N,N'-DIACETYLCHITOBIONIC N-ALKYLAMIDES: SYNTHESIS, AND INTERACTION WITH TWO 2-ACETAMIDO-2-DEOXY-D-GLUCOSE-BINDING LECTINS

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ABSTRACT

Condensation of N,N'-diacetylchitobiono-1,5-lactone, obtained by oxidation of N,N'-diacetylchitobiose, with n-alkylamines gave N,N'-diacetylchitobionic N-alkylamides. The interaction of these glycolipids with wheat-germ agglutinin and Griffonia simplicifolia II lectin is described.

INTRODUCTION

Because they bind to specific, saccharide structures on cell surfaces, lectins have become important tools with which to study membrane structure and function. It has been speculated that the receptors of lectins on cell surfaces are mainly glycoproteins. Indeed, glycoproteins having lectin-binding capacities have been isolated from cell membranes. Since the discovery by Hakomori and his colleagues that wheat-germ agglutinin (WGA) forms a precipitate with a tumor-specific glycolipid, the interaction between lectins and glycolipids (also major components of cell membrane) has been of great interest. Several studies on lectin–glycolipids interaction have been reported. Williams et al. synthesized model glycolipids of neutral sugars in high yield, and investigated the interaction of these glycolipids with lectins. We report here the synthesis of a series of N,N'-diacetylchitobionic N-alkylamides, and their interaction with 2-acetamido-2-deoxy-d-glucose-specific lectins.

RESULTS AND DISCUSSION

The starting material for these studies, N,N'-diacetylchitobiose (1), was prepared from chitin by acetolysis followed by O-deacetylation. N,N'-Diacetylchitobionic N-alkylamides (4-8) were prepared by following the reaction scheme described previously. The oxidation of the disaccharide 1 was conducted with hypoiodite in methanol by the method of Moore and Link. Potassium N,N'-diacetylchitobionate (2) exhibited the i.r.-absorption characteristics of the carboxylate group at 1610 cm⁻¹. After lactonization of 2 by de-ionization with Amberlite IR-120 (H⁺) and repeated addition and evaporation of dry methanol–ethanol, the absorption for
TABLE I

PROPERTIES OF SYNTHETIC GLYCOLIPIDS

<table>
<thead>
<tr>
<th>N,N'-Diacetylchitobionic Compound number</th>
<th>Analyses</th>
<th>Rp valuea in t.l.c.</th>
<th>Yieldb (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Calc.</td>
<td>Found (%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>H</td>
<td>N</td>
</tr>
<tr>
<td>N-Octylamide · 0.5 H2O 4</td>
<td>51.41</td>
<td>8.27</td>
<td>7.49</td>
</tr>
<tr>
<td>N-Decylamide · 0.5 H2O 5</td>
<td>53.04</td>
<td>8.56</td>
<td>7.14</td>
</tr>
<tr>
<td>N-Dodecylamide · H2O 6</td>
<td>53.74</td>
<td>8.86</td>
<td>6.71</td>
</tr>
<tr>
<td>N-Tetradecylamide · H2O 7</td>
<td>55.11</td>
<td>9.09</td>
<td>6.43</td>
</tr>
<tr>
<td>N-Hexadecylamide</td>
<td>57.90</td>
<td>9.26</td>
<td>6.33</td>
</tr>
</tbody>
</table>

aIn 29:10:1 CHCl3–MeOH–H2O. bBased on N,N'-diacetylchitobiose.
carboxylate changed to 1740 cm\(^{-1}\), which is diagnostic of \(\delta\)-lactones. The potassium salt (2) and \(N,N'\)-diacetylchitobiono-1,5-lactone (3) were used without further purification in the following reactions.

The condensation of 3 with \(n\)-alkylamines (8, 10, 12, 14, and 16 carbon atoms) in methanol in the presence of dicyclohexylcarbodiimide gave 4–8 in a yield of 13 to 29%. These glycolipids (4–8) were characterized by strong, i.r. absorptions: Amide I (\(\sim 1650\)), Amide II (\(\sim 1550\)), and the C–H stretching vibration of alkyl groups (\(\sim 2930\) cm\(^{-1}\)). The n.m.r. spectra of 4–8 showed an equivalent number of protons at \(\delta\) 1.24 for methylene in the alkyl chain, three at \(\sim 0.85\) for methyl protons (also in the alkyl chain), and six at \(\sim 1.85\) for protons in the acetyl group. The properties of the synthetic glycolipids are shown in Table I.

