Materials for Faculty Search
Research Statement- Edward A. Berry, Ph.D.

Overview:

My main research interest is in biological energy transduction by membrane protein complexes, with emphasis on oxidative phosphorylation and photo-synthesis. Oxidative phosphorylation is the process by which oxidation of fuel molecules is used to drive phosphorylation of ADP to ATP, the "universal energy currency" of the cell. The oxidative part of oxidative phosphorylation involves transfer of reducing equivalents (electrons) along a chain of redox enzyme of successively lower reducing potential, and eventually to molecular oxygen (reducing it to water). In the process part of the energy available from these reactions is used to pump protons across a membrane, developing an electrochemical gradient in the proton which drives phosphorylation by ATP synthase.

My doctoral thesis was on measurement of the protonmotive force across the (sub)mitochondrial membrane, and relating it to phosphate potential. Since then my emphasis has been mainly on the enzymes of the respiratory chain. In two postdoctoral positions I developed skills in membrane protein purification and biophysics of electron transport applied to respiratory protein complexes.

Current research.

By 1990, the field of mitochondrial bioenergetics had reached the point where further advances really needed some solid structural information about the proteins involved. Furthermore the recent structure of Photosynthetic Reaction Center from Michel et al. showed that it was possible to do crystallography with membrane proteins. In 1990 we attempted to crystallize the bc1 complex, and were lucky to get crystals almost immediately. This success set the direction of research for the next 15 years, during which we crystallized cytochrome oxidase, the bc1 complex, and Complex II. (No one has succeeded in crystallizing Complex I yet).

We have solved numerous structures of the bc_1 complex in different states and with different ligands (1bcc, 2bcc, 3bcc, 1pp9, 1ppj 1zrt, 2a06 are deposited, ten more are being refined now). We discovered the movement of the Rieske iron-sulfur protein in the bc_1 complex, and inferred the involvement of this movement in the catalytic cycle. We solved the antimycin-inhibited complex at high enough resolution to unambiguously show that the bound inhibitor is in a different conformation from its solution state or small-molecule structure, correcting the mistakes of another group's modeling in a lower-resolution structure. (At 2.1 Å, this is the highest resolution of any bc_1 structure to date). We also solved the first structure of a bacterial bc1 complex, 1zrt. Our cytochrome bc1 complex work has been funded by NIH since 1993. We recently submitted a competing renewal along the lines described further below.

Developing a reproducible crystallization protocol for Complex II was surprisingly difficult, but we have it now for the avian enzyme, often yielding resolution beyond 2.0 Å. Occasionally a second crystal form is seen, with a

dimer in the asymmetric unit and resolution as high as 1.7 Å, far better than any other complex II crystals. Recently we succeeded in crystallizing the bovine enzyme, which is what has been used for most of the biochemistry in the literature. We have deposited structures (1yq3, 1yq4, 2fbw, 2h88, 2h89, eight more under refinement) with oxaloacetate, malonate, or 3-nitropropionic acid at the dicarboxylate site. The latter compound is a neurotoxin which causes neurodegeneration *in vivo* with symptoms like Huntington's disease. We showed the compound forms a stable adduct with a catalytic arginine in the dicarboxylate site, irreversibly inhibiting the enzyme. We are preparing a manuscript and structures with seven independent inhibitors of the Q-site (including Carboxin, TTFA, and Atpenin A5), which really define what is important for binding there. This NIH-funded project is now complete, but we plan to apply for a grant to start a new line of Complex II research as outlined below.

Future Plans

1. Take this research program to a more academic environment. While working in a government lab has been very productive, I would prefer a more academic environment. I would like to have more interaction with students in general, and to be able to take on graduate students. My main contact with students here has been with U.C. Berkeley undergraduates who come to do independent research or honors thesis research under my supervision. I would be glad to teach courses in basic biochemistry, cellular bioenergetics, or structural biology. However the teaching load should not be so heavy as to preclude my own research.

2. Further structural work. The days when solving a membrane protein structure was so significant that it could be the final goal of an NIH proposal are gone. In general a structural project should be undertaken to answer specific questions about function, mechanism, regulation, or inhibition. However I feel there are some structural projects significant enough to stand on their own.

One is the structure of Complex I, the remaining unsolved structure of the respiratory chain and a major source of reactive oxygen species in the cell. While the structure of the membrane-extrinsic part has been solved from a bacterial homolog, it is unlikely to answer questions about the mechanism of proton translocation as it does not include the ubiquinone site or any transmembrane portions. The large number of subunits and lack of a strong chromophore such as heme make it more challenging to prepare highly homogeneous samples of Complex I, but we are working to improve the preparations and the methods for characterizing it, including functional assays and RP-HPLC quantitation of the subunits. When we succeed in crystallizing it this will become a major project.

