A PROCEDURE FOR THE RADIOIODINATION OF A GANGLIOSIDE DERIVATIVE

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SUMMARY

A procedure for the preparation of a radioiodinated derivative of ganglioside GM_1 has been devised. Carbon 6 of the terminal galactosyl residue of GM_1 was converted to an aldehyde by galactose oxidase. The adduct formed by this oxidation product of GM_1 with tyramine was reduced with sodium cyanoborohydride. The tyramine moiety covalently attached to GM_1 was then labelled by the conventional iodine chloride procedure. The technique described herein is simple and economical for the preparation of a highly radioactive GM_1 derivative.

Key Words: Tyramine, GM_1, Ganglioside, Radioiodinated

INTRODUCTION

Numerous procedures have been reported for the preparation of tritiated ganglioside *in vitro*. In one approach, tritium is incorporated into its galactosyl moiety (1-3). In another, tritium is introduced directly into the ceramide moiety of gangliosides (4-6). Although these procedures are effective, they are cumbersome. Furthermore, the low energy level and long half life of tritium severely limit the maximal specific activity of tritiated gangliosides. This restiction is particularly acute for the following reason: Gangliosides are capable of expressing some biological activities at low concentrations. In solution, ganglioside can exist as monomer or micelle (7,8). At

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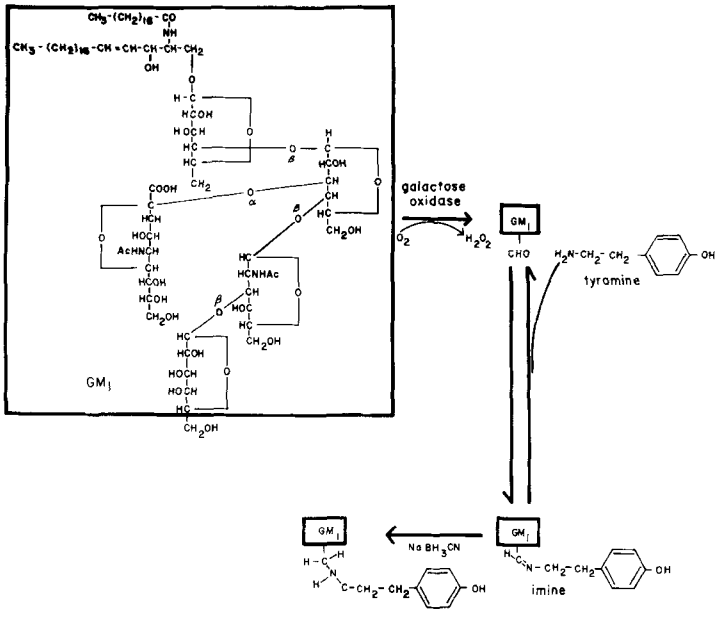
concentrations below critical micellar concentration, the monomer is the reactive species which has completely different properties from the micellar form of ganglioside (9,10). Only concentrations of ganglioside below critical micellar concentration are suitable for studying those problems, but the radioactivity of low concentration of the tritiated ganglioside which has low specific activity falls below the level of detection. To overcome this problem, a procedure for the radioiodination of ganglioside is therefore developed. In addition to high specific activity, the gamma ray can be easily and economically monitored with a gamma counter. The new radioactive compound, whose synthesis is reported here, should be suitable for some metabolic and structural studies of gangliosides that had been impossible in the past.

RESULTS

Since ganglioside GM_1 does not contain a phenolic group which renders itself readily for iodination, we used the following sequence of reactions to attach tyramine to ganglioside GM_1: The first step of the reaction (Fig. 1) is identical to that of Suzuki and Suzuki's method (2). e.g. the C-6 primary hydroxyl group of the galactosyl residue of GM_1 is oxidized to produce an aldehyde. The carbonyl group can then react reversibly with the amino group of tyramine to form an imine. The imine is reduced with sodium cyanoborohydride to form a secondary amine. The tyramine moiety linked to the ganglioside can then be iodinated by conventional procedures.

