THE BIOSYNTHESIS OF N-ACETYLNEURAMINIC ACID

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This communication describes a new enzyme system which catalyzes the synthesis of N-acetylneuraminic acid (NANA) from N-acetylmannosamine and phosphoenolpyruvic acid in the presence of ATP. It differs from N-acetylneuraminic acid aldolase (Comb and Roseman, 1960) which catalyzes the synthesis of NANA from N-acetylmannosamine and pyruvic acid. Whereas equilibrium of the aldolase reaction favors degradation of NANA, this system allows virtually complete conversion of substrates to NANA.

Requirements for the system. It can be seen from the table that N-acetylmannosamine, phosphoenolpyruvate, ATP, magnesium ions and two fractions derived from rat liver extracts are required for synthesis. Potassium ions stimulate synthesis.

N-Acetylmannosamine cannot be replaced by N-acetylglucosamine, N-acetylgalactosamine, ribose, ribose-5-phosphate, glucose, mannose or galactose. UDP-N-Acetylglucosamine is inactive with purer fractions, although in crude extracts UDP-N-acetylglucosamine can substitute for N-acetylmannosamine, presumably because of the known conversion of UDP-N-acetylglucosamine to N-acetylmannosamine in rat liver extracts (Comb and Roseman, 1958; Glaser, 1960). Pyruvic acid cannot substitute for phosphoenolpyruvate. No other di or trinucleotide can replace ATP. It has been found that TPN, and to a lesser extent DPN, in catalytic amounts, show a variable effect on the synthesis of NANA. With some enzyme preparations the yield of NANA has been increased by as much as 7 fold by the addition of both TPN and DPN.

TABLE 1

Requirements for synthesis of N-acetylneuraminic acid

Vessel contents	μMole NANA synthesized
Complete	0.037
No Fraction I	0.000
No Fraction II	0.000
No Mg^{++}	0.000
No KCl	0.022
No N-acetylmannosamine	0.000
No phosphoenolpyruvate	0.004
No phosphoenolpyruvate; + 0.5 μmole pyruvate	0.000
No ATP	0.000

Each vessel contained in a volume of 0.3 ml.: Tris-chloride buffer pH 7.6, 40 μmoles: $MgCl_2$, 10 μmoles: N-acetylmannosamine, 0.35 μmole: phosphoenolpyruvate (tricyclohexylamine salt), 0.5 μmole: ATP, 1 μmole: DPN, 0.02 μmole: TPN, 0.02 μmole: KCl, 10 μmoles. Fraction I, 0.8 mg.: Fraction II, 0.8 mg. Incubation for 2 hours at 37°. Thiobarbituric acid assay (Warren, 1959) on entire vessel contents.

Preparation of enzyme fractions. Extracts which catalyze synthesis of NANA have been partially purified from rat liver[1].

Rat liver extract is prepared by homogenizing one part of liver with two parts of ice cold water in a loose teflon homogenizer. A loose homogenizer is essential in order to obtain active preparations. The homogenate is centrifuged at 105,000 xg for 1 hour in the Spinco preparative ultracentrifuge and the supernatant solution is decanted. The supernatant solution is mixed with 50 mg. of bentonite per ml., and is stirred repeatedly for 10 minutes at 0° C. It is then centrifuged at 5,000 g for 10 minutes at 4° C. The supernatant solution is added to a column of DEAE cellulose-chloride which has been washed with distilled water. One of the required fractions (Fraction I) can be washed through the column with distilled water while a second (Fraction II) is eluted with a sodium chloride gradient (0.02 M to 0.25 M) at pH 7.6. It can be seen

[1] Extracts of Escherichia coli K 235 (Barry, 1958) also form NANA when incubated with the components listed in the table.

from the table that neither fraction is active alone but complete activity is recovered upon recombination. In addition it has been found that Fraction II incubated with N-acetylmannosamine, ATP and Mg^{++} ions forms an intermediate which is converted to NANA in the presence of Fraction I and the remaining reaction components; work is in progress on the identification of this material.

Neither fraction contains N-acetylneuraminic acid aldolase for there is no decrease in NANA concentration when it is incubated with the fractions. In addition, pyruvate is unable to replace phosphoenolpyruvate in the synthesis of NANA.

Equilibrium of the reaction. In the presence of excess ATP and phosphoenolpyruvate, N-acetylmannosamine is almost completely converted to NANA. In one experiment 500 μmoles of N-acetylmannosamine were converted to 497 μmoles of NANA (as measured by the thiobarbituric acid assay) in the presence of 1750 μmoles of ATP and 1150 μmoles of phosphoenolpyruvate.

Identification of product. N-Acetylmannosamine, labelled with C-14 in the acetyl group, has been converted to NANA with the same specific activity. The product, purified on a column of Dowex 1 formate, cochromatographed on Whatman #1 paper with authentic N-acetylneuraminic acid in five solvent systems. Radioactivity and the thiobarbituric acid chromophore of NANA (Warren, 1960) occupied the same area. The biosynthetic material decomposed at 186°, similar to authentic NANA. A mixture of the two also decomposed at 186°. The biosynthetic product gave a positive reaction in the orcinol, diphenylamine, Ehrlich (Werner and Odin, 1958), resorcinol (Svennerholm, 1957), and thiobarbituric acid (Warren, 1959) tests. NANA and the biosynthetic product showed similar absorption spectra and extinction coefficients in each of these tests. The infrared spectrum (KBr pellet) of the product was the same as that of authentic NANA.

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