MAMMALIAN SIALIDASE (NEURAMINIDASE)

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The enzyme sialidase (neuraminidase) selectively removes sialic acids from mucoproteins, mucopolysaccharides (Gottschalk, 1954; Klenk et al., 1955), mucolipids (Rosenberg and Chargaff, 1958) and neuraminlactose (Kuhn and Brossmer, 1956, 1958; Gottschalk, 1957). Sialidase activity was originally observed in the influenza virus in a study of the agglutination of red cells by the virus (Hirst, 1942). However sialidase from the culture medium of Vibrio cholerae, "receptor-destroying enzyme" (RDE), was the first to be studied as an enzymic entity (Burnet, McCrea and Stone, 1946, 1947). The enzyme has also been found in Clostridium perfringens (McCrea 1947, Popenoe and Drew, 1957), type II pneumococcus (Heimer and Meyer, 1956), mumps and Newcastle disease virus (Ada and Stone, 1950). It has hitherto not been described in animal tissues. In this communication, the occurrence of sialidase of mammalian origin is reported. The enzyme has been found in commercial preparations of bovine and human glycoprotein (Cohn Fraction VI)[1].

Since there are sialic acid-containing proteins in these fractions, incubation of the unpurified fractions without additional substrate leads to the release of sialic acid, and the amount released is only slightly greater when sialic acid-containing mucoprotein substrate is added. Under

[1] Purchased from Pentex Incorporated, P. O. Box 248, Kankakee, Illinois.

the condition of the assay described in Table 1, 1 gram of bovine Fraction VI released approximately 16 μmoles (5.0 mg.) of N-acetylneuraminic acid from crude human plasma protein substrate in 24 hours while 1 gram of human Fraction VI released 5 to 7 μmoles in the same period. Activity is low and is not detectable in unprocessed plasma and serum or in solutions of crude plasma protein.

A 30 to 50-fold purification of enzyme has been accomplished by dissolving 20 mg. bovine Fraction VI per ml. of 0.01 M Tris hydroxymethyl-aminomethane chloride (Tris) buffer pH 7.4 at 4° C. and collecting the fraction which precipitates with solid ammonium sulfate between 10 to 35 per cent saturation. The yield of activity is 80 to 100 per cent. The preparation still contains sialoprotein. Virtually all of the sialoprotein in the 10 to 35 per cent ammonium sulfate fraction can be removed by passage through a column of Ecteola-cellulose in 0.01 M Tris buffer pH 7.4 although there is no increase in specific activity of the enzyme and there is only a 50 per cent recovery of activity. With this preparation of the enzyme there is no increase in color without addition of substrate.

It can be seen in Table 1 that the enzyme removes sialic acid from bovine submaxillary gland mucin and fetuin. In other experiments bovine sialidase cleaved sialic acid from human β-globulin, crystalline human transferrin [2], orosomucoid [3], bovine α-globulin, pig submaxillary gland mucin, ovomucoid, beef brain ganglioside [4] and neuramin-lactose. The rate of release of sialic acid is constant when measured at 1, 2 and 4 hours. The enzyme is capable of eliminating the staining of sialomucins in tissue sections in a manner similar to that of cholera sialidase (Spicer and Warren, 1960).

The enzyme is stimulated by calcium ions (Table 1) as is the sialidase of cholera (Burnet and Stone, 1947). In contrast, the activities

2 Kindly supplied by Dr. A. L. Schade.

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TABLE 1

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Additions	µg. Sialic acid released
Purified bovine enzyme	0.2
Bovine submaxillary gland mucin	0.1
Fetuin	0.1
Purified bovine enzyme + bovine submaxillary gland mucin	7.9
Purified bovine enzyme + fetuin	12.4
Purified bovine enzyme + fetuin (Ca^{++} omitted)	5.7
Purified bovine enzyme (heated 80° 5') + fetuin	0.4

All vessels contained in a volume of 0.3 ml. sodium acetate buffer pH 5.5 50 µmoles and $CaCl_2$ 10 µmoles. Where indicated: bovine submaxillary gland mucin 2.3 mg. which contained 39 µg. sialic acid (calculated as N-acetylneuraminic acid), fetuin 2 mg. which contained 156 µg. N-acetylneuraminic acid. In this experiment 0.6 mg. of partially purified bovine enzyme (10-35 per cent ammonium sulfate fraction which has been passed through a column of Ecteola-cellulose) was used. Incubation was carried out for 3 hours at 37° and the thiobarbituric acid assay was carried out on the vessel contents (Warren, 1959).

of viral (McCrea, 1947) and clostridial sialidases (Popenoe and Drew, 1957) are unaffected by calcium ions. Activity is eliminated in 5×10^{-3} M Versene. The pH optimum of the enzyme is 5.5.

Release of sialic acid by bovine sialidase has been measured by the thiobarbituric acid assay (Warren, 1959) and also by the orcinol and Ehrlich assays (Werner and Odin, 1952) after precipitation of proteins by phosphotungstic acid (Popenoe and Drew, 1957). The product released from human plasma protein substrate by the bovine enzyme has been purified on a column of Dowex-1-formate and was eluted by 0.6 M formic acid. It co-chromatographed on Whatman #1 paper with N-acetylneuraminic acid in two solvents. The material was located on paper with thiobarbituric acid spray reagents (Warren, 1960).

Since mammalian sialidase cleaves sialic acid from bovine submaxillary gland, it can break the linkage between the 2-position of sialic acid and the 6-hydroxyl of N-acetylgalactosamine (Gottschalk and Graham, 1959). Similarly it can break the linkage to the 3-hydroxyl of galactose in neuramin-lactose (Kuhn and Brosmer, 1956; Gottschalk, 1957).

Investigations are being made to see whether there are sialidases in organs such as liver and pancreas. However the assay is complicated by the presence in these organs of neuraminic acid aldolase (Comb and Roseman, 1960) which destroys free sialic acid, the product of sialidase activity.

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