s1.m8.p18 **Structural Studies on Human Vascular Adhesion Protein-1.** Heidi Kidron[a], Yvonne Nymalm[1], Annu Söderholm[a], Lenita Viitanen[a], Kimmo Kaukonen[b], Marjo Pihlavisto[b], Tomi T. Airenne[a], Mark S. Johnson[a] and Tiina A. Salminen[a], [a]*Department of Biochemistry and Pharmacy, Åbo Akademi University Finland,* [b]*BioTie Therapies Ltd Finland. E-mail: hkidron@abo.fi*

**Keywords: HVAP-1; Amine oxidase; X-ray structure**

Human vascular adhesion protein-1 (HVAP-1) is a 180 kD homodimeric membrane-bound multifunctional glycoprotein with both adhesive and enzymatic properties. The protein belongs to the copper-containing amine oxidase (CAO) family, which uses 2,4,5-trihydroxyphenylalanine quinone as a cofactor [1].

The CAO enzymes have been isolated from several different organisms, including bacteria, fungi, plants and mammals. In plants CAOs are involved *e.g*. in wound healing, whereas in procaryotes CAOs allow the organsim to utilize various amines metabolically as sources of nitrogen and carbon. However very little is known about the biological function of CAOs in higher eukaryotes besides their role in the metabolism of biogenic amines and the newly proposed function in regulation of glucose uptake and in leukocyte-endothelial cell interactions [3-6].

To this date, CAO crystal structures have been solved from *Escherichia coli*, pea seedling, *Hansenula polymorpha*, *Arthobacter globiformis* and *Pichia pastoris* but none has been solved from the animal kingdom. They all have a very similar structure where two monomers form a heartshaped dimer.

We have solved the structure of a mammalian CAO, HVAP-1 for the first time. It formed hexagonal crystals, which diffracted to 2.9 Å and they belonged to the space group $P6_522$. The structure was determined by molecular replacement with an $R_{factor}$ and $R_{free}$ of 24.1% and 26.6%, respectively.

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s1.m8.p19 **Indirubin and indigo analogues inhibit glycogen phosphorylase b by binding at the inhibitor or/and allosteric site.** Magda N. Kosmopoulou[a], Demetres D. Leonidas[a], Evangelia D. Chrysina[a], Gerhard Eisenbrand[c], Constantinos E. Sakarellos[d] and Nikos G. Oikonomakos[a, b], [a]*Institute of Organic and Pharmaceutical Chemistry and* [b]*Institute of Biological Research & Biotechnology, The National Hellenic Research Foundation, 48 Vas. Constantinou Ave., 11635 Athens, Greece;* [c]*University of Kaiserslautern, Department of Chemistry Food Chemistry & Environmental Toxicology PO Box 3049, D-67653 Kaiserslautern, Germany;* [d]*Department of Chemistry, University of Ioannina, 45110 Ioannina, Greece. E-mail: ngo@eie.gr*

**Keywords: Type 2 diabetes; Glycogen phosphorylase; Inhibitors; X-ray crystallography**

One of the major threats to human health in the 21st century is type 2 diabetes mellitus, potentially reaching a total of 200-300 million cases in 2010 worldwide [1]. At present, treatment of type 2 diabetes relies on diet, exercise and hypoglycaemic drugs. Most of the current antidiabetic drugs have limited efficacy and tolerability and therefore display significant mechanism-based side effects. Thus, there is a continued effort for the discovery of new hypoglycaemic drugs with improved therapeutic properties. A molecular approach aimed at reducing excessive glucose production from liver involves the identification and optimization of novel, potent and selective inhibitors of glycogen phosphorylase (GP), a key enzyme that catalyses the first step in glycogen degradation [2]. Previous studies have shown that flavopiridol, a compound that targets CDKs, a family of key cell cycle regulators, by competitively binding to the ATP binding site, was also inhibiting the activity of GP, by binding at the inhibitor (or nucleoside) site [3]. Compounds such as flavopiridol, that target both CDKs and GP, could have a combined action in tumor cells by disrupting the cell cycle and sending cells to apoptosis while starving cancer cells of glucose. Indigo, indirubin and other indirubin derivatives have been also reported as potent inhibitors of CDKs, and glycogen synthase kinase 3 (GSK3), one of main protein kinases that phosphorylate and inactivate glycogen synthase [4,5,6,7], by binding to the ATP binding site. In an effort to investigate whether indigo and indirubin analogues can inhibit GP activity, kinetic studies of 26 indirubin and 3 indigo analogues were performed with GPb. Measurements of the kinetic parameters at various inhibitor concentrations showed that 5 indirubin and 2 indigo analogues were moderate inhibitors of GPb (with Kis in mM range). X-ray crystallographic analysis has shown strong binding of E226, E243 and E220a at the inhibitor site, by intercalating between the two aromatic rings of Phe285 and Tyr613. Additional density indicated binding of E243 at the allosteric (or nucleotide) site (2 molecules/site). Comparison of the crystal structures with the structures of GPb-caffeine and GPb-flavopiridol complexes provides useful information for the design of new potent inhibitors of the enzyme.

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