P.04.25.9

Acta Cryst. (2005). A**61**, C264

Serendipitous Discovery and X-ray Structure of a Human Phosphate Binding Apolipoprotein

Eric Chabriere[7,3], Renaud Morales[1], Anne Berna[2], Philippe Carpentier[1], Carlos Contreras-Martel[1], Frédérique Renault[3], Murielle Nicodeme[4], Marie-Laure Chesne-Seck[5], François Bernier[2], Christine Schaeffer[6], Hélène Diemer[6], Alain Van-Dorsselaer[6], Juan C. Fontecilla-Camps[1], Patrick Masson[3], Daniel Rochu[3], [1]*Laboratoire de Cristallogenèse et Cristallographie des Protéines, Institut de Biologie Structurale JP EBEL, 38027 Grenoble, France.* [2]*Institut de Biologie Moléculaire des Plantes du CNRS, Université Louis Pasteur, 67083 Strasbourg Cedex, France.* [3]*Unité d'Enzymologie, Département de Toxicologie, Centre de Recherches du Service de Santé des Armées, 38702 La Tronche, France.* [4]*Laboratoire des Biosciences de l'Aliment, Université Henri Poincaré-Nancy 1, 54506 Vandoeuvre-lès-Nancy, France.* [5]*Laboratoire de Cristallographie Macromoléculaire, Institut de Biologie Structurale JP EBEL, 38027 Grenoble, France.* [6]*Laboratoire de Spectrométrie de Masse Bio-Organique, ECPM, 67087 Strasbourg, France.* [7]*Laboratoire de Cristallographie et Modélisation des Matériaux Minéraux et Biologiques, CNRS-Université Henri Poincaré, 54506 Vandoeuvre-lès-Nancy, France.* E-mail: eric.chabriere@lcm3b.uhp-nancy.fr

Solute-binding proteins[1] (SBPs), ubiquitous in archaea and bacteria, play a central role in the substrate uptake mechanism of ATP binding cassette transmembrane transporters[2] (ABC transporters). Despite the existence of ABC transporters in eukaryotes, no SBPs have been characterized or predicted from genome database analyses in this kingdom. Here, we report the discovery of a HDL-associated, phosphate SBP in human plasma and provide the first X-ray structural determination of a eukaryotic SBP. In addition, we conclusively demonstrate that phosphate SBPs are ubiquitous in all taxa including the domain eukaryota. The systematic absence from genome databases of genes associated with eukaryota phosphate SBP, and an astonishing 90% conservation at the nucleotide level of these genes between distant species raise new intriguing questions related to the peculiar features of these genes. Although phosphate is essential to metabolism, this protein is the first identified transporter capable of binding phosphate ions in plasma. This phosphate SBP is similar to another unpredicted human protein, namely the Crystal Adhesion Inhibitor, that could prevent development of kidney stones[3]. Thus these results provide new insights into phosphatemia and related pathologies such as atherosclerosis[4].

[1] Tam R., Saier M.H., *Microbiol. Rev.*, 1993, **57**, 320. [2] Higgins C.F., *Annu. Rev. Cell Biol.* 1992, **8**, 67. [3] Kumar V., Shihui Y., Toback G., Lieske J.C., *Am. J. Physiol*, 2004, **287**, 373. [4] Amann K. et al., *Kidney Int.* 2003, **63**, 1296.

Keywords: ABC transporters, atherosclerosis, missing gene

P.04.25.10

Acta Cryst. (2005). A**61**, C264

Structural Basis for Cyan-Emitting Mechanism in a Cyan Fluorescent Protein

Akihiro Kikuchi[a], Eiko Fukumura[a], Satoshi Karasawa[b], Atsushi Miyawaki[b], Yoshitsugu Shiro[a], [a]*RIKEN Harima Institute at SPring-8, Kouto, Mikazuki-cho, Sayo-gun, Hyogo 679-5148, Japan.* [b]*Brain Science Institute, RIKEN, Hirosawa, Wako city, Saitama 351-0198, Japan.* E-mail: kikuchi@spring8.or.jp

Green fluorescent protein (GFP) from *Aequorea victoria* has become an important tool in molecular and cellular biology and spectral variant proteins with blue, cyan and yellow emissions have been generated from wild-type GFP. They have expanded the range of colours available for application of GFP-based techniques. As far, substitution of tryptophan for tyrosine at the second amino acid of the chromophore-forming tripeptide was the only procedure for generating cyan-emitting fluorescent proteins (CFPs). On the other hand, some CFPs that possess a tyrosinyl-chromophore have been recently cloned from Anthozoa. To understand cyan-emitting mechanism in the CFPs from Anthozoa, it is imperative to have structural information regarding their chromophore environment.

