

channel activity is mainly due to an increased open-time. The results shown here suggest that NBD1 and NBD2 may employ 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 Cystic Fibrosis Transmembrane Conductance Regulator

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The cystic 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 $\Delta 1198$ -CFTR, a mutant that lacks NBD2, indicating that the K978 mutations affect channel gating downstream of NBD dimerization. Interestingly, R domain phosphorylation further stimulated K978/G551D and K978/ $\Delta 1198$ 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 NBD1 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 $\sim 10^5 \text{ M}^{-1} \text{ s}^{-1}$ 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 (trihexammonium-ethyl) and MTS-verapamil, up to $\sim 11 \text{ \AA} \times 16 \text{ \AA} \times 10 \text{ \AA}$, 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

$\Delta F508$ 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. $\Delta F508$ 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 $\Delta F508$ 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 ($\sim 23^\circ\text{C}$) 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