MS7-P9 **Structural Characterization of Trypanosomatids Kinases: Targets to Drug Discovery** Tatiana Souza[1]; Daniel Trindade[1]; Celisa Tonoli[1]; Camila Santos[1]; Richard Ward[2]; Artur Oliveira[2]; Mario Murakami[1] [1]*Laboratório Nacional de Biociencias, CNPEM, Brazil*[2]*USP, Ribeirão Preto, Brazil*
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Leishmaniasis is a global public health problem that affects millions of people worldwide. Nucleoside diphosphate kinase b (NDKb) plays a pivotal role in the nucleotide salvage pathway, and recently it was found in the *Leishmania sp.*secretome, suggesting a new function related to the regulation of ATP levels in host cells. In this work, we describe the ?rst structural characterization of a nucleoside diphosphate kinase b from trypanosomatid parasites (tNDKbs) providing insights into their oligomerization, stability and structural determinants for nucleotide binding. Crystallographic studies of aNDKb from *Leishamania major* in complex with phosphate, AMP or ADP revealed the crucial hydrogen-bonding residues involved in nucleotide. SAXS experiments showed that tNDKbs, like other eukaryotic NDKs, form a hexamer in solution and their oligomeric state does not rely on the presence of nucleotides or mimetics. Fluorescence-based thermal-shift assays demonstrated higher stability of tNDKbs when compared to human NDKb (HsNDKb), which is in agreement with the fact that tNDKbs are secreted and subjected to variations of temperature in the host cells during infection and disease development. Contrasts on the surface electrostatic potential around the nucleotide-binding pocket might be a determinant for nucleotide a?nity and protein stability differentiation. Because of its key roles played in both parasite and host cells, tNDKb from *L. major* was subjected to high-throughput differential scanning fluorimetry in order to identify potential inhibitors. Remarkably, we identified trypanosomatid-specific inhibitors using HsNDKb as control. We confirmed the interaction by isothermal titration calorimetry. Assays of inhibition kinetics and *in vivo* tests are being performed.

Keywords: NDKb, trypanosomatids, structure

MS7-P10 **Structural evidence of anti-HIV lectin actinohivin specific to HMTG of gp120** M. Mominul Hoque[1,4], Jiandong Jiang[2], Kaoru Suzuki[3], Masaru Tsunoda[1], Naomi Ohbayashi[1], Xiaoxue Zhang[2], Takeshi Sekiguchi[3], Haruo Tanaka[1,2,5], and Akio Takénaka[1,2,6] [1]*Faculty of Pharm.,* [2]*Grad. Sch. Sci. & Eng.,* [3]*Coll. Sci & Eng., Iwaki-Meisei Univ., Iwaki 970-8044, Japan,* [4]*Dept. Biochem. & Mol. Biol., Rajshahi Univ., Rajshahi, Bangladesh,* [5]*KIIM Pharm. Lab. Inc., Fukushima 970-8551, Japan,* [6]*Grad. Sch. Biosci. & Biotech., Tokyo Inst. Tech., Yokohama, 226-8501, Japan*
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A new lectin actinohivin (AH) which exhibits a high anti-HIV activity is useful as a microbicide for preventing viral trans-mission, because this lectin specifically binds to high-mannose type glycans (HMTG) of HIV-envelop protein gp120, the target being the three branches attached commonly with α(1,2)-mannobiose (MB) at the respective terminals [1]. To obtain the structural insight of AH bound to the target, X-ray analysis of AH-MB complex was performed. The crystal structure belonging to the $P22_12_1$ space group has been solved at 1.90 Ĺ resolution with an R_{factor} of 0.164.

In the previously reported crystal of AH-MB complex (space group $P2_13$) [2], three AH molecules are disordered around the crystallographic 3-fold axis so that it was difficult to assign the peptide conformations of the peripheral flexible parts on the electron density map. In the present crystal, however, the complete structure has been successifully determined.

The AH structure is composed of the three identical structural modules with high sequence homology to each other, folded according to a 3-fold symmetry. In each module, MB adopting a bracket-shape conformation through the two CH…O hydrogen bonds is accommodated in a pocket of AH through the four hydrogen bonds with Asp, Tyr and Asn residues and hydrophobic interactions, as shown in Fig. 1. In addition, the O^1 atom at the axial configuration of the second mannose residue protrudes from each pocket to an open space surrounded by the conserved hydrophobic residues, suggesting an additional binding site for the third mannose residue of HMTG. These structural features strongly prove that AH is specifically bound to only MB but not to the other types of disaccarides.

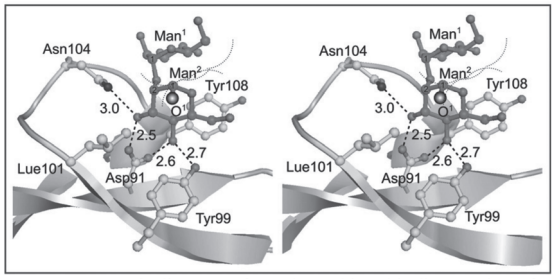

Fig. 1. A stereo-pair diagram of MB bound in a binding pocket of AH.

[1] Tanaka H *et al*., *PNAS*, **106**, 15633-15638 (2009).
[2] Hoque MM *et al*., *PNAS*, **109**, in press (2012).

Keywords: X-ray structure; anti-HIV lectin; actinohivin