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CARTA AL EDITOR

Neuraminidase in Ovine, Equine, Porcine and Bovine Platelets *

In 1964, DASTUGUE *et al.* (4) summarized data on the blood platelets enzymes of mammals; they reported 88 enzyme activities, but neuraminidase (N-acetylneuraminate glycohydrolase, EC 3.2.1.18) is not mentioned in their paper. In the same year, MADOFF *et al.* (6) confirmed that neuraminidase activity has not been reported in platelets. In 1966, MARCUS *et al.* (7) found cytochrome c oxidase, acid phosphatase, β-glucuronidase, cathepsin, catalase and lactic dehydrogenase in human platelets. MAUPIN (8), in 1967, drew attention to the fact that papers on enzymes from animal platelets were less numerous than those on human platelets. More recently, BARBER and JAMIESON (2) purified several enzymes from human platelet membrane fractions (ATPase, Na-K-ATPase, phosphodiesterase, acid phosphatase, esterase, N-acetyl-β-glucosaminidase, β-glucuronidase, β-galactosidase, lactic dehydrogenase, succinic dehydrogenase, alkaline phosphatase and leucine aminopeptidase).

Our present work is concerned with the occurrence of the neuraminidase activity in ovine, equine (horse, donkey and mule), porcine and bovine platelets.

Platelets of the above-named species were prepared from a pool of 5 to 20 animals of each specie following the method of MULLINGER and MANLEY (10), with little modification, *i.e.* the final button (consisting almost entirely of platelets, $> 99.55\ \%$ according to MULLINGER and MANLEY, was washed twice with a 0.9 per cent NaCl solution and twice with distilled water to eliminate contaminating of blood components and salts.

In a first series of assays, 50 mg (dry weight) of donkey platelets were suspended in 10 ml of 0.9 % NaCl solution (containing 0.1 % $CaCl_2$), and equal portion, distributed into 5 tubes. To the first four tubes, 0.2 ml of *Vibrio cholerae* neuraminidase (Behringwerke) was added and 0.2 ml of water to the fifth tube as control. Tubes were incubated for 5, 15, 45 and 120 min at 37°, the control was incubated for the longest time period and the enzyme inactivation was carried out by immersing the tubes in boiling water for 3 min. After cooling, the tubes were centrifuged at 2000 *g* and the supernatants were decanted. Analysis for sialic acids [by the modified resorcinol procedure (9, 11) and the thiobarbituric acid cethod (1, 12)] were carried out and

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positive results were obtained in the supernatants from all tubes, *i.e.* also in the control tube, in which no *Vibrio cholerae* neuraminidase was added.

In a second series of assays, a slightly different procedure was used: 30-40 mg (dry weight) of equine, ovine, porcine or bovine platelets were suspended separately in 6 ml of 0.9 % NaCl solution (containing 0.1 % $CaCl_2$). After homogenization in a tight-fitting, all glass Dounce homogenizer, the contents of the tubes were divided into three equal portions which were treated respectively: *a*) by endogenous neuraminidase; *b*) by *V. cholerae* neuraminidase; and *c*) by 0.1 N H_2SO_4, at 80°, during 45 min (Table I).

Table I. *Sialic acid release from platelets of several species.*
(See experimental conditions in the text)

Specie	mg sialic acid (as NANA)/100 mg platelets (dry weight)		
	Hydrolysis by platelets neuraminidase (a)	Hydrolysis by V. cholerae neuraminidase (b)	Hydrolysis by sulphuric acid (c)
Donkey	0.07	0.94	0.97
Horse	0.09	0.96	1.01
Mule	0.09	0.94	0.98
Lamb	0.10	0.82	0.86
Pig	0.14	0.94	1.00

Tubes (a): Contained an aliquot of platelet homogenate + 0.7 ml H_2O (the blank had the same composition, but was heated at 100° for 5 min.).
Tubes (b): Platelets homogenate + 0.5 ml V. cholerae neuraminidase (250 units) (Behringwerke) with incubation for 60 min. at 37°; then, an additional 0.2 ml V. cholerae neuraminidase was added and the incubation continued for 60 min. more.
Tubes (c): Platelets homogenate + 0.7 ml 0.1 N H_2SO_4, at 80° for 45 min.

In a third series of experiments, neuramin-lactose, pig submaxillary gland mucin and fetuin as well as endogenous substrate were employed in assaying neuraminidase activity of donkey platelets.

Table II shows the results.

It may be deduced that homogenates of donkey, horse, mule, lamb, pig and calf platelets contain neuraminidase, which releases sialic acid from platelet glycoproteins, neuramin-lactose, submaxillary mucin, fetuin and other glycoproteins. (Platelet glycoproteins contain principally N-glycolylneuraminic acid and N-acetylneuraminic acid) (3). Independently of our work, GIELEN *et al.* (5) have detected neuraminidase, which was particlebound, in lysed bovine platelets.

Table II. *Liberation of sialic acid by donkey platelet neuraminidase in comparaison with sulphuric acid hydrolisis.*
Platelets were homogenized in a tight-fitting, all-glass Dounce homogenizer (15 strokes). All vessels contained 2 ml of platelet homogenate (16.2 mg of protein, as dry weight) + 1 ml of substrate containing: 0.2 mg of neuramin-lactose, or 1.5 mg of pig submaxillary mucin, or 2.0 mg of fetuin, or 16.2 of platelets stroma containing glycoproteins, respectively. Incubation was performed at 37° for 3 hrs.
Similar amounts of substrate were submitted to hydrolisis by 0.1 N SO_4H_2 during 3 hrs at 80°. Released sialic acid was determined by the thiobarbituric acid method (1).

Substrate	µmoles sialic acid (as NANA) released by platelets neuraminidase (100 mg of platelets, as dry weight)	Percent of sialic acid liberated as compared with sulphuric acid hydrolysis
Neuramin-lactose	1.4	61
Pig subm. mucin	1.4	61
Fetuin	1.3	55
Platelets glycoproteins	1.3	55

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