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CATABOLISM OF 4-*O*-ACETYLATED SIALIC ACID

Krishna Purnawan Candra

Laboratory of Chemistry and Biochemistry Study Program of Agricultural Product Technology Faculty of Agriculture Mulawarman University, Jl.Tanah Grogot Kampus Gunung Kelua, Samarinda 75123 Tel.0541-749159, Fax.0541-738341, email address: kcandra@telkom.net

ABSTRACT

"Different" sialidases from partially purification of two kinds of horse liver was studied to find out the catabolism of 4-*O*-acetylated *N*-acetylneuraminic acid (Neu4,5Ac_2) with guinea pig serum as substrate. Using fluorimetric HPLC as tools to determine the free sialic acid released from the natural substrate, there was no Neu4,5Ac_2 released by the sialidase detected. This data emphasize that esterase was involved in the released of Neu4,5Ac_2 from sialoglycoconjugate.

INTRODUCTION

Term of sialic acids (*sia*) refers to *N*-acetylneuraminic acid (Neu5Ac) or *N*-glucolylneuraminic acid (Neu5Gc), a pyranose sugar with 9 carbon atoms, having negative charge due to the carboxyl group at C-atom 1 [1]. It usually found as terminal in glycolipid and oligosaccharide. Nowadays more than 40 kinds of *sia* were found from organisms except plants [2]. Enzymes involve in modification of the *sia* are 8-*O*-methyltransferase as well as 4-and 7(9)-*O*-acetyltransferases [3]. Catabolism of 4-*O*-acetyl-*N*-acetylneuraminic acid (Neu4,5Ac_2), which blocked most of sialidase activity, was first elucidated as sialate *O*-acetylesterase was demonstrated involve in the Neu4,5Ac_2 catabolism [4]. However, it is interesting to explore more information from animal rich in Neu4,5Ac_2 like horse, concerning the catabolism of Neu4,5Ac_2 [5]. Here we report that "different" sialidase found in two kinds of horse liver showing no activities on Neu4,5Ac_2.

MATERIALS AND METHODS

Sialidase from partially purification of two kinds of horse liver (A and B) was used in this experiment. The enzyme test and detection of free sialic acid were performed as described by Reuter and Schauer [6]. The enzyme reaction, in a final volume of 100 µl, was incubated at 46 °C in Eppendorf caps (10 to 50 µl sample and 10 µl of guinea pig serum containing 1 mg sialic acids/mL was incubated in 80 mM acetate buffer pH 4.5, while Pefabloc at a final concentration of 1 mM was added as an esterase inhibitor). After 60 min, the reaction was stopped by incubating in ice for 15 min, followed by centrifugal filtration using UF-0.5 with 3 kD cut-off in *Eppendorf* centrifuge at 4 °C. Seventy µl of the filtrate was removed and lyophilised. Sialic acids were derivatized by suspending the lyophilisate in 10 µl of 2 M acetic acid followed by the addition of 49 µl of 1,2-diamino-4,5-methylendioxybenzene (DMB). The mixture was then incubated at 56 °C for 1 h in the dark. The mixture was cooled on ice and was then the free sialic acid was detected by fluorimetric HPLC performed in isocratic mode on an RP-18 cartridge (4x250 mm, 5 µm particle size; Merck) with methanol-acetonitrile-water (7:9:84, v/v/v) as eluent at a flow rate of 1 mL/min at excitation of 373 nm and emission of 448 nm wavelengths. Thirty µl of sample solution was injected. As control the enzyme solution was heated at 95 °C for 5 min prior the enzyme test following addition of buffer and substrate.

RESULTS AND DISCUSSION

Sialidases from two kinds of horse liver (A and B) found to be different in their properties. Sialidase from horse liver A was partially purified using S-Sepharose FF and *p*-aminophenyl oxamic acid agarose, following solubilization and "activation" with the yield of 6 % and purification factor of about 500. Sialidase from horse liver B was partially purified using Fractogel, *p*-Aminophenyl thio-β-D-galactopyranoside agarose, and chromatofocusing with PBE94, following solubilization with the yield of 0.2 % and purification factor of about 20. Sialidase isolated from horse liver A showing no activities of β-galactosidase and could be "activated" by incubating the homogenate at 37 ^{o}C in acidic condition. However, the sialidase isolated from horse liver B showing activities of acid β-galactosidase and could not be "activated".

Sialidase from horse liver A showed no activities of β-galactosidase, it seemed to be single enzyme of sialidase as reported in some different sources [7, 8]. However, sialidase from horse liver B showed activities of β-galactosidase and carboxypeptidase A. This evidence proved that the sialidase from horse liver B is a complex enzyme with β-galactosidase and carboxypeptidase A as reported by [9, 10]**.**

The two sialidases showed no activities on Neu4,5Ac$_2$ when subjected to guinea pig serum as substrates (Figure 1.). This same evidence was also detected when BSM, which rich on Neu5,9Ac$_2$, was used as substrate. The two sialidases were also showed no activities on Neu5,9Ac$_2$ (data not shown).

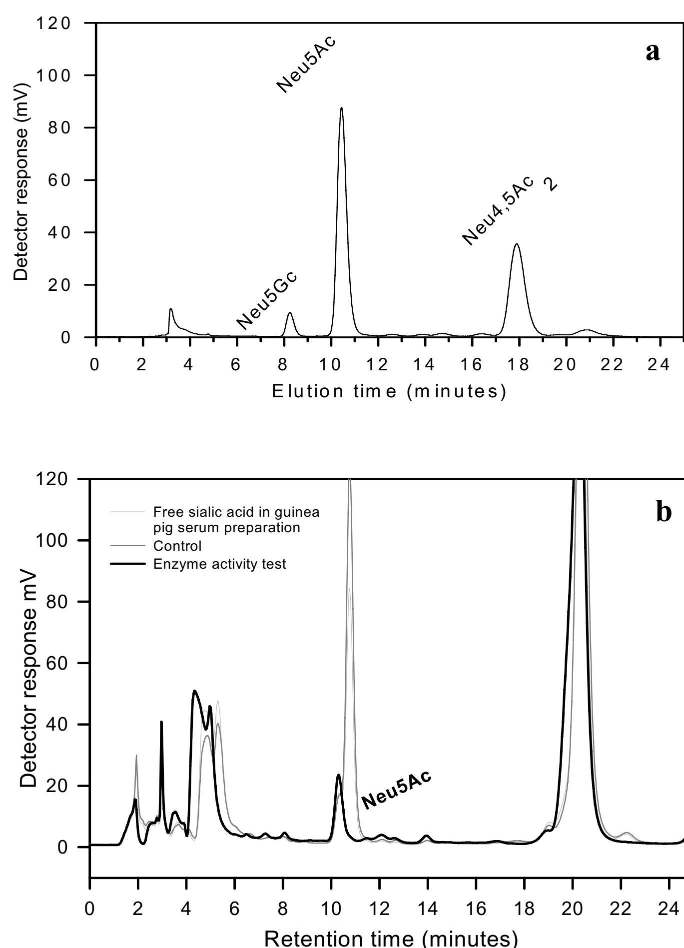

Figure 1. (a) Types of sialic acid in guinea pig serum, (b) sialidase activity on guinea pig serum.

CONCLUSION

The two sialidases showed no activities on Neu4,5Ac_2 when subjected to guinea pig serum as substrate. The HPLC data emphasized that the catabolism of Neu4,5Ac_2 involve esterase activities, the esterase was first modified the acetyl at C-4 allowing the sialidase to hydrolyze the normal sialic acid from sialoglycoconjugate [4].

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