[^{125}I]-iodotyramine can be incorporated directly into ganglioside GM_1 to prepare an iodinated tyramine-ganglioside. However, an excess of [^{125}I]-iodotyramine relative to ganglioside is required for the completion of this reaction. To avoid the waste of expensive radioactive compounds, we first incorporated tyramine into ganglioside and then purified tyramine-ganglioside free of an excess of tyramine before the iodination reaction.

Figure 1 The attachment of tyramine to ganglioside GM1



The specific activity of [^{125}I]-iodotyramine ganglioside GM_1 is 1.3 X 10^7 cpm/nmole. As a reference, the highest specific activity of ^{3}H-ganglioside GM_1, is 1.3 X 10^5 cpm/nmole.

Iodinated tyramine-ganglioside was characterized by precipitability by TCA-PTA. It was found that 95% of the [^{125}I]-iodotyramine-ganglioside was precipitated by TCA-PTA, while Na^{125}I and [^{125}I]-iodotyramine were not ($<$0.5%). Radioactive ganglioside was also analyzed by thin-layer chromatography in three different solvent systems (see Experimental Section for details). [^{125}I]-iodotyramine ganglioside moved slightly slower than [^{125}I]-iodotyramine and moved slightly faster than Na^{125}I, in solvent systems A and B. However, the separation of these three compounds in these two

systems was not complete. In system C, [^{125}I]-iodotyramine ganglioside GM_1 (R_f=0.33) was well separated from authentic ganglioside GM_1 which had a R_f value of 0.17. Finally, [^{125}I]-iodotyramine had an R_f value of 0.58. All these data, taken together, are consistent with the notion that the compound prepared is indeed radioactive tyramine-ganglioside GM_1.

DISCUSSION

There are three major advantages in the use of [^{125}I]-ganglioside: First, tritium gas formation, an undesirable aspect of galactose oxidase-NaB^3H_4 method, is eliminated completely. Second, [^{125}I]-ganglioside without scintillation fluid can be counted directly with a gamma counter. This saves time, money and reduces experimental error. Third, radioactive ganglioside obtained by this method has specific activity at least 100 times greater than that prepared by the improved method reported by Ghidoni et al., (3) and 1,000 times greater than that of the original method of Suzuki and Suzuki (2). For this reason, [^{125}I]-ganglioside may be the tracer of choice for reactions requiring a sensitive assay.

The disadvantage of this method is that the modification of galactosyl residues may alter the properties of the ganglioside, so that this ganglioside derivative can be used only in a system in which the galactosyl residue plays no significant role. The property of iodinated tyramine ganglioside GM_1 has to be compared with the authentic ganglioside GM_1 in individual systems to ensure that its biological activities are not altered. Sonnino et al., (11) reported that the pattern of ganglioside species in cytosolic ganglioside was similar to that of gangliosides bound to membrane, suggesting that the carbohydrate moiety of ganglioside was not directly involved in the interaction of ganglioside with ganglioside binding proteins. [^{125}I]-iodotyramine-ganglioside could be useful in that system.

All experimental results indicate that the carbohydrate moiety of gangliosides in membrane is exposed and may not be involved in the association with other

components of membrane. This is further supported by Toffano's et al., study of the spontaneous insertion of ganglioside into crude neuronal plasma membrane (12). Interestingly, the galactosyl residue of ganglioside in membrane remains accessible to the action of galactose oxidase, indicating the exposure of galactosyl residue. This is consistent with the notion that the carbohydrate moiety of ganglioside does not directly participate in this insertion process. For this reason, [^{125}I]-iodotyramine-ganglioside may also be useful in that system.

In summary, [^{125}I]-iodotyramine-ganglioside should prove useful in some metabolic and structural studies that require a high specific activity of tracer and in which the carbohydrate moiety of ganglioside does not directly participate in the reactions.

