but this static picture alone cannot depict the protein movements that must occur during ion transport. To address this issue, we have utilized fluorine NMR to monitor substrate-induced conformational changes in ClC-ec1. We show that substrate-driven conformational change is not constrained to the Cl^- permeation pathway alone, and that the ClC-ec1 subunit interface participates in protein movement. Furthermore, removal of the protein's H^+ transport ability does not eliminate the H^+-dependent protein movement observed in the intact antiporter. Finally, we observe that a ClC-ec1 ''channel-like'' mutant is not subject to the same substrate-dependent conformational changes that occur in the ClC-ec1 transporters. Together, these results provide new insight into conformational change in ClC-ec1 and lay an essential foundation for future studies on ClC-ec1 protein dynamics.

1668-Pos

Cardioprotective Role For the CLC-3 Chloride Channel in the *mdx* Mouse Model of Duchenne Muscular Dystrophy

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Duchenne Muscular Dystrophy (DMD) is the most common human X-linked disease affecting 1/3,500 male births. DMD patients suffer from severe, progressive muscle wasting with clinical symptoms first detected between 2 to 5 years of age. As the disease progresses patients are confined to a wheelchair in their early teens and die in their early twenties from cardiopulmonary failure. There is currently no effective treatment or cure for DMD. DMD patients and *mdx* mice (the mouse model for DMD) have mutations in the dystrophin gene that result in an absence of the dystrophin protein. Dystrophin is a 427 kDa protein located under the sarcolemma on the inner cytoplasmic membrane of skeletal and cardiac muscle cells and provides structural and functional integrity to muscle. Although therapies have been developed that target skeletal muscle disease, increasing evidence suggests correcting the cardiomyopathy is critical to the survival of DMD patients. As the cardiomyopathy progresses, the hearts of DMD patients and *mdx* mice exhibit arrhythmias, conduction abnormalities and left ventricular dilation. Recent studies have shown that the chloride ion channel ClC-3, a candidate protein responsible for volume-regulated chloride channels in heart, plays a critical cardioprotective role against the development of hypertrophy and failure (*J Mol. Cell. Cardiol.* 2009 Jul 15. [Epub ahead of print]).Using transgenic mice including heart specific ClC-3 knockout *mdx* mice, echocardiography and electrophysiology, we analyzed the role of ClC-3 in modulating cardiac disease progression in dystrophic mice. Our preliminary results indicate ClC-3 may be a major modifier of cardiac disease progression in *mdx* mice and targeting ClC-3 expression or function may provide a novel therapeutic approach for the treatment of dilated cardiomyopathy in DMD (supported by 3P20RR015581 from the National Center for Research Resources).

1669-Pos

Blocking Kinetics of CFTR Channel by Aromatic Carboxylic Acid Positional Isomers Characterised using a Novel Amplitude Distribution Analysis Method

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To investigate the pore structure of the cystic fibrosis transmembrane conductance regulator (CFTR) channel, we performed a systematic pore probing on CFTR channel pore with a series of small aromatic carboxylic acids, including their positional isomers, e.g., 9-anthracene carboxylic acid (9-AC) and 1-anthracene carboxylic acid (1-AC).
Small compounds presumably interacting the channel protein with a few points are sensitive to structural changes of the binding site. However such low affinity blockers show fast - intermediate blocking kinetics which give us the overall affinity, but not on- and off- rates separately. To overcome this problem, we developed an iterative simulation method to estimate the on- and off- rate constants in the 9-AC or 1-AC block from the single channel amplitude distribution.
The newly developed Amplitude Distribution Analysis (ADA) program first generated a single-channel current according to the given kinetic scheme and added a Gaussian noise to the currents for mimicking the background noise. The simulated currents were low-pass filtered and digitized at the same frequencies as those in the experiments and binned into an amplitude histogram. Then the program repeats a direct likelihood comparison between the simulated and experimental current amplitude distributions to find the best fitted values for the blocking kinetic parameters.
The ADA program showed that the off-rate of 1-AC block is 3-fold slower than that of 9-AC and the on-rate of 1-AC is ~3-fold faster than that of 9-AC. The voltage-dependences of on- and off- rates of 1-AC are similar to those of 9-AC, respectively. These suggest that 1-AC and 9-AC block CFTR channel by binding to a common binding site which should be modeled by a combination of a positive charge tightly surrounded by hydrophobic residues.

