

02.1-35 THE X-RAY STRUCTURE OF RICIN AND IMPLICATIONS FOR IMMUNOTOXIN DESIGN. By J.D. Robertus, W. Montfort, J.E. Villafranca, S.R. Ernst, and A.F. Monzingo, Clayton Biochemical Institute, Department of Chemistry, University of Texas, Austin, Texas.

Ricin is a heterodimeric protein toxin of molecular weight 65,000. Its B chain is a lectin which binds to cell surface glycoproteins and facilitates uptake of the toxin. The A chain kills the receptor cell by enzymatically attacking its ribosomes and inhibiting protein synthesis. Although ricin kills tumor transformed cells at least 100 times faster than normal cells, the toxin has been used most effectively when combined with antibodies against target cells to form a complex called an immunotoxin. These conjugates are not completely specific for cancer cells however, and best therapeutic results have been obtained when the immunotoxins are used against cells taken outside the body and placed in 100 mM galactose. The excess galactose retards non-specific binding by the B chain and subsequent immunotoxin uptake. Such a regimen is currently being used in clinical trials against leukemia.

In order to reduce non-specific cell toxicity by immunotoxins it would be desirable to modify the ricin B chain so as to mimic the effect of galactose in retarding cellular uptake. The Cetus Corporation has cloned the ricin B chain gene and a collaboration has been formed to engineer that gene to produce more therapeutically useful immunotoxins. The gene will be modified by oligonucleotide-directed site specific mutagenesis. The X-ray structure will be used to guide that mutagenesis by identifying key residues mediating cell surface binding and uptake.

Particular attention will be paid to those residues in the structure whose side chains form hydrogen bonds to the bound galactose sugars. Mutation at these residues, say a key serine to alanine change, would diminish carbohydrate and cell surface binding. It would probably be better to aim for retarded sugar binding than total abolition of binding, as some surface interaction may be required to trigger endocytosis of the immunotoxin. A poorly binding ricin derivative would eventually be taken up by a cell to which it was tethered by an antibody, whereas untethered molecules should be much less cytotoxic to non-targeted cells than is wild type ricin.

A 2.8 Å MIR map has been obtained for ricin. Difference Fouriers between ricin lactose and no lactose complexes discriminated between the A and B chains. Also these maps revealed that ricin binds two lactose molecules, each on a separate B chain domain.

02.1-36 A LOW RESOLUTION MODEL OF GLUTAMINASE-ASPARAGINASE FROM ACINETOBACTER GLUTAMINASIFICANS By Herman L. Ammon and Kenan C. Murphy, Dept. of Chemistry, University of Maryland, College Park, MD 20742, U.S.A., and Alexander Wlodawer, National Bureau of Standards, Washington, DC 20234, and National Institutes of Health, Bethesda, Maryland 20205, U. S. A., and Lennart Sjölin and Anders Svensson, Dept. of Inorganic Chemistry, Chalmers Polytechnic, Göteborg, Sweden.

The administration of L-asparaginase (L-asparagine amidohydrolase) leads to the regression of certain lymphomas and leukemias in experimental animals and in humans and the E. coli enzyme has been used in the treatment of acute leukemia for about twenty years. The utility of glutaminase in the treatment of leukemia is being evaluated and its activity against asparaginase resistant cells and in asparaginase resistant patients has already been demonstrated.

The bacterial asparaginases and glutaminases are tetrameric molecules with molecular weights in the range of 120,000-147,000 daltons; subunit molecular weights range from 32,000-37,000 daltons. The subunits in a particular enzyme are probably identical and there has been considerable evidence from X-ray studies for an arrangement of subunits with overall 222 symmetry.

We have crystallized glutaminase-asparaginase from acinetobacter glutaminasificans (space group I222, a=96.7, b=112.4, c=70.9 Å) and collected native data to 2.7 Å resolution. Data for three derivatives ranging from 3.2 to 6.0 Å have also been measured. An electron density map based on MIR phases has been calculated. In addition, maps at 3.2 Å resolution have been calculated using B. C. Wang's density modification and phase extension method. Molecular boundary appears to be quite clear and chain tracing is in progress.

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02.1-37 STRUCTURAL STUDIES OF HEMERYTHRIN.

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Crystallographic studies of the oligomeric oxygen-binding protein, hemerythrin, have been carried out to characterize the protein, its binuclear Fe center and its mode of binding of ligands. Refined structures of met and azidomet forms of the protein have been obtained at 2.0 Å resolution and allow a determination of the interactions between the components of the liganded and unliganded states of the macromolecule, i.e., the protein, the metal center, and the exogenous ligands. The structures of the Fe complex in met and azidomet forms of the protein will be summarized to show that one Fe remains 6-coordinate while the other is converted from 5-coordinate to 6-coordinate upon binding of azide. Preliminary structural studies of deoxy (3.9 Å resolution) and oxyhemerythrin (2.2 Å resolution) indicate similarities between the binuclear Fe centers in deoxy and met and between those in oxy and azidomet. The changes in structure associated with binding of dioxygen to deoxy are similar to those found in comparing met and azidomet.

A detailed description of the alpha-helical protein will also be presented with special attention on the conformational changes caused by binding of ligands. The structural differences in the protein observed by comparing the unliganded (met, deoxy) and liganded (azidomet, oxy) structures are mainly located in two regions, near one of the subunit interaction regions and near the C-terminus of the polypeptide. These portions of the protein might provide a molecular connection between the allosteric oxyanion binding site and the metal center, and this aspect of the conformational change will be emphasized.