Trypanosoma evansi Sialidase: Surface Localization, Properties and Hydrolysis of Ghost Red Blood Cells and Brain Cells-Implications in Trypanosomiasis

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A membrane-bound sialidase was isolated from blood stream (BS) *Trypanosoma evansi* partially purified and characterized. The enzyme is a glycosyl phosphatidyl inositol (GPI) membrane anchored protein. It was solubilized from *T.evansi* cells recovered from infected camel blood by detergent treatment with Triton CF 54 and partially purified by a series of chromatography steps. The enzyme was optimally active at pH 5.5 and 37 °C. It had a K_M and V_{max} values of 4.8×10^{-6}M and 3.75×10^{-6} mol/min.mg protein with Neu5Acα2, 3lac as substrate respectively. The K_M and V_{max} values with fetuin (4-nitrophenyl-oxamic acid) as substrate were 2.9×10^{-2}M and 4.2×10^{-3} mol/min.mg protein in the same respect. Kinetic analysis with methly umbelliferyl sialate (MU-Neu5Ac) gave K_M and V_{max} values of 0.17 mM and 0.84 mmol/min.mg protein respectively. The *T. evansi* SD could hydrolyse internally linked sialic acid residues of the ganglioside GM_2 , but was inactive towards colomic acid, and Neu5Ac2, 6. lac. When ghost red blood cell (RBC) was used as substrate, it desialylated the RBC in the following order of efficiency; mouse, rat, camel, goat, and dog. Similarly, cerebral cells isolated from BalbC mouse was desialylated by the *T. evansi* SD.
Inhibition studies using 2-deoxy-2, 3 didehydro-*N*-acetyl neuraminic acid (NeuAc2, 3en) against MU-Neu5Ac revealed a competitive inhibition pattern with K_i of 5.8 μM. The enzyme was also inhibited non-competitively by parahydroxy oxamic acid (pHOA), and competitively by *N*-ethylmaleimide and *N*-bromosuccinate with K_i values of 25, 42, and 53 μM, respectively. It was activated by Mg^{2+} ion and inhibited by Cu^{2+} and Zn^{2+}.

Key words: Trypanosoma evansi, Sialidase, Kinetic Properties