

Evidence for a free N-acetylneuraminic acid-hydroxylating enzyme in pig mandibular gland soluble fraction

Abstract:

The activity of a free N-acetylneuraminic acid (Neu5Ac)-hydroxylating enzyme which converted Neu5Ac into N-glycolyl-neuraminic acid (Neu5Gc) was demonstrated in the soluble fraction of pig mandibular gland. The hydroxylation was possible only with NADPH as the electron donor. The apparent K_m was 4.5 mM Neu5Ac. At 0.5 mM monovalent cations had no effect on the hydroxylation of Neu5Ac whereas bivalent cations gave varied inhibition capacities ranging from 14 to 75%. EDTA gave a time-dependent enhancement of activity. It was concluded that the enzyme does not require an exogenously added inorganic cofactor. Results from salt fractionation of the soluble fraction and the use of inhibitors such as mercurials suggested that the hydroxylation of Neu5Ac to Neu5Gc may involve other, as yet unknown, component(s) and the possibility of electrons donated by NADPH being transferred to activated molecular oxygen (second substrate). We propose to name this enzyme N-acetyl-neuraminic acid hydroxylase.