Tritiated compounds are completely identical to the authentic compounds, while radioiodinated compounds prepared by substitution, are different from original compounds. The nonidentity of radioiodinated compounds is the price we pay for the high specific activity of radioiodinated compounds. For this reason, it is essential that radioiodinated compounds are tested in the biological systems to ensure that the substitution does not alter its activity. It may be pointed out that [^{125}I] -iodotyramine ganglioside contains also nonradioactive GM_1 without tyramine attached. The contamination of authentic compounds does not pose interference problems. We have studied the insertion of radioiodinated ganglioside into crude neuronal membrane and found that it is similar to that of tritiated ganglioside (13).

EXPERIMENTAL

Materials

Ganglioside GM_1 of beef brain was obtained from Supelco, Inc. Galactose oxidase (Polyporus circinatas, 27 int. units/mg protein), tyramine, chloramine T, sodium metabisulfite, catalase, bovine serum albumin and N-acetylneuraminic acid were purchased from Sigma Chemical Co. Carrier free Na^{125}I was obtained from ICN. High-performance silica gel precoated plates were purchased from Merck, GmbH.

Methods

Trichloroacetic acid-phosphotungstic acid precipitation: Fifty micrograms of bovine brain mixed gangliosides and 50 µg bovine serum albumin were added as carriers to samples (200 µl). Then 1 ml of ice cold solution containing 20% TCA and 2% PTA was added to precipitate gangliosides. After 20 min of incubation at 4oC, precipitate was collected by centrifugation with a Sorvall GLC-1 centrifuge at 2,500 rpm for 15 min. The pellets were washed twice with the same solution (1 ml).

Ascending thin layer chromatography: Three solvent systems were used to analyze the gangliosides and their derivatives: A) chloroform:methanol:2.5 mg/ml calcium chloride:58% (w/w) ammonium hydroxide = 60:36:8:0.25. B) 1-butanol:acetic acid:water:pyridine = 75:15:60:50. C) 1-propanol:2-propanol:58% ammonium hydroxide = 35:35:30. Radioactive spots were detected by autoradiography. Tyramine and its derivatives were visualized in ultraviolet light and gangliosides were detected by resorcinol spray (14).

Attachment of tyramine to ganglioside GM_1: A reaction mixture (1 ml) containing a final concentration of 100 mM tyramine, 0.2 mM ganglioside GM_1 (1 mg/ml), 10 mM sodium phosphate, pH 7.4, 200 mM EDTA, 15 units/ml catalase and 14 units/ml galactose oxidase was incubated at 37°C with shaking for 18 h. Sodium cyanoborohydride (1.25 mg) was added and the reaction was incubated for 3 h before the addition of another 5 mg of sodium cyanoborohydride. After 3 h further incubation, another 5 mg of sodium cyanoborohydride was added. After one more hour of incubation at 37°C, the reaction mixture was dried by lyophilization and the ganglioside derivative was extracted twice with 1 ml of chloroform-methanol (3:1, v/v).

This extract was evaporated to dryness under a stream of nitrogen. The residue was dissolved in 2 ml of distilled water and the extract was dialyzed against 1 liter of distilled water for 3 d with daily water changes (to remove unattached tyramine). About 80% of the free ganglioside added in the initial reaction mixture was recovered in tyramine-ganglioside as determined by the resorcinal method. Approximately 20% of the GM_1 molecules contained tyramine as judged by the absorbance at 280 nm.

Iodination of tyramine-ganglioside: Tyramine-ganglioside free of tyramine was iodinated by the procedure of Montelaro and Bolognesi (15): 100 µl of tyramine-ganglioside (0.4 mg/ml) was diluted with 200 µl glycine (1 M, pH=10.0) and 2 µl of $Na^{125}I$ (1 mCi) (Carrier free, 17 Ci/mg) was added to it. The reaction was initiated with 100 µl of iodine chloride solution (72 mg/ml) (13). After 1 min of incubation at room temperature, the reaction mixture was then dialyzed for 2 d against distilled water with two daily changes of solution. Alternatively, [^{125}I]-iodotyramine-ganglioside GM_1 can be separated from $Na^{125}I$ by preparative thin layer chromatography (solvent: chloroform:methanol:water 60:30:4.5 v/v/v).

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