1670-Pos

Effects of Aromatic Carboxylic Acids on Genistein- and Curcumin- Potentiated G551D-CFTR

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The Cystic fibrosis transmembrane conductance regulator (CFTR) chloride channel plays an important role in salt and water transport across epithelia and defective function due to mutations in the CFTR gene cause cystic fibrosis (CF). The glycine-to-aspartate missense mutation at position 551 (G551D) is the third most common CF-associated mutation and G551D-CFTR is characterized by a very low open probability despite of its normal trafficking to the plasma membrane.
Numerous small molecules have been shown to increase the activity of G551D-CFTR presumably by binding to the CFTR protein. Among the many G551D-CFTR potentiators, a bioflavonoid found in legumes, genistein is perhaps the most extensively studied. More recently it was reported that a component of the spice turmeric, curcumin strongly activated G551D-CFTR. However, The mechanism through which these compounds increase G551D-CFTR activity is still unclear. On the other hand, we have previously reported that anthracene-9-carboxylate (9-AC) showed an inhibitory effect and an potentiation effect on CFTR channels by binding to two chemically distinct sites for each effect (Ai *et al,* 2004).
In this study, we made a functional probing on genistein-potentiated G551D-CFTR and curcumin-potentiated G551D-CFTR using 9-AC and its positional isomer, anthracene-1-carboxylate (1-AC). In wild type- (WT-) CFTR, 9-AC induced a large voltage-independent enhancement and a voltage-dependent inhibition in the whole-cell (WC) current. 1-AC induced a smaller enhancement and a larger voltage-dependent block in WT-CFTR WC currents in compared with 9-AC. In the other hand, both 9-AC and 1-AC induced only a weak voltage-dependent inhibitions in genistein-potentiated G551D-CFTR WC currents whereas, in curcumin-potentiated G551D-CFTR WC currents, 9-AC and 1-AC induced similar effects to those in WT-CFTR. These suggest that curcumin and genistein potentiated G551D-CFTR via different mechanisms. We speculate that genistein-potentiated and curcumin-potentiated G551D-CFTRs might have different protein conformations.

1671-Pos

Optimization of the NBD1 Site Improves the Function of G551D-CFTR Channels

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CFTR chloride channels comprise two nucleotide binding domains (NBD1 and NBD2). Mutations (for example, G551D) that impair CFTR function result in the lethal genetic disease cystic fibrosis (CF). It's established that the opening and closing of CFTR are mainly controlled by ATP binding and hydrolysis respectively in NBD2 while ATP binding at NBD1 can modulate the stability of the open state. Using a non-hydrolytic ligand MgPPi, we locked open CFTR channels with mutations at NBD1. We found that two W401 mutations significantly increased the lock-open time (W401F: 72 ± 3s; W401Y: 51 ± 6s; WT: 27 ± 2s). These gain-of-function mutations are unexpected since the W401-equivalent residue Y1219 in NBD2 can be effectively replaced by tryptophan but not phenylalanine. As the ATP molecule bound in NBD1 may interact with NBD2's signature sequence (LSHGH), we extended our study to this region. We found that reverting the histidine residue 1348 to the canonical glycine (H1348G) similarly stabilized the lock-open state (τ=67 ± 8s). Once W401F, H1348G, or W401F/H1348G mutations were incorporated into G551D channels which no longer respond to ATP, the mutant channels become ATP-responsive with basal activity increased by ~10-fold for W401F/G551D-, ~4-fold for H1348G/5G551D-, and ~25-fold for W401F/H1348G/G551D-channels. The increased

channel activity is mainly due to an increased open-time. The results shown here suggest that NBD1 and NBD2 may employee different chemical mechanisms in binding ATP and that NBD1 can be a potential molecular target for developing CFTR potentiators for CF-related mutants. The effects of different nucleotides (for example, GTP and UTP) on NBD1 and NBD2 will be studied to gain a better understanding of the chemical mechanism underlying nucleotide-NBD interaction.

