Development of novel methods for rapid and efficient extraction of naturally occurring bioactive sphingoid bases

Title

Author(s)
SIDDABASAVEGOWDA, B.

Issue Date
2016-03-24

Doc URL
http://hdl.handle.net/2115/61774

Rights(URL)
http://creativecommons.org/licenses/by-nc-sa/2.1/jp/

Type
theses (doctoral - abstract and summary of review)

Additional Information
There are other files related to this item in HUSCAP. Check the above URL.

File Information
SIDDABASAVEGOWDA_B_abstract.pdf (論文内容の要旨)
Sphingolipids were initially isolated as chemical constituents of brain in 1884 by German physician J. L. W. Thudichum, and named after the mythological Sphinx owing to their enigmatic natures. They are found in essentially all animals, plants, and fungi as well as some prokaryotic organism and viruses. Sphingolipids emerged as bio-effector molecules, controlling various aspects of cell growth, extracellular signals in addition to being major constituents of lipoproteins and the multi-lamellar water barrier of skin. Their chemical structure composed of a sphingoid base backbone, amide linked fatty acids, and various moieties at the primary hydroxyl group. Many of the naturally occurring and synthetic sphingoid bases are cytotoxic for cancer cells and pathogenic microorganisms or have other potentially useful bioactivities; hence, they offer a promise as pharmaceutical leads. Even though sphingoid bases have of greater chemotherapeutic importance, studies focusing on the efficient extraction of sphingoid base from natural resources were limited due to several drawbacks, including complex extraction protocols, poor extraction efficiency and low abundance.

The aim of this work is to develop a methodology for the efficient extraction of sphingoid bases; such methodology is a crucial requirement for the discovery of novel sphingoid bases from natural resources, to evaluate their biological activity. Structurally, all sphingoid bases possess a common 2-amino 1,3 diol moiety, and these functional group know to react chemoselectively with glutaraldehyde at room temperature. Based on these key idea, the novel glutaraldehyde resin (GR) was designed and synthesised in the laboratory after optimization of several reaction conditions. The GR was synthesized in gram-scale starting from commercially vailable trans-p-coumaric acid in eight steps and the resin was found to be stable at room temperature for more than a year, which is confirmed from IR-spectroscopy. Using GR, a novel protocol for extraction of sphingipid bases from biological samples was developed. Finally, GR was applied for the extraction of endogenous sphingosine from human serum sample as well as sphingoid bases (9-methyl sphingadienine and glucosyl-9-methyl-sphingadienine) from a golden oyster fungi (Tamogitake in Japanese). Present GR extraction method is highly efficient (>80%) and rapid compared to classical solvent extraction. Also, this will provide the opportunity to establish an analytical method for rapid analysis of sphingoid bases in nanoscale with ultra purity. Hence GR based extraction is very promising technology for the discovery of new sphingoid bases, based chemotherapeutics.

While searching for large-scale production of sphingoid bases, we found that glucosylceramides (GlcCer), are abundant in natural resources (~ 20 % of total lipids) whereas sphingoid bases are present as minor components. GlcCer are a class of glycosphingolipids, consisting of ceramide and a single sugar residue (glucose) at C-1. Since sphingoid bases are naturally less abundant, if one can find a method to convert GlcCers into sphingoid bases, it will be an efficient way to supply sphingoid bases in large-scale. Eventhough, there are many reports on sphingoid base preparation from GlcCer, they all use mainly, either acid or base hydrolysis, which is laborious and less efficient. In the case of acid-catalyzed hydrolysis reaction, isomerization and degradation were frequently observed. This undesired reaction is caused by its allylic alcohol group, which readily react with solvents such as
methanol leading to introduction a methoxy group at C3 position, also, which reacts as an ester via \(N\)-acyl migration, and more allylic rearrangements. While in the case of base-catalyzed hydrolysis, the \(\beta\)-glycosidic bond is much more resistant than \(N\)-acylated bond against alkali hydrolysis and thus, the reaction does not produce sphingoid bases.

To overcome these limitations, in this study, a novel chemoenzymatic method was developed to prepare sphingoid bases from various kinds of glucosyleramides (GlcCers) with high efficiency. The first step is performed by an alkali-catalyzed hydrolysis reaction accelerated by microwave irradiation leading selective and efficient cleave of the amide bond to obtain lysoGlcCers. The second step is accomplished by an enzymatic hydrolysis of the \(\beta\)-glycosidic bond in GlcCers by almond \(\beta\)-glucosidase for industrial use. The removal of the acyl chain will increase the hydrophilic character to increase accessibility toward its enzymatic active center of \(\beta\)-glucosidase. In fact, hydrolysis of original GlcCer from golden oyster mushroom (\(P.\ citrinopileatus\)) by almond \(\beta\)-glucosidase didn’t work even after adding Triton X-100. Our strategy to increase accessibility toward the enzyme by removing the hydrophobic fatty acid chain by microwave-assisted efficient basic hydrolysis is quite unique and practical. Several sphingoid bases were successfully prepared in high yield by employing the designed method from a wide variety of GlcCers. This novel method provides a new way to prepare various kinds of sphingoid bases from GlcCers of dietary natural resources such as rice, wheat, and soy suitable for practical use.

Analysis and functional studies of naturally occurring sphingoid bases are an emerging area of lipid research and are promising drug leads. In this view a practically efficient method for the preparation of sphingoid bases starting from naturally abundant glucosyleramides was provided. In the current study, the chemoenzymatic method for preparation of sphingoid bases was performed on a medium and small-scale. However, this procedure has a potential to be expanded to large-scale experiments. This method is a simple, economical, and reliable, which is not required a limited amount of starting material and providing a suitable tool for convenient preparation and the proper analysis of sphingoids without interference of any artifacts. Also, as an extension of this study, to evaluate the biological activity of sphingoid bases. Sphingoid bases and their derivatives such as ceramides, \(N,N\) dimethyl sphingoids, were prepared and MCF-7/ADR cell were established from parent MCF-7 by sequential treatment of adriamycin. The preliminary result from the cell viability assay suggests that, these natural sphingoid bases and their derivatives are cytotoxic to MCF-7 and MCF-7/ADR.

In conclusion, compared to the reported extraction/preparation methods these novel methods are more simple, efficient and practically applicable. Hence adopting these methodologies for extracting sphingoid bases by lipid chemists may solve the crisis for sphingoid bases. Henceforth, it may open the door for exploring their useful bioactivities in various fields.