CRYSTAL STRUCTURE OF PS8/ERGIC-53, A PROTEIN INVOLVED IN GLYCOPROTEIN EXPORT FROM THE ENDOPLASMIC RETICULUM

L. Velloso 1 K. Svensson 2 G. Schneider 1 R. F. Pettersson 1 Y. Lindqvist 1 S. Watanabe 1 M. Yao 1 Y. Takada 1 N. Fukunaga 1 I. Tanaka 1 J. Chevalier 1 B. Langlois d'Estaintot 1 B. Gallois 1 G. Precigoux 1 B. Corsi 3 S. Levi 2 P. Arosio 3 J. Drysdale 4

1 Karolinska Institute Medical Biochemistry and Biophysics Division of Molecular Structural Biology Tomtebodavagen 6, 4tr STOCKHOLM S-17177 SWEDEN 2 Ludwig Institute for Cancer Research, Stockholm Branch, Karolinska Institute, Box 240, S17177, Stockholm, Sweden

Proper folding, maturation and export of glycoproteins from the endoplasmic reticulum (ER) is an important cellular process in higher eukaryotes. Recently it has been shown that some glycoproteins need sorting receptors in order to exit the ER efficiently. p58/ERGIC-53 was the first ER cargo receptor identified. It has a carbohydrate recognition domain (CRD) with low homology to plant lectins which is highly conserved in eukaryotes. The gene for this protein has been identified independently as the locus for the bleeding disorder combined deficiency of coagulation factors V and VIII. We have overexpressed the CRD of p58/ERGIC-53 in insect cells, crystallized it and solved its structure to 1.46Å by MIR. The structure of the monomeric protein displays striking resemblance to leguminous lectins and the ER folding chaperone calnexin, representing one of the first observations of this domain fold in the animal kingdom. The putative ligand binding site of p58/ERGIC-53 has residues which are conserved when compared to the leguminous lectins occupying equivalent positions. The binding site lies in a negatively charged cleft on the surface of the protein. By mapping the sequence conservation onto the surface of the p58/ERGIC-53 structure, we have identified a conserved surface patch which is likely to be involved in oligomerization, which is important for the cargo receptor activity of this protein. Our data together with previous functional studies and the recently determined structure of calnexin define a new class of animal calcium dependent lectins with roles in the quality control mechanisms in the ER.

Keywords: LECTIN, GLYCOPROTEIN, ENDOPLASMIC RETICULUM


STRUCTURE ANALYSIS OF THE MONOMERIC IDH USING Mn-MAD METHOD - DIMER MIMICRY BY A DOMAIN DUPLICATION

Y. Yasutake 1 S. Watanabe 1 M. Yae 1 T. Akada 1 N. Fukunaga 1 I. Tanaka 1
Hokkaido University Division of Biological Sciences Graduate School of Science Kita-10, Nishi-8, Kitau-Ku SAPPORO HOKKAIDO 060-0810 JAPAN

NADP-dependent isocitrate dehydrogenase is a member of the β-decarboxylating dehydrogenase family, and catalyzes the oxidative decarboxylation reaction from 2R,3S-isocitrate to yield 2-oxoglutarate and CO2 in the Krebs cycle. Although most prokaryotic NADP-dependent IDHs are dimeric enzymes composed of two identical subunits with a molecular weight of 40-50 kDa, the monomeric IDH with a molecular weight of 80-100 kDa has been found in a few species of bacteria. We successfully determined the 1.95 Å crystal structure of the monomeric IDH in complex with isocitrate and Mn from the nitrogen-fixing bacterium Azotobacter vinelandii by the multiwavelength anomalous diffraction (MAD) method. The MAD phase calculation was performed with the anomalous signal of two Mn atoms bound to two independent IDH molecules in an asymmetric unit. The final refined model of the monomeric IDH revealed that it consists of two distinct domains, and its folding topology is related to the homodimeric form of the dimeric IDH. The structure of the large domain repeats a motif observed in the dimeric IDH, and the motifs are related by a pseudo 2-fold axis. This pseudo symmetry coincides with a crystallographic 2-fold axis that relates identical subunits of the E. coli dimeric IDH. Such a fusional structure by a domain duplication enables a single polypeptide chain to form a structure at the catalytic site that is homologous to the dimeric IDH, the catalytic site of which is located at the interface of two identical subunits and is formed with residues from both subunits.

