



Title	IDENTIFICATION OF N-GLYCOLYLNEURAMINIC ACID AS A HANGANUTZIU-DEIDHER ANTIGEN DETERMINANT IN CHICKEN MAREK'S DISEASE LYMPHOMA-DERIVED CELL LINES BY GASCHROMATOGRAPHY-MASSFRAGMENTGRAPHY
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IDENTIFICATION OF N-GLYCOLYLNEURAMINIC ACID
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Chickens do not synthesize N-glycolylneuraminic acid (NeuGc) and show immune response against Hanganrtziu-Deicher (HD) antigens, the epitope of which is NeuGc. Normal tissues and lymphocytes from chickens do not express HD antigen; however, lymphoma tissues of chickens suffering from Marek's disease and the lymphoma-derived cell lines (MSB1 and HP1) do (Ikuta et al., (1981), *Biken J.*, 25, 47-50).

In this report, the author attempted to quantitate directly NeuGc to demonstrate HD antigen in cell lines derived from Marek's disease by the use of gaschromatography-massfragmentgraphy (GC-MF). As for the separation of sialic acid from the cells, whole cells were hydrolyzed under a mild acid condition and they released sialic acids from both glycoproteins and glycolipids. The contents were then analyzed by GC-MF.

The preparation method was simplified to eliminate the purification procedure, and thus the analysis could be completed within one day.

Prior to using the cell lines, the most suitable condition was determined by hydrolyzing various mixtures of goat stroma and human stroma, which contained NeuGc and N-acetylneuraminic acid (NeuAc) at a molar ratio of 1.4 to 1, and NeuAc alone, respectively.

The detection limit using a 2% OV-17 column with 1m length was found to be 0.01% of NeuGc (absolute amount 30pg) when 30ng of total sialic acid was injected. The sensitivity was lowered to 1/10 times in comparison with that for pure sialic acid because of impurities resulting from the hydrolysis of whole cells.

Under this condition, NeuGc was detected in Marek's disease lymphoma cell lines (MBS1, HP1, HP2, and JP2), except HP1, and the amounts were 0.05% to 0.12% among the total sialic acid content. NeuGc was also detected in a leukemia lymphoma-derived 1104B1 line, of which the amount was 0.14% of total sialic acids. In the case of 1104X5, which is a subline of 1104B1, NeuGc was not detected. Therefore, it is necessary to clarify the difference between these two lines in future research. In RP1, which was established from transplantable Marek's disease lymphoma, NeuGc was not detected. This result agreed with Ikuta's report in which an immunological method was used.

To confirm these results immunologically, the thin-layer chromatography/enzyme immunostaining method was used for the total lipids extracted from the JP2, but from the results obtained only from JP2, the sensitivity was negligible.