An Unusual Glucocerebroside in the Crustacean Nervous System

Abstract. *High concentrations of glucocerebroside (glucosylceramide) were found in the ventral nerve cord, brain, optic nerve, and antenna, but not in the nonneural tissue, of the brown shrimp* Penaeus aztecus aztecus. *This lipid contained unusual sphingoid bases consisting of 14-, 15-, and 16-carbon sphinganines and sphingenines. The fatty acids were mainly nonhydroxylated homologs 22 carbons long and longer, similar to those found in galactocerebroside but differing from those in glucocerebroside in mammalian nervous systems.*

A distinguishing feature of vertebrate nervous systems is that they contain myelin, the membrane that ensheaths axons and permits saltatory conduction of nervous impulses. Invertebrate nervous systems, however, do not contain true myelin, although axons in higher invertebrates such as shrimp are surrounded by several layers of loosely packed sheath (*1*). Nothing is known about the chemical nature of the sheath and sheath-forming cells found in such higher invertebrates, whereas the chemical composition of the higher vertebrate myelin has been well studied (*2*). Myelin is characterized by a high concentration of cerebrosides (*3*) containing galactose as the sole sugar moiety. We have used a recently developed highly sensitive and specific high-performance liquid chromatography (HPLC) method (*4*) to examine the nervous systems of shrimp and other crustaceans. We have found that these organisms contain high concentrations of glucocerebroside with unusual sphingoid bases.

Freshly caught shore shrimp, *Penaeus aztecus aztecus*, were obtained from a local wholesaler. The tissues were removed and cleaned under a stereoscope, homogenized in three volumes of cold water with a Polytron homogenizer, and lyophilized. The total lipids were extracted, washed (*5*), and analyzed as described below.

After benzoylation and desulfation, a portion of the total lipids was analyzed by HPLC (*4*). In a typical chromatogram of ventral nerve cord lipids (Fig. 1a) no galactocerebroside or sulfatide was detected, but a large peak with a retention time corresponding to glucocerebroside was present. Fractionation of the remaining total lipids on a silica gel (Unisil, 100 to 200 mesh) column (*6*) produced neutral lipids, glycolipids, and phospholipids with yields of 35, 6, and 59 percent (by weight) of the starting material, respectively. The glycolipid fraction was further fractionated with preparative thin-layer chromatography (TLC) on a Silica Gel G plate with a mixture of chloroform and methanol (9:1). A major band (located by bromthymol blue spray) migrated with a relative mobility (R_f) similar to that of the glucocerebroside standard. The powder containing the band was scraped and eluted with a 1:1 mixture of chloroform and methanol. The eluant was then washed by the Folch procedure (*5*) and evaporated to dryness. Analysis of the residue with HPLC showed a single peak identical to that seen with HPLC of the total lipid. The infrared spectrum of a KBr disk of this material (not shown) was essentially identical to that of authentic glucocerebroside (purchased from Miles Laboratories) and the published spectrum (*7*). The absorption at 10.4 μm (*trans* double bond) was much smaller than that of the reference compound. The absorption at 11.1 μm by the shrimp glucocerebroside indicated that it had a β-glucosidic linkage.

The glycolipid was methanolyzed with anhydrous 0.5*N* methanolic HCl (*8*), and the fatty acid methyl esters obtained were analyzed by gas-liquid chromatography (GLC) on a fused silica capillary column coated with OV-1. The major fatty acid was 22:0 (approximately 40 percent), followed by 24:1 (26 percent), 24:0 and 23:0 (8 percent each), 18:0 (5 percent), 22:1 (3 percent), and 23:1 (2 percent), with other minor acids ranging from 14:0 to 26:0. The carbohydrate component was confirmed as glucose by analyzing the material remaining in the methanolic layer after converting it to trimethylsilyl ether by GLC on a 3 percent SE-30 column (*9*).

