A-helices of the  $\beta$ -subunits, differences in the interactions of dimerdimer interface hinge region and the constrained heme environment associated with  $\beta_1$ -subunit are the most significant structural differences which presumably play a remarkable hampering effect on oxygen affinity of sheep hemoglobin.

Keywords: methemoglobin, allosteric mechanism, low-oxygen affinity

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## Purification, crytallization and X-ray structure determination of cocosin from *Cocos nucifera*

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Plant proteins are the cheapest available sources of nutrition in many countries; hence form an important part of human diet. Large quantities of storage proteins are accumulated in developing seeds of leguminous plants that function as a reserve of carbon and nitrogen used during germination and early growth. These proteins are deposited in an aggregated form within specialized organelles of the seed called protein bodies. There are two major types of storage proteins in legume seeds, known as vicilins and legumins, which are distinguishable by their sedimentation coefficients (7S/11S), oligomeric organization (trimeric/hexameric) and polypeptide chain structure (single chain/disulfide linked pair of chains). 11S globulins are non-glycosylated proteins, each of the subunits in the hexamer itself is composed of an acidic and a basic chain derived from a single precursor and linked by a disulphide bond. Globulin (11S) is one of the major storage proteins of many legume and nonlegume seeds. Coconut (Cocus nucifera), is a major source of plant protein in most tropical and subtropical regions of the world. The globulin cocosin is a legume class reserve protein in coconut. It is important to understand the biosynthesis, targeting and biological functions of seed proteins as a prerequisite to their rational manipulation in improving nutritional value. However, such information is still in paucity for seed storage proteins of coconut. Therefore, the cocosin is purified from the coconut endosperm and the crystal structure was determined at 2Å resolution. The detailed structural description is to be presented.

Keywords: seed storage protein, cocosin, crystal structure determination

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# Structural analysis of a giant cell wall-associated adhesion protein Ebh from *Staphylococcus aureus*

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Staphylococcus aureus is a major cause of hospital- and communityacquired infections. S. aureus causes serious and fatal diseases, such as toxic shock syndrome or septicemia. Genome analyses of several strains of S. aureus revealed the presence of a giant gene 31494 bp in length. The putative protein of ca. 1.1 MDa encoded by this gene shows homology to the major adhesion protein of Streptococcus defectivus, Emb, a protein that binds to the extracellular matrix (ECM) of host cells, and therefore the giant protein was named Ebh (ECM binding protein homologue). Ebh consists of several distinct regions, including a large central region with 52 imperfect repeats of 126 amino acid residues. In the present study, we investigated the structure of this giant molecule by X-ray crystallography, CD spectrometry, and small-angle X-ray scattering (SAXS). The crystal structure of two repeats showed that each repeat consists of two distinct three-helix bundles, and two such repeats are connected along the long axis resulting in a rod-like structure 120 Å in length. CD and SAXS analyses of the samples with longer repeats suggested that each repeat has an identical structure and such repeats are connected tandemly to form a rod-like structure in solution the length of which increased proportionately to the number of repeating units. On the basis of these results, it was proposed that Ebh is a rod-like molecule 320 nm in length with some plasticity at module junctions.

Keywords: small-angle X-ray scattering, *ab-initio structure* determination, circular dichroism

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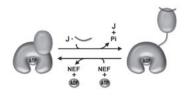
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## Crystal structures of the 70-kDa heat shock proteins in domain disjoining conformation

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The 70-kDa heat shock proteins (Hsp70s) are highly conserved ATP-dependent molecular chaperones composed of an N-terminal nucleotide binding domain (NBD) and a C-terminal protein substrate binding domain (SBD) in a bilobate structure. Interdomain communication and nucleotide-dependent structural motions are critical for Hsp70 chaperone functions. Our understanding of these functions remains elusive due to insufficient structural information of functionally intact Hsp70s in different chaperone cycle states. We report here the crystal structures of DnaK from Geobacillus kaustophilus HTA426 bound with ADP-Mg<sup>2+</sup>-Pi at 2.37 Å and 70-kDa heat shock cognate protein from Rattus norvegicus bound with ADP-Pi at 3.5 Å. The NBD and SBD in these structures are significantly separated from each other and they may be corresponding to the ADP-bound conformation. Moreover, a Trp reporter was introduced at the potential interface region between NBD and interdomain linker of GkDnaK to probe the environmental

changes. The result of fluorescence measurement further supports that the substrate binding enhanced domain disjoining behavior for Hsp70 chaperone family.



Keywords: Hsp70, chaperone, heat shock protein