Isolation and Properties of a Sialidase from *Trypanosoma rangeli*

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In the culture supernatant of *Trypanosoma rangeli*, strain El Salvador, a sialidase was present with an activity of 0.1 U/mg protein as determined with the 4-methylumbelliferyl glycoside of α-N-acetylneuraminic acid as substrate. This enzyme was purified about 700-fold almost to homogeneity by gel chromatography on Sephadex G-100 and Blue Sepharose, and affinity chromatographies on 2-deoxy-2,3-didehydroneuraminic acid and horse submandibular gland mucin, both immobilized on Sepharose. The pH optimum is at 5.4-5.6, and the molecular weight was determined by gel chromatography, high performance liquid chromatography and sodium dodecyl sulphate gel electrophoresis to be 70 000. The substrate specificity of the enzyme is comparable to bacterial, viral and mammalian sialidases with cleavage rates for the following substrates in decreasing order:

*N*-acetylneuraminyl-α(2-3)-lactose > *N*-glycoloylneuraminyl-α(2-3)-lactose > *N*-acetylneuraminyl-α(2-6)-lactose > sialoglycoproteins > gangliosides > 9-O-acetylated sialoglycoproteins.

4-O-Acetylated derivatives are resistant towards the action of this sialidase. The enzyme activity can be inhibited by 2-deoxy-2,3-didehydro-*N*-acetylneuraminic acid, Hg²⁺ ions, and *p*-nitrophenyloxamic acid; it is not dependent on the presence of Ca²⁺, Mn²⁺ or Mg²⁺ ions.

**Abbreviations:** BSA, bovine serum albumin; BSM, bovine submandibular gland mucin; CMP, cytidine monophosphate; EDTA, ethylenediaminetetraacetic acid; ESM, equine submandibular gland mucin; HEPES, N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid; HPLC, high performance liquid chromatography; Lac, lactose; MU-Neu5Ac, 4-methylumbelliferyl glycoside of α-N-acetylneuraminic acid; Neu5Ac, N-acetyleneuraminic acid; Neu5Ac2en, 2-deoxy-2,3-didehydro-*N*-acetylneuraminic acid; Neu4Ac5Gc, N-glycoloyl-4-O-acetylneuraminic acid; Neu2en, 2-deoxy-2,3-didehydroneuraminic acid; Neu2en, 2-deoxy-2,3-didehydroneuraminic acid; Neu5Gc, N-glycoloylneuraminic acid; PMSF, phenylmethylsulfonyl fluoride; PSM, pig submandibular gland mucin; SDS, sodium dodecyl sulfate; Tris, tris-(hydroxymethyl)aminomethane.

*Dedicated to Professor Dr. Heinz Mühlpfordt on the occasion of his 65th birthday.*

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The South American trypanosomiasis "Chagas' disease" is a human disease caused by *Trypanosoma cruzi*. As this parasite often occurs together with the non-pathogenic *T. rangeli*, parameters are needed to discriminate between both forms by reliable, specific, and sensitive methods. As has been described earlier, *T. cruzi* has sialic acids on the cell surface. The nature of this sugar seems to depend on the medium in which it was grown [1]. This finding led to the suggestion that sialic acids might be taken up by the parasites from the surrounding medium, especially as *T. cruzi* is unable to biosynthesize this sugar from its well-known precursors [1]. The occurrence of sialidase activity that might be helpful for sialic acid acquisition from the environment has been reported for *T. cruzi* [2] and *T. rangeli* [3]. The localization of the enzyme in these parasites is, however, different. Whereas *T. cruzi* has a low and membrane-bound sialidase activity, *T. rangeli* releases high amounts of this glycosidase into the surrounding medium. As an isolation and a detailed analysis of the sialidase of *T. rangeli* has not yet been carried out, the present study describes the purification and characterization of this enzyme.

Materials and Methods

If not otherwise stated all procedures were carried out at 4°C, with the exception of enzyme incubations, which were performed at 37°C. Chemicals were purchased from Merck (Darmstadt, W. Germany) or Sigma (Munich, W. Germany). Sephadex, Blue-Sepharose and Sepharose 4B were from Pharmacia (Freiburg, W. Germany). ESM was prepared and coupled to Sepharose (35 μg sialic acids/ml gel) as described earlier [4, 5]. Neu5Ac2en was purchased from Boehringer (Mannheim, W. Germany) and, after de-N-acetylation, linked to Sepharose [6] (1.2 mg Neu2en/ml gel).

MU-Neu5Ac was prepared according to the method of Warner and O'Brien [7] and p-nitrophenyloxamic acid according to [8]. Trasylol was obtained from Bayer (Leverkusen, W. Germany). A ganglioside mixture from bovine brain was prepared as described in the literature [9], and Neu5Gc-GM3 and Neu4Ac5Gc-GM3 were isolated from horse erythrocytes according to [10]. GM3 was a generous gift from Professor Ghidoni, Milan. Neu5Gc-α(2-3)-Lac was obtained by ozonolysis of Neu5Gc-GM3 as described earlier [11]. Neu5Ac-α(2-3)-Lac and Neu5Ac-α(2-6)-Lac were prepared from bovine colostrum as reported [12]. BSM and PSM were isolated as described earlier [13, 14]. Oligosaccharides from PSM (PSM'ol) were produced by β-elimination according to the method of Aminoff et al. [15]. BSM was de-O-acetylated by alkaline treatment [16].

Culture of Trypanosomes

*Trypanosoma rangeli*, strain El Salvador, was grown in liver infusion tryptose medium (LIT) supplemented with 10% fetal calf serum as described [3, 17]. The pH value was monitored and kept at 7.2 throughout the culture time by addition of 20 mM HEPES buffer. The LIT medium consists of 5.0 g NaCl, 0.4 g KCl, 5.0 g Na₂HPO₄, 2.0 g glucose, 5.0 g tryptose, 5.0 g liver infusion, made up with H₂O to a total volume of 1 l, and the pH buffered to 7.2 with HEPES. After 10-14 days of culture at 37°C parasites were harvested by centrifugation at 1500 × g for 10 min at 4°C. The supernatant was filtered through nitrocellulose filters (0.22 μm) and lyophilized.