Keywords: ISOCITRATE DEHYDROGENASE MOLECULAR EVOLUTION MN-MAD


CRYSTALLIZATION AND X-RAY ANALYSIS OF MYCOBACTERIUM TUBERCULOSIS HSP65

N. Adir 1 E. Dobrovetsky 1 Y. Kashir 1
1 Department of Chemistry and Institute of Catalysis, Science and Technology, Technion, Technion City 32000, Haifa, ISRAEL 2 Dept. of Food Engineering & Biotechnology, Technion - Israel Institute of Technology Haifa 32000, Israel

Rheumatoid arthritis is a common chronic inflammatory disease of the joints that leads to severe deformation and incapacitation of affected extremities. Adjuvant arthritis (AA) in the Lewis rat resembles in many aspects the human disease and, therefore, serves as a useful experimental model. It is an autoimmune disease that is driven by T-cells against specific epitopes on the Mycobacterium tuberculosis HSP65 (MT65) molecule. The role of the 65 kDa heat shock protein of Mt (MT65) in the pathogenesis of autoimmune arthritis, both in experimental animals as well as in humans has been investigated intensively in the past several years. In patients suffering from rheumatoid arthritis, an association between T-cell responses to MT65 and early stages of joint inflammation has been found. Pre-immunization of rats with the mycobacterial MT65 leads to resistance to induction of the disease by Mt, and this protective effect is believed to be mediated by T-cells specific for epitopes on the Mycobacterium tuberculosis HSP65 (MT65) molecule. The role of the 65 kDa heat shock protein of Mt (MT65) in the pathogenesis of autoimmune arthritis, both in experimental animals as well as in humans has been investigated intensively in the past several years. In patients suffering from rheumatoid arthritis, an association between T-cell responses to MT65 and early stages of joint inflammation has been found. Pre-immunization of rats with the mycobacterial MT65 leads to resistance to induction of the disease by Mt, and this protective effect is believed to be mediated by T-cells specific for epitopes on the Mycobacterium tuberculosis HSP65 (MT65) molecule. The role of the 65 kDa heat shock protein of Mt (MT65) in the pathogenesis of autoimmune arthritis, both in experimental animals as well as in humans has been investigated intensively in the past several years.

Up to now, the observed mammalian ferritins are heteropolymers (24 subunits) with variable amounts of both subunits H and L. The H chain possesses a ferroxidase activity responsible for the cytoprotective action of ferritin. The L chain has a nucleation centre which favours the mineral growth of ferric hydroxide in the cavity. Recently, a new human ferritin (MfH), encoded by an intronless gene and targeted to mitochondria, was identified and characterized (Levi et al. J. Biol. Chem., 2001, 276(27):24437-24440). It is expressed as a 30 kD precursor further processed into a 22 kD subunit, the sequence of which overlaps that of recombinant human H chain ferritin (rFHA). Both sequences share 81% identity and a conserved ferroxidase centre. MfH is the first example of a homopolymer mammalian ferritin specifically targeted to an organelle. Crystals of MfH were obtained using MgCl2. The structure was solved by molecular replacement (starting model FHA, pdb entry code 2FHA). To investigate the iron binding sites, several crystals were soaked with various metal salts, MnCl2, TbCl3, and further cryo-cooled at 100 K. The corresponding structures were refined at 1.7 - 2.2 Å resolution. The following results will be discussed: - the binding geometries of aquated Mg2+ ions observed at the 3-fold symmetry pores suggest Fe2+ ions undergo a dehydration process when crossing the protein shell. - Contrary to Tb3+ which only binds the 3-fold symmetry axes, both Mg2+ and Mn2+ ions bind at the ferroxidase center though with noticeable differences (site occupancy, µ - oxo bridge formation).

Keywords: MOLECULAR CHAPERONS, HEAT SHOCK PROTEINS, RHEUMATOID ARTHRITIS


IRON STORAGE ANOMALOUS SCATTERING CRYSTALLOGRAPHY


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N. Adir 1 E. Dobrovetsky 1 Y. Kashir 1
1 Department of Chemistry and Institute of Catalysis, Science and Technology, Technion, Technion City 32000, Haifa, ISRAEL 2 Dept. of Food Engineering & Biotechnology, Technion - Israel Institute of Technology Haifa 32000, Israel

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