (23%), τc1 = 18 ms (56%), and τo = 455 ms (44%) at +80 mV. B, ATP channels from inside-out patch in symmetrical ATP solution (Solution B) with PKA (180 nM) in the bath. In single-channel patches (n = 4) at positive potentials (+80, +90, and +100 mV), Po = 0.4, τc1 = 17 ms (28%), τc2 = 247 ms (72%), τo = 7 ms (81%), and τo = 1350 ms (19%). This figure is representative of 13 experiments. C, IV relationship for CFTR Cl− (triangles) and ATP (circles) channels under symmetrical (●) and asymmetrical (○) conditions. Slope conductance for symmetrical Cl− and ATP solutions was 6.1 ± 0.7 pS (n = 3 patches) and 4.5 ± 0.3 pS (n = 3 patches), respectively. In asymmetrical conditions the pipette solution was Solution B, whereas 100 mM Na2ATP, 5 mM KCl, 1 mM MgSO4, 0.1 mM CaCl2, 1.1 mM EGTA, 10 mM HEPES, and 200 mM glucose, pH 7.3 with NaOH (n = 6 patches).
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![Diagram](image-url)

**FIG. 2.** Properties of ATP channels in inside-out patches of plasma membrane. **A**, PKA dependence of ATP channels. No channel activities were observed in the absence of PKA (upper trace). PKA (180 nM) activated multiple channels after 2 min (lower trace), recorded from the same patch at the potentials indicated. This figure is representative of five experiments. **B**, insensitivity of ATP channels to DIDS (0.1–0.3 mM). Data filtered at 200 Hz. This figure is representative of eight experiments. **C**, reduction of P in the absence of PKA (180 nM added to bath) activated channels in the same patch after 1 min. **D**, absence of ATP channels in control cells despite the presence of PKA (180 nM). This figure is representative of 10 experiments.

**FIG. 3.** ATP channel activities in inside-out plasma membrane patches in solution containing reduced Cl⁻. **A**, ATP channels were recorded in symmetrical ATP solution (Solution B) with Cl⁻ reduced from 5.2 to 0.7 mM by the omission of 5 mM KCl. Activities were absent in unstimulated patches (upper trace). PKA (180 nM added to bath) activated channels after 2 min (lower trace). Traces are from the same patch. This figure is representative of 10 experiments. **B**, ATP channels recorded in symmetrical Tris-ATP solutions (replacing Na⁺). No activities were observed in the absence of PKA (upper trace). PKA (180 nM added to bath) activated channels in the same patch after 1 min (lower trace). This figure is representative of eight experiments. **C**, lack of channel activities in symmetrical solutions containing 100 mM sodium gluconate in lieu of Na₂ATP in the absence or presence of PKA (180 nM) during a 22-min recording at the potentiostats indicated. Current traces were recorded from same patch. This figure is representative of four experiments. **D**, I/V relationships for ATP channels shown in A (cation = Na⁺; A) and B (cation = Tris⁺; D). Slope conductance was 4.6 ± 0.2 (n = 4 patches; dashed line) and 4.6 ± 0.1 (n = 4 patches; solid line) pS in symmetrical solutions in A and B, respectively.

Na₂ATP was replaced with sodium gluconate (zero of four patches; Fig. 3C). Importantly, no ATP channels were observed in control cells (0 of 10 patches; Fig. 2D).

In inside-out plasma membrane patches with Cl⁻ and ATP in the pipette and bath solutions, respectively, currents at +70 mV had slope conductances of 6–8 pS, whereas at −70 mV they were 4–5 pS (6 of 6 patches; Fig. 4), consistent with the currents being carried by Cl⁻ and ATP, respectively. Replacement of bath ATP by perfusion with 120 mM NaCl solution restored 6–8 pS activities at −70 mV (Fig. 4A). Under reversed biionic conditions in inside-out patches (i.e., pipette, ATP; bath, Cl⁻), 6–8 pS Cl⁻ currents were observed at −70 mV, whereas 4–5 pS ATP currents were present at +70 mV (8 of 9 patches; data not shown).

We previously demonstrated by patch-clamp of the outer membrane of nuclei isolated from CFTR-expressing CHO cells that ER-localized CFTR was functional as a Cl⁻ channel, with properties similar to those exhibited when it resided in the plasma membrane (38). Using a similar approach, we evaluated the ATP conductance of the ER, using nuclei isolated from the same CHO cells. With PKA and MgATP in the pipette (because the cytosolic face of the membrane patch faces into the pipette in this configuration), PKA-dependent channels with linear I/V relation and 4.6 ± 0.5 pS conductance were recorded in symmetrical ATP solutions (33 of 35 patches; Fig. 5). Other properties of the ATP channels in the nuclear/ER membrane were also similar to those observed for wild-type CFTR in the plasma membrane (Fig. 5).

