SubBioen Su-PM-1

EXTENSION OF STRUCTURAL ANALYSIS OF THE BACTERIAL REACTION CENTER BY SITE-DIRECTED AND COMPENSATORY MUTATIONS. ((M. Schiffer, Y.-L. Deng, C. Ainsworth, F.H. Pascoe* and D.K. Hanson)) CMB, Argonne National Laboratory, Argonne, IL 60439. *College of St. Francis, Joliet, IL 60435

The photosynthetic reaction center complex is composed of three protein subunits: the intermembrane L and M chains, and the mostly cytoplasmic H chain. The cofactors are located within the membrane, surrounded by the transmembrane helices of the L and M chains. The homologous L and M chains are related by approximate twofold symmetry; they provide the scaffolding for the finely tuned environment in which electrons and protons are transferred to the cofactors. Though the cofactors are also related by a twofold axis, the electron transfer is unidirectional. We used site-directed mutagenesis to probe the function of residues in symmetrical positions near the primary donor and the quinone sites. We found that the stability and/or folding of the complex was affected in some of the double mutants carrying smaller amino acid replacements near the primary donor, yielding a strain that was incapable of photosynthetic growth. Photocompetent derivatives of this strain were isolated that carried an Ala to Pro suppressor mutation in a transmembrane helix at a distant third site that partially compensated for this defect. (Supported by U.S. Dept. of Energy, OHER, Contract No. W-31-109-ENG-38 and Public Health Service Grant GM36598.)

SubBioen Su-PM-2

CRYSTALS AND STRUCTURE OF PHOTOSYSTEM I
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Water-oxidizing photosynthesis in higher plants and cyanobacteria is realized by the cooperation of two photosystems PS I and PS II. The structure of PS II is unknown but is in part related to the known structure of the reaction center of the purple bacteria. PS I is of quite different structural organization. The method is described how the trimer of PS I, with a mass of 1020 kDa has been crystallized with a resolution of ca. 4 Å. By X-ray analysis of the crystals at 6 Å, 28 helices and 40 antenna chlorophyll a have been located. The electron transfer chain of the reaction center is characterized by 1. two chlorophyll a, attributed to the primary electron donor P700 as a dimer; 2. two new Chl a in the vicinity of P700; 3. one Chl a assigned to the electron acceptor, A_0, and 4. a contour corresponding to the head group of vitamin K_1. 5. As terminal electron acceptors the organization of 3 iron-sulphur clusters has been determined.

Literature: Witt et al., Ber. Bunsenges. Phys. Chem. (1988) 92, 1503-1506; Witt et al., Research in Photosynthesis, 1992, I, 521-528 (ed. N. Murata), Kluwer Acad, Publ.; N. Krauß et al., Nature, 1993, 361, 326-330

SubBioen Su-PM-3

An atomic model of plant light-harvesting complex determined by electron crystallography

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We have determined the three-dimensional structure of plant light-harvesting complex (LHC-II) at 3.4 Å resolution by electron cryo-microscopy and electron diffraction of two-dimensional crystals. LHC-II is the most abundant membrane protein in chloroplasts and binds about 50% of all chlorophyll in plants. The three-dimensional map shows clear density for the three membrane-spanning α-helices, one short helix at the membrane surface, much of the connecting loops, 13 to 14 chlorophyll molecules and two carotenoids. The polypeptide chain has been traced in the helices and the loop regions which has enabled us to identify most of the chlorophyll-binding amino acid side chains. The two carotenoids lie between the chlorophylls to protect the system from being photo-damaged by singlet oxygen.

SubBioen Su-PM-4

CRYSTAL STRUCTURE OF THE CHLOROPLAST CYTOCHROME f REVEALS A NOVEL CYTOCHROME FOLD AND UNEXPECTED HEME LIGATION
((Sergio E. Martinez, Deru Huang, Andrzej Szczepaniak, William A. Cramer, and Janet L. Smith)) Dept. of Biological Sciences, Purdue University, West Lafayette, IN 47907