Although the \(N\)-alkylaldobionamides of neutral sugars had been synthesized in high yields, the yields of 4–8, synthesized by the same procedure, were low. The reason for the low yields of both the oxidation and amidation reactions are believed to be due to diminution of the partial positive charge on C-1 of the reducing 2-acetamido-2-deoxy-D-glucose residue by interaction with the neighboring carbonyl group of the acetamido group on C-2. This results in a weakening of the nucleophilic attack at C-1 by hypoiodite in the oxidation reaction, and by the nitrogen atom of the alkylamines at C-1 of the lactone in the amidation reaction.

Having long-chain alkyl groups, the glycolipids (4–8) form micelles, and precipitate with lectins that have a specificity for 2-acetamido-2-deoxy-D-glucose. The precipitation curves of WGA with micelles of these glycolipids are shown in Fig. 1. The initial points of precipitation depend on the critical concentration of micelles of the glycolipids. The concentrations of \(N\)-hexadecyl (8), \(N\)-tetradecyl-
Fig. 2. Quantitative precipitin curves of \(N,N'\)-diacetylchitobionic \(N\)-alkylamides with GS II lectin. Increasing quantities of glycolipid were added to GS II lectin (850 \(\mu\)g) in salt-Pi buffer (pH 7.2, total volume 0.6 mL). △, 6; ○, 7; □, 8.

Fig. 3. The effect of 2-acetamido-2-deoxy-\(\alpha\)-glucose on the amount of nonspecific precipitation of GS II lectin. [GS II lectin (850 \(\mu\)g) in salt-Pi buffer (pH 7.2, 0.6 mL) was incubated with increasing amounts of 2-acetamido-2-deoxy-\(\alpha\)-glucose at 25\(^\circ\).]

\(7\), and \(N\)-dodecyl- (6) amides of \(N,N'\)-diacetylchitobionic acid at the point of initial precipitation were <1.6, 11, and 100 \(\mu\)g/mL. These values are very similar to the concentrations of the corresponding \(N\)-alkylmaltobionamides at which precipitation of concanavalin A first occurred\(^9\). This similarity indicates that the critical micelle-concentration is governed by the number of carbon atoms in the alkyl chain, and is independent of the nature of the sugar moiety, a finding that was noted in a previous study\(^9\).
Fig. 4. Inhibition of GS II lectin-N,N'-diacetylchitobionic N-hexadecylamide interaction by amino sugars. [Each tube contained BS II lectin (850 μg), N,N'-diacetylchitobionic N-hexadecylamide (10 μg), and inhibitor as noted, in a total volume of 0.6 mL. Δ, 2-acetamido-2-deoxy-D-glucose; ○, 2-acetamido-2-deoxy-D-galactose.]

The precipitation of the *Griffonia simplicifolia* II lectin (GS II)* with 8 and 7 (see Fig. 2) commenced at concentrations of <1.6 and 15 μg/mL, respectively, but precipitation with 6 did not occur, even at a concentration of 600 μg per tube.

The precipitin curve of N,N'-diacetylchitobionic N-tetradecylamide with the GS II lectin (see Fig. 2) represents the sum of both nonspecific and specific precipitation. The amount of precipitate at the low concentration points of glycolipid may be attributed principally to nonspecific precipitation of the lectin, whereas the precipitated complex at high concentration points of glycolipid is composed mostly of specific precipitate. These conclusions derive from the following observations: GS II (850 μg) gradually precipitates from buffer solution. Increasing concentrations of 2-acetamido-2-deoxy-D-glucose specifically protect the GS II lectin from nonspecific precipitation, as is evident from Fig. 3. It is probable that interaction of the GS II lectin with high concentrations of glycolipid enhances specific, and diminishes nonspecific, precipitate formation. That the precipitation reaction between N,N'-diacetylchitobionic N-hexadecylamide and GS II is specific is indicated by the demonstration that 2-acetamido-2-deoxy-D-glucose inhibits, whereas 2-acetamido-2-deoxy-D-galactose fails to inhibit, the precipitation reaction (see Fig. 4).

**EXPERIMENTAL**

*General.* — I.r. spectra of compounds in potassium bromide discs were recorded with a Perkin–Elmer 283 spectrophotometer. N.m.r. spectra for carbohydrates dissolved in dimethyl sulfoxide-d<sub>6</sub> were recorded with a Varian T-60 n.m.r. spectrometer, with tetramethylsilane as the internal standard. Silica gel G (E. Merck, Darmstadt, Germany) was used for column chromatography. T.i.c. was conducted

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*The correct designation for the plant we have previously referred to as *Bandeiraea simplicifolia* is *Griffonia simplicifolia.*
on plates precoated with silica gel G (layer thickness 0.25 mm; E. Merck). Micro-
analyses were performed by Spang Microanalytical Laboratory (Eagle Harbor, MI).