Our studies suggest that CFTR is associated with a regulated ATP channel in the plasma membrane and ER, and by extension in membranes in intermediate compartments including Golgi. The single ATP channel conductance, I/V relation, PKA sensitivity, pharmacological sensitivities, and dependence
on expression of CFTR we have observed in the present study are similar to those reported previously (33, 34) in different cell types, suggesting that CFTR is closely associated with or is in fact an ATP conductance. We do not think that the Cl− conductance pathway in CFTR is the ATP conductance pathway, although the resemblance of the biophysical, kinetic, and pharmacological properties of the ATP conductance to those of the CFTR Cl− conductance is striking and suggests that gating of the ATP permeability is strongly tied to CFTR gating. The reasons why some investigators have observed nucleotide conductances associated with CFTR (33, 34) although others have not (35–37) are not addressed by our study, and our results provide no insights into this issue. We would point out, however, that we have also observed CFTR Cl− channels that were not associated with ATP conductances in a different CHO cell clone,2 suggesting that coupling of the two permeabilities may be cell-type dependent. The clear channel activities we observe will enable us in future studies to define variables that might regulate the manifestation of the ATP conductances and therefore resolve these conflicting observations.

The presence of a CFTR-associated ATP conductance in intracellular membranes raises the possibility that altered ATP permeability in intracellular compartments in CF may contribute to disease pathogenesis. ATP-translocase activity has been detected in ER and Golgi membranes (44), and ATP binding to ER proteins, ATP-dependence of the structure or activities of ER-resident proteins, and lumenal ATP requirement for protein translocation in the ER (45–47) suggest that intralumenal ATP content is physiologically regulated. ATP binding by ER-localized molecular chaperones, including BiP and calnexin, is necessary for ATPase activity, structural changes, and oligomerization required for chaperone functions (45–47). These observations may have significance for CF because CFTR and ΔF508-CFTR both associate with calnexin in the ER membrane (40), and the trafficking defect of ΔF508-CFTR is likely a chaperone-assisted process (12). If ATP permeability through ER- or Golgi-localized CFTR affected intraluminal ATP concentrations, mutant CFTRs could conceivably modify lumenal ATP concentrations and consequently affect protein processing. However, measurements of lumenal ATP concentrations will be required to test this hypothesis.

It was proposed that CFTR may normally provide a Cl− conductance in Golgi membranes to facilitate lumenal acidification by a proton pump and that lack of such activity in CF, by compromising Golgi pH, might account for altered glycoprotein processing (26). Nevertheless, a direct test of this model failed to provide support for it (48). Nor can altered protein sulfation

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2 M. Sugita and J. K. Foskett, unpublished results.
in CF airway epithelial cells be accounted for by changes in plasma membrane sulfate transport or cell content of inorganic sulfate (31, 49). Intracellular sulfation reactions utilize sulfate that has been “activated” by its incorporation into PAPS (50). PAPS is synthesized in the cytoplasm by a reaction involving ATP and sulfate, catalyzed by a bifunctional ATP-sulfurylase-adenosine 5′-phosphosulfate kinase, and transported into the Golgi lumen by a Golgi-specific saturable process that is temperature-dependent, independent of pH gradients or ATP hydrolysis, and inhibited by stilbenes (51, 52). Once transported into the Golgi lumen, PAPS donates sulfate to sulfotransferases for protein sulfation. PAPS is an intermediate for synthesis of all naturally occurring sulfated compounds, including sulfated glycoproteins, fibronectins, sulfatides, and glycosaminoglycans (51, 52). We have considered whether PAPS, as an adenosine nucleotide, might also be permeable through CFTR.

With high PAPS on both sides of excised plasma membrane patches from CFTR-expressing CHO cells, channels were observed in a linear I/V relation, had a 4.7 mV/pA conductance (Fig. 6C), and were DIDS-insensitive (eight of eight patches; Fig. 7B). They were not observed in the presence of PKA (20 of 28 patches; Fig. 7A) but were not observed in its absence (0 of 7 patches; Fig. 7A). The channels were PAPS-selective because a 10-fold PAPS gradient (100 mM PAPSout/10 mM PAPScyt) shifted the reversal potential from 0 mV to approximately −15 mV (6 of 7 patches; Fig. 6, B and C) (the expected Nernstian potential is −15 mV because PAPS, like the ATP in these experiments, has a valence of −4). Li+ was excluded as a current carrier because no channel activities were observed when currents were recorded in inside-out patches in symmetrical Li2SO4 solution (Solution C with 150 mM Li2SO4 replacing Li2PAPS) (0 of 13 patches; data not shown). In control cells, no activities were observed in inside-out patches in Solution C in the presence or absence of PKA (zero of seven patches; data not shown).

By demonstrating that PAPS permeability is associated with CFTR, our results raise the possibility that the concentration of PAPS in the Golgi lumen may be regulated in part by CFTR. In a simple model, activity of CFTR in this intracellular compartment would constitute a PAPS “leak” in parallel with the PAPS pump and would tend to lower the PAPS concentration in the Golgi lumen. Lack of CFTR in this compartment in CF would shift the balance of these activities in favor of the pump, resulting in a higher Golgi lumenal PAPS concentration. Because the PAPS concentration in the Golgi lumen is likely rate-limiting for sulfation reactions (50, 53, 54), this model predicts hypersulfation of proteins in CF, consistent with observations. The physiological consequences of hypersulfation may include altered visco-elastic properties of airway secre-
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