The cytochrome *b6f* complex connects the two reaction center complexes in oxygenic photosynthesis. The crystal structure of the reduced redox-active 252 residue lumen-side domain of the 285 residue cytochrome *f* of the *b6f* complex has been solved by multiple isomorphous replacement and anomalous scattering to a resolution of 2.3 Å. In addition to being the first polypeptide of the *b6f* or bc_1 complexes to be solved crystallographically, the structure has unique aspects and major features: (i) Unlike all other *c*-type cytochrome structures that consist of one predominantly alpha helical domain, cyt *f* contains two domains in an elongate (75 Å x 35 Å x 24 Å) structure whose defined secondary structure is mostly beta sheet. (ii) The covalently bound heme lies within the larger domain near the interface between the two domains, its iron 45 Å from the C-terminal residue that is connected to the trans-membrane alpha-helix. (iii) The smaller domain (residues 169 - 231) contains a positively charged region that includes Lys 187, 28 Å from the heme Fe, which was previously shown to cross-link to plastocyanin. (iv) The axial sixth heme ligand is the alpha amino group of the N-terminal tyrosine residue. This implies that the redox center cannot be assembled until translocation of cyt *f* has proceeded sufficiently through the thylakoid membrane for the signal peptide to be cleaved. (v) The larger domain has the fibronectin type III domain fold found in many animal proteins. This fold has not been previously found in a plant protein.

MEMBRANE BIOPHYSICS SUBGROUP - CHLORIDE CHANNELS: DIVERSITY IN FORM AND FUNCTION

SubMemBio Su-PM-1

REGULATORY RELATIONSHIP BETWEEN CFTR AND OUTWARDLY RECTIFYING CHLORIDE CHANNELS IN AIRWAY EPITHELIAL CELLS.((Erik M. Schwiebert, *Marie E. Egan, and William B. Guggino,)) Department of Physiology, Johns Hopkins University School of Medicine, Baltimore, MD 21205 and *Dept. of Pediatrics, Yale University School of Medicine, New Haven CT 06510

Previously, we showed that regulation of both CFTR and outwardly rectifying (ORCC) chloride channels by the cyclic AMP/protein kinase A (PKA) signalling pathway was defective in cystic fibrosis (CF) airway epithelial cells. Complementation of CF airway cells with the wild-type CF gene restored cAMP-activated chloride transport, induced the appearance of low conductance CFTR chloride channels, and corrected the defective regulation of ORCC chloride channels by PKA {Egan et al. *Nature* 358:581, 1992}. More recently, whole cell patch-clamp recordings at physiological temperature utilizing nystatin perforation or conventional methods with 5 mM ATP-Mg^{2+} in the pipette solution provided similar results. Both CFTR and ORCC chloride channels contribute to cyclic AMP-activated whole cell chloride currents in normal airway epithelial cells and in cystic fibrosis (CF) airway epithelial cells complemented with the wild-type CF gene {Schwiebert et al. *Ped. Pulmonol* Suppl, 9:224, 1993}. These results were obtained in whole cell recordings by exploiting dissimilar biophysical properties of CFTR and ORCC currents such as the degree of rectification of the I-V relationship, the difference in sensitivity to chloride channel blocking drugs such as DIDS, calizarenes, and DPC, and the opposing chloride relative to iodide permeabilities of the two channels. Taken together, we conclude that CFTR not only functions as a cAMP-regulated chloride channel in airway epithelial cells but also controls the regulation of ORCC's. The cellular mechanisms involved in the regulatory relationship between CFTR and ORCC channels, especially with respect to the role of ATP, are being investigated.

SubMemBio Su-PM-2

REGULATION OF CFTR Cl CHANNEL GATING BY PKA PHOSPHORYLATION AND ATP HYDROLYSIS. ((D.C. Gadsby, T.-C. Hwang, G. Nagel, T. Baukrowitz, M. Horie & *A.C. Nairn)) Laboratories of Cardiac/Membrane Physiology and *Molecular and Cellular Neuroscience, The Rockefeller University, New York, NY 10021.

