Inhibition of the biosynthesis of N-acetyleneuraminic acid by metal ions and selenium in vitro*

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In liver homogenate the biosynthesis of N-acetyleneuraminic acid using N-acetylglucosamine as precursor can be followed stepwise by applying different chromatographic procedures. In this cell-free system 16 metal ions (Zn$^{2+}$, Mn$^{2+}$, La$^{3+}$, Co$^{2+}$, Cu$^{2+}$, Hg$^{2+}$, VO$_{2}^{+}$, Pb$^{2+}$, Ce$^{3+}$, Cd$^{2+}$, Fe$^{3+}$, Fe$^{3+}$, Al$^{3+}$, Sn$^{2+}$, Cs$^{+}$ and Li$^{+}$) and the selenium compounds, selenium(IV) oxide and sodium selenite, have been checked with respect to their ability to influence a single or possibly several steps of the biosynthesis of N-acetyleneuraminic acid. It could be shown that the following enzymes are sensitive to these metal ions (usually applied at a concentration of 1 mmol l$^{-1}$): N-acetylglucosamine kinase (inhibited by Zn$^{2+}$ and vandate), UDP-N-acetylglucosamine-2'-epimerase (inhibited by Zn$^{2+}$, Co$^{2+}$, Cu$^{2+}$, Hg$^{2+}$, VO$_{2}^{+}$, Pb$^{2+}$, Cd$^{2+}$, Fe$^{3+}$, Cs$^{+}$, Li$^{+}$, selenium(IV) oxide and selenite), and N-acetylmannosamine kinase (inhibited by Zn$^{2+}$, Cu$^{2+}$, Cd$^{2+}$ and Co$^{2+}$). Dose dependent measurements have shown that Zn$^{2+}$, Cu$^{2+}$ and selenite are more efficient inhibitors of UDP-N-acetylgulcosamine-2'-epimerase than vandate. As for the N-acetylmannosamine kinase inhibition, a decreasing inhibitory effect exists in the following order Zn$^{2+}$, Cd$^{2+}$, Co$^{2+}$ and Cu$^{2+}$. In contrast, La$^{3+}$, Al$^{3+}$ and Mn$^{2+}$ (1 mmol l$^{-1}$) did not interfere with the biosynthesis of N-acetyleneuraminic acid. Thus, the conclusion that the inhibitory effect of the metal ions investigated cannot be regarded as simply unspecific is justified.

Keywords: N-acetyleneuraminic acid biosynthesis, metal ions, selenium, rat liver, inhibition of enzymes

Introduction

The biosynthesis of N-acetyleneuraminic acid is well established, especially due to studies performed by the groups of Roseman and Warren (for review see Warren & Felsenfeld 1962, Corfield & Schauer 1982). In Figure 1 the metabolic pathway is represented which involves 10 different enzymatic reactions. Co-substrate of most enzymes is either ATP, UTP or finally CTP, leading to CMP-N-acetyleneuraminic acid, an unusual sugar nucleotide. The singularity of this biosynthetic pathway in carbohydrate biochemistry can be deduced from two reactions: (i) the direct formation of N-acetylmannosamine from UDP-N-acetylgulcosamine, which comprises 2'-epimerization as well as splitting off of UDP, and (ii) the condensation of N-acetylmannosamine-6-phosphate with phosphoenolpyruvate leading to N-acetyleneuraminic acid-9-phosphate. N-Acetyleneuraminic acid is involved in a variety of biological processes including antigenicity, cellular adhesion, transport, stability of glycoproteins, receptor function, synaptic transmission, the action of some hormones and hemostasis. With regard to the diversity of biological processes in which N-acetyleneuraminic acid is involved, it is likely that impairment of its biosynthesis may lead to marked consequences. In previous studies it could be shown that the biosynthesis of N-acetyleneuraminic acid can be inhibited by some sugar analogs such as N-propionyl-d-glucosamine or N-dimethyl-d-mannosamine (Grünholz et al. 1981, Bauer et al. 1983, Reutter & Bauer 1986). In this paper different metal ions, together with selenium, have been checked as potential inhibitors of one or several enzymes involved in the biosynthesis of N-acetyleneuraminic acid. Furthermore, some attempts are made to explain the mechanism of inhibition in vitro.

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Figure 1. Biosynthetic pathway of CMP-N-acetylneuraminic acid from the precursor N-acetylglucosamine. The respective enzymes involved are: N-acetylglucosamine kinase (2.7.1.59), N-acetylglucosamine-6-phosphate mutase (2.7.5.2), UDP-N-acetylglucosamine pyrophosphorylase (2.7.7.23), UDP-N-acetylglucosamine-2'-epimerase (5.1.3.14), N-acetylmannosamine kinase (2.7.1.60), N-acetylneuraminic acid 9-phosphate synthase (4.1.3.20), N-acetylneuraminic acid 9-phosphate phosphatase (3.1.3.29) and CMP-N-acetylneuraminic acid synthase (2.7.7.43).

Materials and methods

Preparation of the homogenate

Wistar rats, weighing 170–200 g each, were perfused with 40 ml of 0.15 mol·l⁻¹ NaCl under light ether anesthesia. Livers were then removed, squeezed through a sieve and the tissue was mixed with 2.5 volumes of chilled buffer, containing 0.2 mol·l⁻¹ Tris–HCl, 75 mmol·l⁻¹ nicotinamide and 2 mmol·l⁻¹ MgCl₂ (pH 7.4). Final homogenization was achieved with a dounce type homogenizer (10 strokes with the loosely fitting pestle) and the homogenate thus obtained was centrifuged for 10 min at 48 000 × g at 4 °C. The protein concentration was 25 mg ml⁻¹.

Freezing of homogenates should be avoided, because this would lead to a substantial loss of enzyme activity.

Enzyme assay

The enzyme assays contained in a final volume of 350 μl: liver supernatant fraction (250 μl), ATP (1.14 mmol·l⁻¹), UTP (1.14 mmol·l⁻¹), CTP (1.14 mmol·l⁻¹), phosphoenolpyruvate (2.28 mmol·l⁻¹) and pyruvate kinase (57 mU μl⁻¹). The reaction performed at 37 °C under aerobic conditions was started by adding [¹⁴C]N-acetyl-D-glucosamine (100 nCi; incubation time: 5 min) or [¹⁴C]UDP-N-acetyl-D-glucosamine (100 nCi; incubation time: 10 min) or [³H]N-acetyl-D-mannosamine (1 μCi; incubation time: 10 min). The reaction was stopped by addition of 500 μl ethanol (97%); the mixture was then heated for 5 min at 65 °C to stimulate the precipitation of proteins, followed by centrifugation at 5000 × g for 3 min.

Paperchromatographic separation of the metabolites

Deproteinized samples (usually 50 μl) together with 5 μl of 2% UMP as an internal standard were applied on Whatman 3MM paper and chromatographed for 22 h with n-propanol:1 mol·l⁻¹ sodium acetate, pH 5.0:H₂O (7:1:2) as solvent (Lewin & Wei 1966). Radioactivity of the separated metabolites was determined by counting 0.5–2.0 cm wide stripes in 10 ml liquid scintillation cocktail Quickszint 501 from Zinsser Analytic (Frankfurt, Germany) by using a Packard (Zürich, Switzerland) liquid scintillation counter. Individual metabolites were identified as described previously (Hultsch et al. 1972).