Materials. — *Griffonia simplicifolia* II lectin was isolated by the method of Shankar Iyer et al.\textsuperscript{14}. Wheat-germ agglutinin was purchased from Calbiochem (San Diego, CA). Octylamine, decylamine, dodecylamine, tetradecylamine, and dicyclo-
hexylcarbodiimide were obtained from the Aldrich Chemical Company (Milwaukee, WI). Hexadecylamine was obtained from Eastman Kodak Company (Rochester, NY). Amberlite IR-120 cation-exchange resin was obtained from the Mallinkrodt Chemical Company (St. Louis, MO).

Precipitin reaction. — A modification of the precipitin reaction described by So and Goldstein\textsuperscript{15} was employed. Increasing quantities of glycolipid dissolved in salt-Pi buffer (0.01 M phosphate, pH 7.2; 0.5 M NaCl; 0.1 mM MnCl\(_2\); and 0.1 mM CaCl\(_2\)) were added to tubes containing buffer and lectin (WGA, 460 µg; GS II, 850 µg) in a total volume of 0.6 mL. Tubes were incubated for 48 h at 25\(^\circ\), and centrifuged. The precipitates were dissolved in 0.05 M NaOH, and protein was determined by a semimicro, Lowry procedure\textsuperscript{16}.

N,N'‐Diacyctetylchitobionic N‐dodecylamide (6). — A solution of N,N'‐diacetylchitobiose (424 mg, 1 mmol) in 95% methanol (10 mL) was added to a solution of iodine (508 mg, 2 mmol) in methanol (10 mL) at 40\(^\circ\). After removal of the heat source, a solution of potassium hydroxide (336 mg, 6 mmol) in methanol (12 mL) was added dropwise, with stirring, during 30 min. After a further 15 min, diethyl ether (50 mL) was added to the mixture, and the potassium N,N'-diacetylchitobionate (2) which was precipitated was filtered off, washed with ethanol (3 × 8 mL), and dried *in vacuo*; yield, 390 mg (0.82 mmol).

The potassium salt (390 mg) was dissolved in a mixture of water (1 mL) and methanol (5 mL), and Amberlite IR-120 (H\(^+\)) ion-exchange resin (2 mL) was added to the solution. The mixture was stirred for 5 min, the resin was filtered off, and the filtrate was evaporated to dryness under diminished pressure. Repeated addition and evaporation of methanol and ethanol gave N,N'-diacetylchitobiono-1,5-lactone (3); yield, 320 mg (0.76 mmol).

A mixture of 3 (320 mg, 0.76 mmol), dodecylamine (154 mg, 0.83 mmol), and dicyclohexylcarbodiimide (187 mg, 0.91 mmol) in absolute methanol (3 mL) was kept for 18 h at room temperature. The mixture was concentrated, and the concentrate was applied to a column of silica gel (6 × 600 mm). The column was washed successively with chloroform (100 mL), and chloroform–methanol 99:1 (v/v) (100 mL), 49:1 (100 mL), and 97:3 (100 mL). Fractions containing 6, \(R_f 0.24\) in t.l.c. in 29:10:1 (v/v) chloroform–methanol–water, were combined, and evaporated, to give 125 mg (0.20 mmol) of product. The residue was dissolved in a small volume of hot methanol, and hot acetone was added. The precipitate that formed upon cooling was collected by centrifugation; it showed \(\nu_{\text{max}}^{\text{KBr}}\) 2935 (C–H), 1653 (Amide I), and 1552 cm\(^{-1}\) (Amide II); \(^1H\)-n.m.r. (60 MHz; Me\(_2\)SO-d\(_6\)): δ ~0.85 (3 H, CH\(_3\)), 1.24 (22 H, 11 CH\(_2\) in alkyl chain), and ~1.85 (6 H, 2 COCH\(_3\)).
N,N'-diacetylcitobionionic N-alkylamides 

Anal. Calc. for C\textsubscript{28}H\textsubscript{53}N\textsubscript{3}O\textsubscript{11} · H\textsubscript{2}O: C, 53.74; H, 8.86; N, 6.71. Found: C, 54.04; H, 8.58; N, 6.79.

The remaining glycolipids were prepared by a similar method. The products, \( R_F \) values in t.l.c., elemental analyses, and yields are given in Table 1.

REFERENCES