1672-Pos

The Inhibition Mechanisms of the Regulatory Domain of Cyctic Fibrosis Transmembrane Conductance Regulator

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The cyctic fibrosis transmembrane conductance regulator (CFTR) is a member of the human C subfamily of ATP-binding cassette (ABC) transporters but functions as a chloride channel. Activity of CFTR is tightly controlled not only by ATP binding-induced NBD1-NBD2 dimerization but also by phosphorylation of the unique regulatory (R) domain by protein kinase A (PKA). The R domain has multiple phosphorylation sites for which only Ser737 and Ser768 are inhibitory. The underlying mechanisms are unclear because neither the structure of the R domain nor its interactions with other parts of CFTR have been fully illuminated. Here I applied the crystal structure of bacterial transporter Sav1866 and sulfhydryl-specific crosslinking strategy to determine which part of CFTR interacts with the R domain regulating channel activity. The results show that diamide-induced disulfide bond crosslinking of S768C to H950C, K951C, H954C or S955C from cytoplasmic loop 3 (CL3) inhibited the channel activity and inhibition was reversed by DTT. Similarly, disulfide crosslinking of S737C to H954C, S955C or Q958C also suppressed the channel activity. Furthermore, mutation of these residues to alanines weakened the curcumin-induced relative PKA-dependence which was completely removed by deletion of the R domain. Finally, activation of a double mutant H950R/S768R CFTR did not need any PKA while either H950R or S768R construct needed it. These results suggest that S768 and S737 may form putative H-bonds with hydrophilic residues of CL3 and thus inhibit the channel activity in the unphosphorylated state. In the phosphorylated state, a putative ferrous bridge involving H950, H954, C832, D836, H775 and H667 at the CL3-R interface may inhibit the channel activity. All these observations are consistent with the recent electron cryomicroscopy-based structural model on which the R domain is closed to cytoplasmic loops regulating channel gating.

1673-Pos

CFTR Cytosolic Loop Mutations Allosterically Promote ATP Independent Channel Gating

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CFTR channel gating normally depends on ATP binding and NBD dimerization. Optimal CFTR channel activation further requires phosphorylation of the R domain. How ATP binding at the NBDs and phosphorylation of the R domain regulate CFTR channel gating are not fully understood. In the present study, we demonstrate that mutations in the CFTR Cytosolic Loops (CL) markedly promote channel opening in the absence of ATP and NBD2, presumably by an allosteric mechanism. In excised inside-out patches, we observed that single or double mutations of K978 and K190 in CL 3 and 1 induced large ATP independent currents (5-70% of current before removing ATP). These mutant channels deactivated with a slow time constant (49.11 ± 4.58 sec) when ATP was removed by Hexokinase/glucose and subsequent bath perfusion. A K978 point mutation greatly increased the ATP sensitivity of channel activation by decreasing the EC_{50} (by 8-fold) for ATP activation, which is consistent with the slow deactivation following ATP removal. K978 mutations markedly enhanced G551D channel activity, a disease mutant that fails to respond to ATP, and Δ1198-CFTR, a mutant that lacks NBD2, indicating that the K978 mutations affect channel gating downstream of NBD dimerization. Interestedly, R domain phosphorylation further stimulated K978/G551D and K978/Δ1198 combined mutants, indicating that the R domain regulates channel activity independently of NBD dimerization. Similarly, K978 mutations also increased the activation rate at low dose (3 U/ml) of PKA, indicating that K978 mutations also enhance the PKA sensitivity of channel activation. Our results support an allosteric gating mechanism in which loops 1 and 3 functionally link ATP binding and NBD dimerization to CFTR channel opening.