3. Application of structural methods to functional problems

Another project which uses structural studies to answer functional questions is aimed at understanding what causes the Rieske Iron-Sulfur protein extrinsic domain of the cytochrome bc_1 complex to move to different positions in the

presence of different inhibitors (and by extension at different stages of the catalytic cycle). Early results suggested that the H-bond between the substrate or inhibitor in the Qo site and the cluster-ligand histidine of the Rieske were important in holding it in that position. Xia and coworkers found an example in which that cannot be the case, and proposed that conformational changes in the docking surface on cyt b were responsible. However the differences in surface complimentarity and buried surface area which they documented were clearly due to the different positions of the Rieske and not to changes in cyt b; and the conformational changes in cyt b (which we also have seen) are as likely to be the result of pressure from docking the Rieske as they are to be the cause of the docking. In a proposal currently up for review at NIH, we propose four different ways to look at inhibitor binding and the resultant conformational changes under circumstances where the Rieske is absent or prevented from docking.

Another project we are currently initiating is development of a mutatable source of human Complex II for crystallization and functional studies. Up until now we have avoided problems of protein expression because we are working with high-copy-number proteins available from slaughterhouse material. This has the advantage that we are working with vertebrate sources, essentially the same as the human enzymes. However it has the disadvantage that mutagenesis is not practical. A number of human hereditary or aging-related diseases have been traced to mutations in Complex II. Some of these are not interesting from a structural point of view, for example frameshift mutations or deletions that eliminate the major part of one of the subunits. Others however involve a single amino acid change and understanding their affects may require seeing if there are far-reaching structural effects, and how the newly-introduced amino acid is arranged.

In general heterologous expression of multisubunit mitochondrial complexes is complicated by the fact that assembly of the complexes is tied up with import into the mitochondrial and insertion into the bilayer, and may involve translocases and processing proteases not present in the heterologous host. Insertion of the iron-sulfur clusters and heme are additional problems in the case of Complex II. However mitochondria and many of their proteins are derived from α-proteobacteria, and in the case of complex II bacteria such as *Paracoccus denitrificans* and *Rhodobacter capsulatus* have a closely related 4-subunit Complex II. It seems quite likely that putting the human mature sequences in the bacterial operon, and introducing it in a high-copy-number plasmid in a strain from which the native operon has been eliminated, would lead to an efficient expression system. Furthermore growth with succinate as a carbon source would allow an easy assessment of functionality of the enzyme, both in initial preparation of the substituted strain and later in characterizing the effect of mutations. These bugs are very versatile metabolically so it should be possible to find growth conditions not depending on Complex II in order produce the "lethal" mutations. Once good expression is verified chromatophore membranes will be prepared by disruption with the French press, activity will be characterized in the

membranes and after purification, and the purified protein will be crystallized for structural studies.

I also have considerable interest in photosynthetic systems. Although my time as a post-doc in Tony Croft's lab was spent mainly on the bc1 complex and oxidases of Rhodobacter, I had a lot of exposure to work on reaction centers, b6f, and PS II by others in the Crofts and Govindjee Labs, as well as photophysical techniques and their interpretation: flash activation, fluorescence yield, phosphorescence. After moving to Berkeley, I worked for several years at a bench in the old Calvin Lab adjacent to members of the Klein/Sauer/Yachandra group. At that time as a side project I developed a chromatographic b6f preparation starting with a Triton-X-100 extract of chloroplasts which was a side product of their Spinach PS II preparation. This could easily be scaled up to provide material for crystallization. Although structures are available for algal and cyanobacterial b6f complexes, the resolution leaves much to be desired and these structures lack the Ferredoxin:NADP+ Reductase subunit known to be associated with the spinach b6f complex and believed to be involved in cyclic photosynthetic electron transport. I would like to extend this project as well as working on structural biology of the chloroplast reaction centers and light-harvesting complexes.

Another long-time interest of mine, unrelated except that it has grown out of our work with colorful heme proteins of the mitochondria and their spectral changes with redox state, is the development of software to apply linear algebraic methods to spectroscopic data. The first type of problem is analyzing a spectrum or a series of spectra at different time points, from a mixture containing many chromophores whose individual spectra are known, to determine the concentration and changes in concentration of each. This greatly extends the power of vis/UV spectroscopy in the direction of the Warburg and Christian determination of protein in the presence of nucleic acid, or the Arnon method for determining chlorophylls a and b in extracts containing both.

The second problem is determining the spectra of individual components of a mixture when it is impractical to separate them- for example the four chromophores of the bc1 complex, which cannot be isolated without spectral alterations. Given a set of spectra in different redox states, and a kinetic or thermodynamic model explaining those redox levels in terms of adjustable parameters (rate constants, midpoint potentials), the difference extinction coefficients for each reaction at each wavelength become linear parameters in a nonlinear minimization problem, and so solving the minimization problem provides the individual spectra. Application of the Singular Value Decomposition reduces the dimension of the problem and provides a guide to the number of components present. We have developed a powerful set of software tools for these problems, and I am initiating a project at sourceforge.org to further develop the suite and make it available to all researchers.