Another portion of the glycolipid was methanolyzed with aqueous methanolic HCl (*10*). The sphingoid base obtained was purified by silica gel chromatography (*10*). On analysis by TLC (*11*), the base provided a major ninhydrin-positive spot corresponding to authentic sphinganine (purchased from Miles Laboratories) and a minor spot identical to standard sphingenine. Analysis with GLC of the trimethylsilyl derivative of

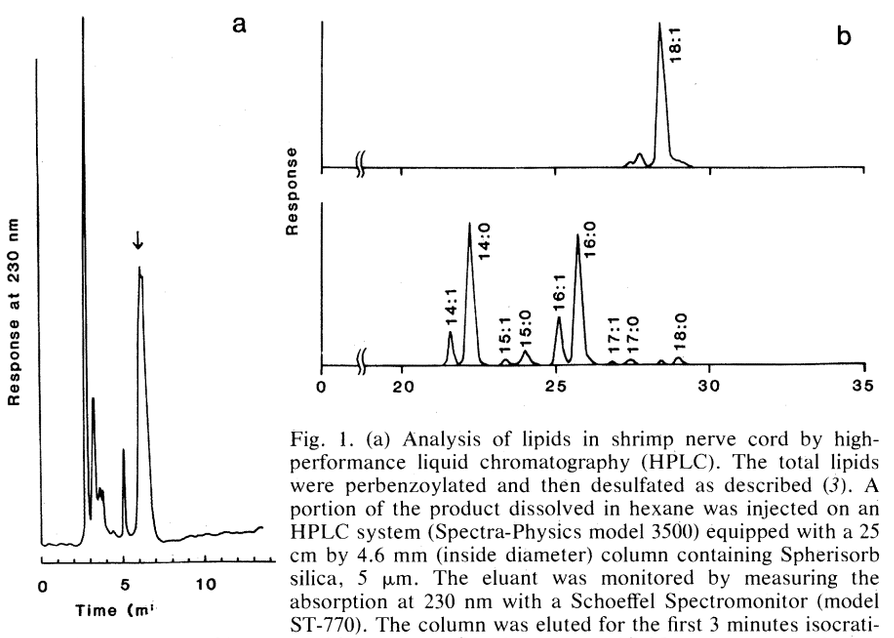

Fig. 1. (a) Analysis of lipids in shrimp nerve cord by high-performance liquid chromatography (HPLC). The total lipids were perbenzoylated and then desulfated as described (*3*). A portion of the product dissolved in hexane was injected on an HPLC system (Spectra-Physics model 3500) equipped with a 25 cm by 4.6 mm (inside diameter) column containing Spherisorb silica, 5 μm. The eluant was monitored by measuring the absorption at 230 nm with a Schoeffel Spectromonitor (model ST-770). The column was eluted for the first 3 minutes isocratically with a mixture of hexane and isopropanol (99.15 percent and 0.85 percent, respectively). The isopropanol concentration was increased linearly to 5 percent in 10 minutes. The flow rate was 1.1 ml/min throughout. All solvents included 0.04 percent concentrated ammonium hydroxide. The peak of a glucocerebroside derivative is indicated by an arrow. Under these conditions, the derivatives of galactocerebrosides containing nonhydroxy and hydroxy fatty acids appeared at 8.0 and 11.5 minutes, respectively. (b) Gas-liquid chromatogram of sphingoid base of glucocerebroside from Gaucher's spleen (top) and shrimp nerve cord (bottom). Both preparations were derivatized to trimethylsilyl ethers and analyzed by a gas chromatograph (Hewlett-Packard model 5880 A) equipped with a 2 m by 3 mm packed column containing 3 percent SE-30. The initial temperature was set at 100°C, and a temperature gradient of 5 degrees per minute was applied. Identity of each peak, which is shown by number of carbon atoms followed by number of double bonds, is confirmed by GLC-MS.

the sphingoid base (*10*) revealed a number of peaks that were completely different from the chromatogram of the sphingoid base obtained from glucocerebroside from the spleen of a patient with Gaucher's disease (Fig. 1b). Analysis with GLC–mass spectrometry (MS) established that these peaks were composed of pairs of saturated and unsaturated 14-, 15-, 16-, 17-, and 18-carbon sphingoid bases, all of which had a 3-alkyl-3-hydroxyl-2-amino-1-propanol structure. Occurrence of 14- to 16-carbon sphingoid bases has been reported in phospholipids of several species of invertebrates, including crayfish (*12*). The ratio of sphingoid base [determined by fluorescamine (*13*)] to glucose (determined by anthrone–sulfuric acid) was 1:1. Furthermore, glucose was of the D configuration, determined by quantitatively evaluating its reaction with hexokinase and glucose-6-phosphate dehydrogenase (*14*). Analysis (GLC-MS) of the methanolysis product of permethylated material (*15*) from shrimp glucocerebroside indicated that the glucose is linked to primary alcohol of the sphingoid base by a glycosidic linkage.