Cardiac CFTR Cl channels closely resemble those in epithelia. Both require phosphorylation by PKA followed by exposure to ATP before they will open. In cardiac myocytes, complete inhibition of types 1 and 2A cellular phosphatases (PPases) with okadaic acid or microcystin prevents full dephosphorylation of CFTR channels following inhibition of PKA. The resulting, partially deactivated Cl conductance is smaller than that observed during PKA stimulation, corresponding to the lower open probability (P_o) of partially, than of fully, phosphorylated channels in excised patches. In the absence of ATP, neither partially nor fully phosphorylated CFTR channels can be opened by AMP-PNP. But, in the presence of ATP, AMP-PNP acts on fully phosphorylated CFTR channels (with high P_o) to markedly delay channel closing, but has no effect on partially phosphorylated channels (with low P_o). This suggests that CFTR's two nucleotide binding domains (NBDs) are differentially regulated by incremental phosphorylation: PKA phosphorylation at sites dephosphorylated only by PPases 1 or 2A confers function on NBD-A at which ATP, but not AMP-PNP, can act. Additional phosphorylation, at sites dephosphorylated by other PPases, confers function also on NBD-B, which can interact with either ATP or AMP-PNP. Because ATP binding at NBD-B appears to cause a 2-3 fold stabilization of the channel open state, whereas AMP-PNP binding there causes a >100-fold stabilization, we conclude that ATP hydrolysis at NBD-B normally prompts channel closing. The inorganic phosphate (P_i) analogue, orthovanadate, also markedly stabilizes channels opened by ATP, but does so regardless of their P_o or degree of phosphorylation. P_i analogues inhibit ATPases by binding tightly at the site from which P_i dissociates after ATP hydrolysis. We therefore conclude that the free energy of ATP hydrolysis (at NBD-A) is used to open CFTR channels, which close following release of the hydrolysis products, ADP and P_i. We propose that when channels are fully phosphorylated, ATP binding at NBD-B delays release of the hydrolysis products at NBD-A and so delays channel closing. Hydrolysis of the ATP bound at NBD-B then relieves that inhibition, enabling release of the hydrolysis products at NBD-A and so permitting channel closing. Supported by NIH, NYHA and the CF Foundation.

SubMemBio Su-PM-3

GABA-GATED CHLORIDE CHANNELS IN NERVE: SEX, DRUGS, AND MOLECULAR BIOLOGY. **((Meyer B. Jackson)), Dept. Physiology, University of Wisconsin, Madison, WI.**

GABA activates $GABA_A$ receptors throughout the nervous system to gate a Cl^- channel, and usually to inhibit electrical activity. We have recently found that nerve terminals in the posterior pituitary contain a $GABA_A$ receptor which closely resembles the GABA receptor of nerve cell bodies (Zhang and Jackson, Science 259, 531, 1993). Activation of this receptor depolarizes the nerve terminal membrane by about 15 mV, resulting in an inactivation of Na^+ channels and block of action potential propagation. We found that the $GABA_A$ receptor of the posterior pituitary is modulated by neuroactive derivatives of gonadal steroids. By regulating oxytocin release this steroid action is capable of playing a role in the orchestration of reproductive endocrine function.

GABA also functions as an inhibitory transmitter in arthropods, by activating an invertebrate homologue of the $GABA_A$ receptor. This receptor has recently been cloned and sequenced in *Drosophila* by ffrench - Constant and coworkers. We have studied a picrotoxin-resistant mutant in *Drosophila*, and found that mutant receptor differs both in terms of ligand binding and desensitization behavior. We hypothesize that both alterations contribute to the resistance phenotype of the mutant receptor. 1) The mutant receptor binds picrotoxin less tightly. 2) The reduction of desensitization destabilizes the drug-preferred conformation. This interpretation helps explain why only one amino acid location has been associated with resistance to this class of drugs.

SubMemBio Su-PM-4

CHANNEL FUNCTION OF THE P-GLYCOPROTEIN (Pgp) OF MULTIDRUG RESISTANT CANCER CELLS **L. Reuss, G. A. Altenberg, C. G. Vanoye and E. Han, Department of Physiology and Biophysics, University of Texas Medical Branch, Galveston, Texas, USA.**

