The FnIII3-FnIII4 tandem has a radius of gyration (Rg), calculated from the SAXS data, of 21 Å, which is significant smaller than the Rg of the FnIII1-FnIII2 (~29 Å) indicating that the second pair of FnIII domains adopts a more compact structure than the first pair of FnIII domains. We have modelled the low resolution structure of the FnIII3-FnIII4 region by using the SAXS data and *ab initio* methods. This region has a heart-shaped structure. The limited resolution of the SAXS-based model hinders the unequivocal docking of the high resolution structures into the molecular envelope. Thus, we have used structure-based Cys-scanning mutagenesis combined with crosslinking experiments to obtain distance restraints that will help us to elucidate the relative orientation of the FnIII3 and FnIII4 in solution. Our results have implications for the organization of the integrin β4 subunit and for its mechanisms of auto-inhibition and activation.

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Keywords: integrin, SAXS, macromolecular-crystallography

MS51.P10

Acta Cryst. (2011) A**67,** C553

Raman assisted X-Ray crystallographic study of nitric oxide binding to deoxygenated hemoglobins

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Nitric oxide (NO) is a signaling molecule that regulates essential physiological processes, including neurotransmission, vasodilatation, and blood clotting [1].

The heme groups of deoxy hemoglobin (Hb) bind NO very strongly, almost irreversibly, with a $K_{diss} = 0.9 \times 10^{-12}$ M. The kinetic constant of NO dissociation from $Hb(NO)_4$ increases as the reaction progresses, indicating that partially NO-saturated T-state Hb has a lower NO affinity than fully bound, R-state, Hb [2].

Previous spectroscopic and crystallographic studies have shown that NO binding to the heme groups of T-state human hemoglobin (HbA) produces the breakage of Fe-proximal histidine bonds at the α-subunits but not at the β-subunits [3]. NO can also react with the thiol group of the Cys93β of HbA [4].

Until now there are very few crystallographic structures of nitrosyl-hemoglobin, due to the high and various reactivity of these species.

Here we report a Raman-assisted crystallographic study of the NO binding to the hemoglobin isolated from the Antarctic fish Trematomus bernacchii (HbTb). HbTb is endowed with the Root effect, i.e. a drastic drop of cooperativity at acidic pH [5,6]. The crystal structures of the nitrosylated form of T-state HbTb, crystallized at pH 6.2 and 8.4 (HbTb6NO and HbTb8NO), have been solved. These structures and the Raman spectra have been compared to those of nitrosylated HbA, reported in literature [3,7]. The main results of the analysis reveal a different behavior of α and β chains. In particular, in both HbTb6NO and HbTb8NO, the α-heme is nitrosylated and shows a six-coordination, whereas the iron ion at β-heme is clearly oxidized in high spin form.

This work was financially supported by PNRA (Italian National Program for Antarctic Research).

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Keywords: hemoglobin, binding, raman

MS51.P11

Acta Cryst. (2011) A**67,** C553

Small Angle X-ray Scattering Studies of Effect of pH and Temperature on the Global Compactness on Cellobiohydrolase I from *Trichoderma harzianum*

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Cellulases produced by filamentous fungi are widely used in biotechnological applications, including biomass depolymerization and second generation bioethanol production [1]. To make possible the use of biomass it is necessary to degrade cellulose, a constituent of the cell wall, to fermentable sugars [2]. One form of degradation is the enzymatic hydrolysis. The complete enzymatic cellulose hydrolysis involves synergistic actions of endoglucanases (EC 3.2.1.4), exoglucanases/cellobiohydrolases (EC 3.2.1.91) and β-glucosidases (EC 3.2.1.21) [3]The structural knowledge of the enzymes as well as the interaction of these with the pre-treated bagasse from sugarcane during the hydrolysis is extremely important. However, stability of cellulases in defined pH and temperature ranges sets limits to their industrial utilizations. SAXS is able to give structural parameters about intact structures, as radius of gyration, maximum diameter, shape and relative position of the domains. SAXS studies have been done with cellobiohydrolase I from *T. harzianum* (*Th*CBHI). These study aims at understanding the molecular basis of the functioning of enzymes for application in the production of bioethanol from sugarcane bagasse. Our results show that pH and temperature perturbations affect *Th*CBHI stability by two different mechanisms. Variations of pH modify protonation of the enzyme residues, directly affecting its activity, but leading to structural destabilization only at extreme pH limits. Temperature, on the other hand, has direct influence on mobility, fold and compactness of the enzyme, causing irreversible unfolding of *Th*CBHI just above optimum temperature limit. Thus, our studies might provide insights into understanding, of the interplay between structure and activity of *Th*CBH1 at different pH and temperature conditions, which can be useful for possible biotechnological applications of the enzyme as bioethanol production.

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Keywords: cellobiohydrolase_I, bioethanol, SAXS