Glucocerebroside was highly concentrated in the nerve cord, brain, optic nerves, and antenna (Table 1). The concentrations in these tissues are similar to the amounts of galactocerebroside found in vertebrate nervous systems (*2, 16*). The lipid from the antenna contained the highest concentration of glucocerebroside. The hepatopancreas and shells had no detectable glucocerebroside. The small amounts of glucocerebroside found in gills and muscle may be due to nerves present in these tissues. Our preliminary investigation showed that glucocerebroside was also found in other crustaceans, but in smaller concentrations. In lobster the ventral nerve cord was separated into ganglia and the nerve fibers connecting them. The fibers had an almost ten times greater concentration of glucocerebroside than the ganglia. Glucocerebrosides were also detected in the ventral nerve cord and brain of crayfish. Although such a distribution suggests that this lipid is localized in the sheath and sheath-forming cells rather than in the neuronal cell bodies of these crustaceans, more defined analysis is needed.

Since shrimp nerve has an unusually high-conduction velocity (*17*), it is interesting that the concentration of glucocerebroside in the shrimp nervous system is much higher than that in lobster or crayfish. Moreover, the highest concentration of glucocerebroside is in the shrimp antenna, an organ solely designed for tactile sensing. This finding further supports the concept that glucocerebroside is important for nerve conduction. Mammalian epidermis, which contains tactile sensing nerves, also contains relatively high concentrations of glucocerebroside and its derivatives (*18*).

Table 1. Glucocerebroside concentrations (measured as nanomoles per milligram of total lipids) in various tissues of the brown shrimp *Penaeus aztecus aztecus*. Total lipids are measured as milligrams per gram of dry tissue. N.D., none detected.

Tissue	Total lipids (mg/g)	Glucocerebroside (nmole/mg)
Ventral nerve cord, including ganglia	405	78
Brain*	125	37
Optic nerve	605	46
Antenna	42	96
Gill	117	6.9
Muscle	71	8.7
Hepatopancreas	444	N.D.
Shell	16	N.D.

*Cephaloganglia.

Vertebrate myelin is characterized by high concentrations of galactocerebroside, but the presence of glucocerebroside has not been confirmed (*2*). Glucocerebrosides, in contrast, are considered to be neuronal lipids and the precursors of gangliosides that are enriched at neuronal synaptic junctions. Moreover, in the rat brain, these lipids contain mainly 16- and 18-carbon fatty acids and 18-carbon sphingoid bases (*19*), whereas in shrimp glucocerebroside, most of the fatty acids have chains longer than 22 carbons, and the sphingoid bases contain 14, 15, and 16 carbons. In fact, the composition of the ceramide portion of shrimp glucocerebroside resembles the composition of the ceramide in mammalian brain galactocerebroside more closely than that in glucocerebroside. It has also been observed that the concentration of glucocerebroside is higher in immature mammalian brain than in mature brain (*16*). In view of these findings, the role of glucocerebroside in immature mammalian brain requires reexamination.

On the basis of embryological designations that refer to the formation of a blastopore, animals can be separated into two subkingdoms: protostomes and deuterostomes. Crustaceans, the most highly evolved animals of the protostome groups, contain glucocerebroside as a major nerve glycolipid, whereas mammals, which head the deuterostome group, contain galactolipids in the nervous system almost exclusively. Even in lower vertebrates such as amphibians, galactocerebroside is the major glycolipid in the nervous system (*20*). Furthermore, in the more highly evolved nervous system of the deuterostome group, both sulfate derivatives and α-hydroxy fatty acid–containing glycolipids are found almost exclusively in galactolipids rather than glucolipids (*2*). These observations suggest that galactolipid may have contributed significantly to the evolution of the more advanced nervous system.

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References and Notes

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3. Trivial names used: sphinganine is a long-alkyl chain 2-amino-1,3,-diol, and sphingenine is its 4,5-unsaturated derivative. Cerebroside is *N*-acyl-1-*O*-β-hexosyl derivative of the sphingoid base, and sulfatide is the 3-sulfate ester of cerebroside. Cerebroside containing galactose or glucose is called galacto- or glucocerebroside, respectively. Fatty acids are abbreviated as the number of carbon atoms followed by the number of double bonds.
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