SHORT COMMUNICATIONS

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Transfer of *N*-acetylneuraminic acid to neuraminidase-treated alkaline phosphatase from sheep brain*

In an earlier paper[1], we have reported that the two alkaline phosphatase (orthophosphoric monoester phosphohydrolase, EC 3.1.3.1) fractions isolated from sheep brain are associated with different concentrations of *N*-acetylneuraminic acid (NANA) and that the neuraminidase (mucopolysaccharide *N*-acetylneuraminylhydrolase, EC 3.2.1.18) treatment of the alkaline phosphatase fraction which contains the higher concentration of NANA (Enzyme II) makes it chromatographically similar to Enzyme I which has a low concentration of NANA. Since this change in the elution pattern of neuraminidase-treated Enzyme II does not alter the difference in kinetics between these two alkaline phosphatase fractions, it has been concluded that NANA is not the only factor responsible for the heterogeneity of alkaline phosphatase in brain.

In the present communication, evidence has been presented to show that in the presence of rat liver microsomes, NANA can be transferred from CMP–NANA to the neuraminidase-treated alkaline phosphatase fractions of sheep brain. The effect of the transfer of NANA on the kinetics of the alkaline phosphatase fractions has been investigated.

Rat liver microsomes prepared by the method of SCHNEIDER[2] were used as the source of sialyltransferase enzyme, as reported by O'BRIEN *et al.*[3]. CMP–NANA was prepared by the method of SHOYAB AND BACHHAWAT[4].

The alkaline phosphatase was extracted from sheep brain by the butanol method of MORTON[5] as described in our earlier paper[1]. Further purification of the enzyme fractions was achieved by the following steps. The alkaline phosphatase from the aqueous butanol fraction was precipitated by the addition of an equal volume of ethanol and the solution was allowed to stand overnight at 3–4°. The precipitate was suspended in 0.02 M Tris–HCl, pH 7.4. $(NH_4)_2SO_4$ was added to this solution up to 60% saturation and the precipitate was discarded. The supernatant was brought to 90% saturation with $(NH_4)_2SO_4$. The precipitate was collected, dissolved in 0.02 M Tris–HCl, pH 7.4, and dialysed overnight. Enzyme I and Enzyme II were separated by chromatography on DEAE-cellulose as described earlier[1].

Neuraminidase-treated alkaline phosphatase fractions were prepared by incubation with *Vibrio cholerae* neuraminidase. Gel filtration on Sephadex G-200 was done to remove the neuraminidase and free NANA, and chromatography on DEAE-cellulose was done as described earlier[1], to isolate the alkaline phosphatase fractions from which NANA has been removed.

The incubation mixture for the transfer of NANA contained in a total volume of 0.6 ml: 0.1 μmole of CMP–NANA, 3 μmoles of $MgCl_2$, 0.3 mg of rat liver microsomes suspended in 0.3 ml of 0.1 M Tris–maleate buffer, pH 7.4, and either 2 mg of neuram-

Abbreviation: NANA, *N*-acetylneuraminic acid.

* A preliminary report of a part of this work has been presented at the Meeting of the New York Academy of Sciences, Conference on Phosphohydrolases, 1968.

inidase-treated Enzyme II or 3 mg of Enzyme I. After incubation for 2 h, the reaction mixture was dialysed and chromatographed on a column of DEAE-cellulose with gradient elution as described in our earlier paper[1].

When neuraminidase-treated Enzyme II was used as the substrate, about 40% of the enzyme activity was eluted at a much higher salt concentration corresponding to that of the original Enzyme II, indicating that the transfer of NANA to the above enzyme has taken place. This alkaline phosphatase peak was called as 'resialylated Enzyme II'.

No change in the elution pattern was observed when Enzyme II prior to neuraminidase treatment was used as the substrate.

The above changes in the elution pattern caused by the removal or transfer of NANA was accompanied by similar changes in the mobilities during acrylamide disc electrophoresis. While neuraminidase treatment caused a marked decrease in the anodal mobility of Enzyme II, the resialylated enzyme had identical mobility as the original Enzyme II. In the case of Enzyme I, the shift in the elution pattern indicating resialylation was observed even without any prior neuraminidase treatment. However, the resialylated Enzyme I was not identical to resialylated Enzyme II in its elution pattern.

Estimation of the NANA content of the resialylated enzymes has confirmed that the above shift in the elution pattern of the alkaline phosphatase fractions is

TABLE I

CONCENTRATION OF NANA IN THE NEURAMINIDASE-TREATED AND RESIALYLATED ALKALINE PHOSPHATASE FRACTIONS

The neuraminidase-treated enzymes were prepared as mentioned in the text and the concentrations mentioned were used as substratrs in the NANA transfer system. The resialylated enzymes were isolated from the NANA transfer system by means of DEAE-cellulose column chromatography as described in the text. The percentage of the total activity recovered in the resialylated fractions are given. Assay of the enzyme activity and estimation of protein and NANA concentration were done as described earlier[1].

Alkaline phosphatase fraction	*Total activity (units)*	*Percentage recovery*	*Total protein (mg)*	*NANA content (nmoles/mg)*
Neuraminidase-treated Enzyme II	20	100	2	9
Resialylated Enzyme II	7	35	1	30
Neuraminidase-treated Enzyme I	15	100	3	7.5
Resialylated Enzyme I	5	33	1.5	16

associated with the transfer of NANA, since the resialylated enzymes contain 2–3 times as much NANA as the neuraminidase-treated enzymes (Table I).

The resialylated Enzyme II was similar to the original Enzyme II in its kinetics and the kinetics of the resialylated Enzyme I was similar to that of the original Enzyme I.

The difference in kinetics between the Enzyme II and Enzyme I with respect to their affinity towards pyridoxal phosphate is maintained even after the transfer of NANA to these alkaline phosphatase fractions.

Even though the transfer of NANA to neuraminidase-treated glycoproteins has been well described[3,6–8], the present results demonstrate for the first time that NANA can be transferred to a neuraminidase-treated glycoprotein enzyme without affecting its activity or kinetics. This is in agreement with our earlier report that NANA can be removed from the alkaline phosphatase glycoprotein without affecting its kinetics and thus substantiates our conclusion that the difference in the NANA content between different alkaline phosphatase fractions is not the only factor for the heterogeneity of the enzyme in brain.

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