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PURIFICATION AND CHARACTERIZATION OF A MUSCLE CYTOSOLIC CHAPERONIN.

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Folding of the heavy meromyosin (HMM) subfragment of myosin synthesized *de novo* in a rabbit reticulocyte lysate is mediated by an eukaryotic cytosolic chaperonin, (CCT). The CCT is a 16-18 mer composed of up to nine different TCP-1 subunits. Analysis of the folding intermediates reveals incomplete folding of the motor domain. However, the heavy chains form homodimers and associate with the light chains stochiometrically. Addition of a cytoplasmic extract prepared from myogenic cells to the reactions dramatically improves the folding of HMM, suggesting a role for muscle-specific factors in the folding pathway. These may represent additional cofactors that enhance the activity of the existing reticulocyte CCT, or simply another chaperonin enriched in the subunits suitable for myosin folding. To distinguish between these possibilities and to understand the folding of myosin *in vivo*, we purified cytosolic chaperonin from chicken skeletal muscle. Using an anti-TCP-1β antibody, we identified two CCT complexes with similar molecular mass, but distinct ionic properties. We developed a functional assay, in which nascent HMM synthesized in reticulocyte lysate is incubated with purified muscle chaperonin(s) and analyzed for its ability to bind and release from actin in an ATP dependent manner. The chaperonins are also being characterized by electron microscopy, subunit composition, ATPase activity, and ATPase activation by various target proteins. Supported by NIH grant AR38454.

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A COPPER CHAPERONE THAT IS SPECIFIC FOR CU_A, BUT NOT FOR CU_B, ASSEMBLY IN CYTOCHROME OXIDASE FROM *BACILLUS SUBTILIS* 168.

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Bacillus subtilis 168 expresses two members of the cytochrome oxidase family of integral membrane proteins. One enzyme is a cytochrome *c* oxidase, which uses a dinuclear copper centre called Cu_A as the initial electron acceptor from reduced cytochrome *c*, and the second enzyme is a menaquinol oxidase that does not have a Cu_A centre. Both enzymes have a second copper centre, Cu_B, which is physically associated with the site of oxygen binding. We have cloned the gene *ypmQ* from *B. subtilis* that is homologous to cytochrome oxidase copper chaperone Sco1, which was first identified in yeast. When *ypmQ* was deleted from the chromosome of *B. subtilis*, cytochrome *c* oxidase activity was absent when the strain was grown on solid growth media; the menaquinol oxidase was apparently unaffected. This deletion can be complemented by the expression of *ypmQ*, with or without a poly-histidine tag, from a plasmid. Interestingly, the deletion was not complemented by a mutated form of *ypmQ*, in which cysteine residues 64 and 68, proposed to be functional in copper insertion, were replaced with serine residues. The wild-type and C64S, C68S YpmQ proteins were present in about equal amounts when detected by western blotting. (This work was supported by an MRC grant to BCH.)

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NEURONAL TAU ACTS AS ANTI-CHAPERONE LIKE FUNCTION TO GAPDH

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Tau, a member of microtubule associated proteins, functions in promoting the assembly and stabilization of microtubule, and plays an important role in neuronal morphogenesis, maintenance of axonal shape and axonal transportation. Recently, *D*-glyceraldehyde-3-phosphate dehydrogenase (GAPDH, EC 1.2.1.12) was used as a substrate to research the effect of some chaperones, for example, protein disulfide isomerase on the refolding and reactivation. Human neuronal tau (Tau-40) has been used to study on denaturation and renaturation of rabbit muscle GAPDH. Tau suppressed refolding and reactivation of GAPDH when this enzyme was reactivated by dilution of GuHCl. Tau did not influence the activity of GAPDH at room temperature or in the solution absence of the denaturant. Furthermore, tau improved aggregation of non-native GAPDH either in GuHCl solution at low concentration or by heating. It suggested that tau may act as an anti-chaperone to GAPDH. However, tau lost the anti-chaperone-like function when it was aggregated or phosphorylated by neuronal cdc2-like protein kinase. The anti-chaperone-like function depends on tau native conformation.

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SYNCHROTRON X-RAY FOOTPRINTING OF THE GELSOLIN-ACTIN COMPLEX

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Synchrotron X-ray footprinting methods are applied to structural studies of gelsolin segment-1 and actin, as a model protein-ligand system. This high affinity (nM) macromolecular complex is an ideal system as its crystal structure has been determined, identifying specific amino acids participating in the binding interface. Radiolysis of water by synchrotron radiation generates a high flux of hydroxyl radicals that oxidize amino acid side chains of proteins according to their solvent accessibility. As the solvent accessibilities of discrete residues are altered upon interactions of a protein with ligand, synchrotron footprinting provides a method to probe these changes, and through mass spectrometric analysis, identify the residues involved in ligand binding. A number of amino acids in gelsolin undergo substantial changes in solvent accessibility upon interaction with actin, and we predict that these gelsolin residues will have different rates of oxidation, in the presence and absence of actin. The extent of radiolytic oxidation of gelsolin actin binding residues, after millisecond exposure to synchrotron X-rays, has been quantitated by mass spectrometry. The rates of oxidation of these residues have been established in the absence of actin, and will be compared to the rates in the presence of actin. The change in the extent of oxidation of these amino acids in the complex will be representative of an actin "footprint" on gelsolin.

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SYNCHROTRON X-RAY FOOTPRINTING OF PROTEIN ANTIGENS AND THEIR COMPLEXES

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A combination of synchrotron radiolysis and mass spectrometry is described to study the structure of proteins and the dynamics of protein-protein interactions. Hydroxyl radicals, produced by radiolysis of protein solutions with synchrotron light, react with aromatic and sulfur-containing amino acids within several milliseconds. The oxidation sites are identified by tandem mass spectrometry. The rate of oxidation at these reactive sites is measured as a function of exposure time based on a measure of the relative proportion of oxidized and unoxidized proteolytic peptide ions by mass spectrometry. We found that oxidation rates correlate with a theoretical measure of the solvent accessibility of residue side chains to provide a quantitative description of the three-dimensional surface of a protein. In the case of the lysozyme model the approach is able, for example, to distinguish the relative accessibility of the tryptophan residue side chains at positions 62 and 123 from each other and all other tryptophan residues based upon their rates of oxidation. We have applied the technique to a number of protein antigens to show that synchrotron X-ray footprinting can be applied to examine the nature and conformational dynamics of antigen-antibody interactions. Antibody bound to the surface of an antigen affords a degree of protection from oxidation at the reactive residues to enable the binding site to be identified.