# STUDIES ON THE STRUCTURE OF GANGLIOSIDES I. ON THE LINKAGE OF N-ACETYL NEURAMINIC ACID IN MONOSIALOGANGLIOSIDE

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Purified ganglioside preparations appear to contain at least four different compounds (Kuhn et al., 1961; Dain et al., 1962; Klenk and Gielen, 1961), which are believed to be due to the occurrence of mono- di- and trisialogangliosides (Kuhn et al., 1961). These authors have demonstrated that the di- and trisialogangliosides can be degraded to the monosialoganglioside by treatment with the enzyme neuraminidase. This enzyme specifically cleaves terminal N-acetylneuraminic acid (NANA) residues having an α-ketosidic linkage (Gottschalk, 1960a).

It has been observed by several investigators that 35 - 60% of the NANA present in a mixture of gangliosides is resistant to hydrolysis by neuraminidase (Trams and Lauter, 1962; Svennerholm, 1962; Dain et al., 1962). However, 90% of the total NANA can be liberated by hydrolysis with dilute mineral acids (Klenk and Gielen, 1960; Trams and Lauter, 1962) and the remainder with more concentrated acid. Therefore, although nearly all of the NANA linkages have similar acid lability, approximately only one-half of the NANA is neuraminidase-labile.

The present study was undertaken in order to gain some insight into the nature of the linkage of the neuraminidase-resistant NANA. In contrast to the other NANA residues, it was assumed that the keto group on carbon 2 of the NANA molecule was not ketosidically linked. Borohydride reduction has been used by several investigators to distinguish between ketosidically linked and free NANA (Roseman, 1962; Warren and Blacklow, 1962). Under these conditions free NANA is reduced and no longer reactive in the chemical determination of Warren (1959) while ketosidically linked NANA is unaffected by borohydride treatment. We wished to learn if the keto group of the NANA residue in monosialoganglioside was reduced by borohydride. Orosomucoid was employed as a control compound because all of the NANA in this material has been demonstrated to be neuraminidase-labile and therefore ketosidically linked (Gottschalk, 1960b).

Materials and Methods: The ganglioside preparation employed was obtained from the Sigma Chemical Company prepared according to the method of Trams and Lauter (1962), and shown to be predominantly monosialoganglioside. A sample of orosomucoid was

generously provided by Dr. L. Warren of N.I.H. Neuraminidase was obtained from Boehringer and Soehne.

NANA was estimated by the procedure of Warren (1959) after acid treatment according to Trams and Lauter (1962). Total hexose was estimated by a phenol-sulfuric acid method of Dubois et al.,(1956). $LiBH_4$ reduction was carried out by the procedure of Gottschalk et al., (1962).

Neuraminidase Treatment: Treatment of the monosialoganglioside with neuraminidase according to the procedure of Trams and Lauter (1962) resulted in the release of no more than 5% of the total NANA.

Silicic Acid Column Chromatography: The ganglioside preparation separated into two somewhat overlapping peaks on silicic acid column chromatography carried out according to a procedure of L. Svennerholm (personal communication). Each peak was shown to have one major component and an additional trace component by thin layer chromatography on silica gel.

Borohydride Reduction: The use of $NaBH_4$ as the reducing agent resulted in some loss of total NANA. Under the conditions employed by Gottschalk et al., (1962) using $LiBH_4$ dissolved in redistilled tetrahydrofuran there was nearly a quantitative disappearance of NANA in the ganglioside preparations (Table I). There was no loss of NANA from orosomucoid. In neither case was there any appreciable loss of total hexose.

Table I

$LiBH_4$ Treatment of Monosialoganglioside and Orosomucoid

| | $LiBH_4$ Treated | μmoles/mg. Hexose | μmoles/mg. NANA* |
|---|---|---|---|
| Orosomucoid | - | 1.1 | 0.23 |
| " | + | 1.0 | 0.24 |
| Ganglioside Fraction A | - | 1.92 | 0.95 |
| " " " | + | 1.65 | 0.09 |
| " " B | - | 1.3 | 0.88 |
| " " " | + | 1.12 | 0.05 |

* All values are corrected for the chemical degradation of NANA which occurred during the acid hydrolysis required for the liberation of bound NANA.

Other Determinations: Because of the assumption that this NANA had a free α-keto group several additional chemical assays were employed in attempts to obtain verification of this configuration. The acidic conditions for the O-phenylene-

diamine reaction for 2-keto acids (Lanning and Cohen, 1951) led to the liberation of all the bound NANA from both ganglioside and orosomucoid, therefore a positive reaction was obtained. In the semicarbazide reaction (Umbarger and Magasanik, 1952) carried out as suggested by Levin and Racker (1959) for 2-keto-3-deoxy aldonic acids, there was only negligible reaction with ganglioside and orosomucoid. We have no satisfactory explanation at this time for the failure of the monosialoganglioside to form the semicarbazide derivative.

The results of this study should not be taken as definitive evidence but are consistent with the conclusion that the NANA in the monosialoganglioside is linked at some position other than ketosidically at carbon 2. We should like to suggest that the NANA in this compound may be linked through carbon 4. This suggestion is supported by the fact that a direct thiobarbituric acid reaction is not obtained with monosialoganglioside. This assay is based upon the formation of β-formyl pyruvate after periodate treatment (Warren, 1959). In order for β-formyl pyruvate to be formed carbons 1 through 5 must be unbound. Since our data indicate that carbon 2 is free, it appears most likely that carbon 4 is linked to the rest of the ganglioside molecule. This suggestion is also consistent with the observation of Rosenberg and Chargaff (1960) that periodate treatment of ganglioside led to the loss of only 50% of the total NANA as determined by the direct Ehrlich reaction. This assay also requires that carbons 1 through 5 be available for formation of pyrrole-2-carboxylic acid (Gottschalk, 1960).

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