We have cloned and subsequently solved the crystal structure at 1.4 Å resolution of a new CFP from *Fungia* coral. The structure reveled that the chromophore is formed by cyclization reaction of three residues (Ser-Tyr-Gly), which is the same as that of GFP, and that the direction of –OH group in the Ser residue should be of importance for the nature of cyan-emitting property. Theoretical calculation supports the proposed mechanism.

Keywords: fluorescent proteins, structural biochemistry, structure-properties relationships

P.04.25.11

Acta Cryst. (2005). A**61**, C264

Structural Basis of Glycogen Synthesis

Marcelo E. Guerin[a], Alejandro Buschiazzo[a], Samuel Lara Gonzalez[a], Gwenaelle André[a], Juan E. Ugalde[c], William Shepard[b], Rodolfo A. Ugalde[c], Pedro M. Alzari[a], [a]*Unité de Biochimie Structurale, Institut Pasteur, Paris.* [b]*ESRF, Grenoble, France.* [c]*IIB-UNSAM, Buenos Aires, Argentine.* E-mail: mguerin@pasteur.fr

Glycogen and starch are the major carbon and energy storage compounds in most living organisms. Glycogen synthase (GS) [EC 2.4.1.21] is a key component of the enzymatic machinery involved in glycogen metabolism, catalyzing the successive addition of α-1,4-linked glucose residues to the non-reducing end of glycogen. The other components of this machinery are glycogen phosphorylase (GP) and the branching/debranching enzymes. GSs from bacteria and higher plants (starch synthases) are α-retaining family GT5 that use ADP-glucose as sugar donor and have MW around 50 kDa. We now report the first 3D structure of GS at 2.3 Å resolution in the presence and absence of adenosine diphosphate [1]. The recombinant enzyme from *A. tumefaciens* was purified to homogeneity and crystallized [2]. The overall fold and the active site architecture of the protein are remarkably similar to those of GP, indicating a common catalytic mechanism and comparable substrate-binding properties.

[1] Buschiazzo A., et al., *EMBO J*, 2004, **23**, 3196. [2] Guerin M.E., et al., *Acta Crystallogr. D*, 2003, **59**, 526.

Keywords: glycogen, glycosyltransferase, X-ray crystallography

P.04.25.12

Acta Cryst. (2005). A**61**, C264

Dramatic Structural Change in CLIC1: Globular Protein that Forms an Ion Channel

Paul Curmi[a,b], Dene Littler[a,b], Stephen Harrop[a,b], Louise Brown[a,b], Greg Pankhurst[b], Raffaella Tonini[c], Michele Mazzanti[c], Samuel Breit[b], [a]*School of Physics, University of New South Wales, Sydney, Australia.* [b]*Centre for Immunology, St Vincent's Hospital, Sydney, Australia.* [c]*University of Rome 'La Sapienza', Rome, Italy.* E-mail: p.curmi@unsw.edu.au

The CLIC proteins are an unusual family of mainly cytosolic proteins that can integrate into membranes to form ion channels. The family consists of six members that are highly conserved in vertebrates. The crystal structure of the soluble form of CLIC1 belongs to the GST fold family. On oxidation, CLIC1 undergoes a dramatic structural change, which is coincident with non-covalent dimerisation and the formation of an intramolecular disulphide bond.

In the oxidized form, the characteristic 4 stranded beta sheet of the GST fold has been transformed into helical and loop segments, resulting in the formation of a large hydrophobic surface which forms the dimer interface. We postulate that this altered structure represents the membrane docking form. Surface plasmon resonance, liposome chloride efflux experiments and tip dip electrophysiology show that CLIC1 binds to membranes to form a chloride ion channel under oxidizing conditions. Reducing agents inhibit or prevent channel activity. The electrophysiological characteristics of the channel form by CLIC1 alone in an artificial bilayer are identical to those observed in cells overexpressing CLIC1. Thus, we believe that oxidation triggers CLIC1 membrane insertion and that the dramatic structural rearrangement of the N-terminal domain is on pathway to channel formation.

Keywords: conformational change, ion channel proteins, redox proteins