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Synthesis and Natural Occurrence of 2-Deoxy-2,3-Didehydro-N-Glycoloylneuraminic Acid

2-Deoxy-2,3-didehydro-*N*-glycoloylneuraminic acid (Neu5Gc2en) was synthesized in three different ways:

(i) according to the synthesis of 2-deoxy-2,3-didehydro-*N*-acetylneuraminic acid (Neu5Ac2en)[1] using *N*-glycoloylneuraminic acid (Neu5Gc) as precursor,

(ii) according to the preparation of Neu5Ac2en by enzymic synthesis of the CMP glycoside of Neu5Gc followed by treatment with triethylamine at pH 13[2] (50% yield) and

(iii) by preparation of 2-deoxy-2,3-didehydroneuraminic acid[3] followed by glycoloylation of the free amino group with 1,3-dioxolane-2,4-dione[4] (80% yield in the latter reaction).

Since Neu5Gc is known to occur as the main sialic acid in porcine tissues, it was speculated that Neu5Gc2en may also be present. We therefore analysed the free sialic acids from porcine urine and submandibular gland by gel filtration, anion-exchange chromatography, high performance liquid chromatography[5], preparative thin-layer chromatography and capillary gas-liquid chromatography/mass spectrometry on OV-17 and OV-101 (fused silica)[6] and identified Neu5Gc2en in an amount of 1–3% relative to the whole sialic acid fraction. Neu5Gc2en was also detected in horse urine.

The inhibitory effect of Neu5Gc2en on *Vibrio cholerae* sialidase with NeuAc(α2–3)Gal(β1–4)Glc as substrate was slightly stronger than with Neu5Ac2en (50% inhibition at 10^{-5}M Neu5Gc2en and at 1.5×10^{-5}M Neu5Ac2en), confirming an increase of the inhibitory effect in dependence on the electronegativity of the *N*-acyl chain[7].

Although the chemical origin of Neu5Gc2en (as well as that of Neu5Ac2en[2]) was proved to be an elimination reaction of CMP-Neu5Gc yielding about 3% Neu5Gc2en after treatment of an 1.6mM solution of CMP-Neu5Gc overnight at pH 7 and 37 °C, a possible biological origin has still to be elucidated.

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Partial Purification and Characterization of β-D-Mannosidase from Human Placenta

β-D-Mannosidase (EC 3.2.1.25) was purified from human placenta by ammonium sulfate precipitation, dialysis, affinity chromatography on Con A-Sepharose 4B, gel filtration and hydroxylapatite chromatography. The specific activity increased 108-fold, the yield of enzyme activity was 6%. The purification was not complete but could be documented by a drastic increase of the intensity of the β-D-mannosidase protein band in disc gel electrophoresis.

The β-D-mannosidase activity against the synthetic substrate 4-nitrophenyl β-D-mannopyranoside is quite labile in acid buffers and even at pH 8 decreased to about 60% of its starting activity within 20 days at 4 °C. It is stabilized by EDTA and 2-mercaptoethanol and inhibited by Cu(II), Hg(II), mannosylamine and D-mannono-1,4-lactone. Its pH optimum is 4.2, its IP around 5.1 and its K_m about 2.4mM.

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Sialidase Production in Different Media of Clostridia Causing Myonecrosis

Most of the *Clostridia* causing gas gangrene have been observed to produce sialidase. This enzyme is discussed to enhance the toxic effects of other agents produced by *Clostridia* in host tissues. Furthermore, sialidase activity is considered to be useful as a diagnostic tool in gas gangrene. The first aim was to find a medium which leads to optimal production of sialidase (EC 3.2.1.18) in the species *Cl. perfringens, Cl. septicum, Cl. histolyticum* and *Cl. sordellii.* Thus, liver-liver broth (Tarozzi) and chopped meat medium, both home-made, were applied as well as Todd Hewitt broth (OXOID) and brain-heart infusion (DIFCO). The four media were used with or without 0.1mM sialic acid bound to glycopeptides from edible bird nest substance. These glycopeptides are known to induce sialidase production[1]. Five strains of each species were grown anaerobically at 35 °C in the dark. Samples (2 m*l*) were taken from the inoculated (1 : 10) media every 24 h in the course of 5 days. After centrifugation (5000 × *g*, 20 min, 20 °C) 1 m*l* of the supernatant was used for the estimation of sialidase activity with NeuAc(α2–3)Gal(β1–4)-Glc[2] or with tritium-labelled fetuin[3] as substrates. The *Clostridia* produced the greatest quantity of sialidase in chopped meat medium, followed by Todd Hewitt broth, brain-heart infusion and liver-liver broth. The addition of sialoglycopeptides resulted in only little higher values. Two of the most active strains of each species were cultivated in chopped meat medium with sialoglycopeptides and samples were taken every hour during 12 h.

The clostridial species markedly differed in the amount of sialidase produced. The highest value of sialidase activity was observed for *Cl. septicum*, followed in a decreasing order by *Cl. perfringens, Cl. histolyticum* (one half of the activity of *Cl. septicum*) and *Cl. sordellii* (one third). *Cl. novyi* produced no sialidase under the described conditions.

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