1674-Pos

Accessibility of Cysteines Within the NBD Interface in a CFTR Channel

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Opening and closing of a CFTR channel is accompanied by ATP-driven formation and hydrolysis-triggered disruption of a head-to-tail NBD1-NBD2 heterodimer where composite interfacial sites, between the Walker motifs of one NBD and the LSGGQ-like (ABC signature) sequence of the other, each enclose an ATP molecule. Only the ''NBD2'' composite site (containing NBD2 Walker motifs) is catalytically competent. The ATP-bound tight NBD1-NBD2 heterodimer is linked to the open-channel state, but the disposition of the NBDs in the closed-channel state of CFTR, in the absence of ATP or after its hydrolysis, remains unknown. To address this, we assess accessibility to various size MTS reagents of single interfacial target cysteines introduced into the ABC signature sequence of the competent site (at NDB1 position S549), or of the dead site (at NBD2 position S1347), or at mid-interface positions S605 of NBD1 or A1374 of NBD2, in full-length cys-depleted CFTR-C(832-1458)S,expressed in *Xenopus* oocytes and examined in excised patches. Cysteines at all four positions were readily accessible to MTSET in closed channels in the absence of ATP. For channels opening and closing in 3 mM ATP, the reaction rate depended on MTSET concentration and was ~10^5 $M^{-1}s^{-1}$ at 5 μM at position 549, and was similarly rapid at corresponding position 1347. In closed channels without ATP, cysteines at 549, 605, 1347, and 1374 were all also readily accessible to MTS reagents of increasing size, e.g., MTS-biotin, MTS-THAE (trihexylammonium-ethyl) and MTS-verapamil, up to ~11Åx16Åx10Å, suggesting substantial separation between NBD1 and NBD2, throughout the NBD interface, in closed CFTR channels. (Supported by NIH DK51767).

1675-Pos

Strict Coupling Between CFTR's Catalytic Cycle and Gating of its Channel Pore Revealed by Distributions of Open Burst Durations

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CFTR, the ABC protein defective in cystic fibrosis, functions as an anion channel. Once phosphorylated by protein kinase A, a CFTR channel is opened and closed by events at its two cytosolic nucleotide binding domains (NBDs). Formation of a head-to-tail NBD1/NBD2 heterodimer, by ATP binding in two interfacial composite sites between conserved Walker A and B motifs of one NBD and the ABC-specific signature sequence of the other, has been proposed to trigger channel opening. ATP hydrolysis at the only catalytically competent interfacial site is suggested to then destabilize the NBD dimer and prompt channel closure. But this gating mechanism, and how tightly CFTR channel opening and closing are coupled to its catalytic cycle, remain controversial. Here we determine the distributions of open burst durations of individual CFTR channels, and use maximum likelihood to evaluate fits to equilibrium and non-equilibrium mechanisms and estimate the rate constants that govern channel closure. We examine partially- and fully-phosphorylated, wild-type CFTR channels, and two mutant CFTR channels each bearing a deleterious mutation in one or other composite ATP binding site. We show that the wild-type CFTR channel gating cycle is essentially irreversible and tightly coupled to the ATPase cycle, and that this coupling is completely destroyed by the NBD2 Walker-B mutation D1370N but only partially disrupted by the NBD1 Walker-A mutation K464A. [NIH R01-DK051767, NIH FIC R03-TW007829, Wellcome Trust 081298/Z/06/Z]

1676-Pos

ΔF508 CFTR Expressed In *Xenopus* Oocytes Exhibits Unique Thermal Sensitivity

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The deletion of a phenylalanine at position 508 is the most common, disease-related mutation in the CFTR protein. ΔF508 CFTR channels are assembled in mammalian cells, but exhibit two deficiencies thought to underlie the disease phenotype: impaired trafficking and defective gating. The CFTR trafficking defect is partially mitigated at low temperature so that *Xenopus* oocytes are ideal for studying the physical properties of ΔF508 channels under physiological conditions. In order to investigate possible effects of the Phe deletion on the intrinsic stability of CFTR channel function in the plasma membrane, we monitored channel activity at room temperature (~23°C) and during a brief (10-12 minute) period of elevated temperature (28°C to 37°C). In oocytes expressing wild type CFTR, a temperature challenge resulted in a reversible increase in the conductance; a result of a simultaneous increase in single-channel conductance and open probability. Both parameters, however, returned to baseline values