The channel function of P-glycoprotein (Pgp) was studied by measurements of single-channel currents, whole-cell currents, membrane voltage and intracellular ionic concentrations. We used a human breast cancer cell line with negligible Pgp expression (MCF-7) and a cell line transfected with human *mdr1* cDNA (BC19/3). BC19/3 cells express a Cl^- current activated by osmotic or hydrostatic cell swelling ($I_{Cl}(s)$) not present in MCF-7 cells, which reflects activation of at least two Cl^- channels (in symmetric 140 mM-Cl^- solutions, 10 pS and 25 pS, respectively). Activation of $I_{Cl}(s)$ is prevented by 100 μM vinblastine in the pipette (but not by other Pgp substrates) and by the mAb HYB-241 in the bath. Conductive Cl^- transport has no role in drug transport and does not contribute to cell volume regulation. The selectivity of the Cl^- channels for monovalent anions argues against significant permeation of ATP or other large organic anions. We conclude that Pgp expression is associated with a swelling-activated Cl^- current related to activation of two distinct ion channels whose precise relationship to Pgp is unclear. In human breast cancer cells, the channels are not involved in drug resistance or cell volume regulation. Supported by The John Sealy Memorial Endowment Fund.

SubMemBio Su-PM-5

VOLUME-REGULATED CL^- CHANNELS IN LYMPHOCYTES. ((M.D. Cahalan)) Dept. of Physiology & Biophysics, UCI, Irvine, CA 92717.

Lymphocytes and fibroblasts express a class of volume-activated Cl^- channel that is controlled by cell swelling. In response to an osmotic gradient (hypotonic outside or hypertonic inside), chloride conductance (g_{Cl}) can reach values of several nS, representing the opening of thousands of low-conductance Cl^- channels. In Jurkat T cells exposed to a 100-mOsM stimulus, Cl^- channel activation begins ~60 sec following the onset of swelling. During this period, the cell volume increases by about 20%. If Cl^- channels were inserted into the plasma membrane from an intracellular vesicular pool, an increase in membrane area and hence C_m would be expected to accompany the increase in g_{Cl}. However, C_m actually decreases slightly during the induction of g_{Cl}, arguing that the Cl^- channels pre-exist in the plasma membrane. In addition to swelling, channel opening also requires a source of intracellular ATP. Once opened, the channel rectifies outwardly and selects among different anions with the permeability sequence I^- (1.35) > SCN^- (1.23) > NO_3^-, Br^- (1.17) > Cl^- (1.0) > $MeSO_3^-$ (0.43) > acetate (0.32), propionate (0.27) > ascorbate (0.19) > aspartate (0.11) and gluconate (0.10). The estimated single-channel conductance is 2-3 pS. P-glycoprotein has been proposed to function as a drug-efflux pump and as a volume-activated chloride channel. However, control fibroblasts lacking P-glycoprotein express g_{Cl} channels at the same levels as *mdr-1* transfectants. We conclude that a wide range of cell types express these channels. The opening of Cl^- channels may provide the initial trigger for regulatory volume decrease in lymphocytes. Supported by NIH NS14609.

SubMemBio Su-PM-6

STRUCTURE AND FUNCTION OF CLC CHLORIDE CHANNELS: MULTIMERIC STRUCTURE OF CLC-11 REVEALED BY MUTATIONS IN DOMINANT MYOTONIA CONGENITA (THOMSEN). **((Michael Pusch, Klaus Steinmeyer, Claudius Lorenz, and Thomas Jentsch)) Centre for Molecular Neurobiology (ZMNH), Hamburg University, Martinistrasse 52, D-20246 Hamburg, Germany.**

Voltage-gated ClC chloride channels play important roles in cell volume regulation, control of muscle excitability, and probably transepithelial transport. First insights into structure-function relationship has been obtained for the structures involved in opening the volume activated ClC-2 chloride channel. ClC channels can be functionally expressed without other subunits, but it is unknown whether they function as monomers. We now exploit the properties of human mutations in the muscle chloride channel ClC-1 to explore its multimeric structure. This is based on analysis of the dominant negative effects of ClC-1 mutations causing Myotonia Congenita (MC, Thomsen's disease), including a newly identified mutation (P480L) in Thomsen's own family. In a co-expression assay, Thomsen's mutation dramatically inhibits normal ClC-1 function. A mutation found in Canadian MC families (G230E) has a less pronounced dominant negative effect, which can be explained by functional dominant negative effect, which can be explained by functional WT/G230E heterooligomeric channels with altered kinetics and selectivity. Analysis of both mutants show independently that ClC-1 functions as a homooligomer with most likely four subunits.