

行政院國家科學委員會補助專題研究計畫成果報告

鼠李醣轉移酵素之反應機制研究與其在藥物研發之應用

計畫類別： 個別型計畫 整合型計畫

計畫編號：NSC - 90-2113-M-009-014

執行期間：90年08月01日至91年07月31日

計畫主持人：邱 顯 泰

本成果報告包括以下應繳交之附件：

赴國外出差或研習心得報告一份

赴大陸地區出差或研習心得報告一份

出席國際學術會議心得報告及發表之論文各一份

國際合作研究計畫國外研究報告書一份

執行單位：國立交通大學生物科技學系

中 華 民 國 91 年 07 月 31 日

行政院國家科學委員會專題研究計畫成果報告

鼠李糖轉移酵素之反應機制研究與其在藥物研發之應用

計畫編號：NSC - 90-2113-M-009-014

執行期限：90年08月01日至91年07月31日

主持人：邱顯泰 國立交通大學生物科技學系

計畫參與人員：陳億霖、陳宜嫻 國立交通大學生物科技研究所

一、中文摘要

本研究的目標在探討重要生物合成途徑中，負責將特殊去氧糖類轉接至天然物糖？(aglycone) 之糖基轉移酵素 (glycosyltransferase)。許多這些重要天然二次代謝物若無特殊去氧糖的存在，便無法呈現其所報導之生物活性，這突顯了天然物糖基轉移酵素之活性表現與反應機制研究的重要性。因此，本研究以斯拜諾辛 (spinosyn) 生物合成中之鼠李糖轉移酵素為研究標的，希望為天然物之糖接 polyketide 之此類酵素提供研究與示範的重要資訊。再者，鼠李糖二磷酸核糖？為一特殊去氧糖二磷酸核糖？，不易由化學合成得來。本計畫因此利用微生物合成途徑來合成，並提供為轉移酵素之受質。

本研究成果包括：(一)成功在大腸桿菌 (*E. coli*) 中表達獲得大量鼠李糖轉移酵素，進行酵素動力學與活性測試，觀察到酵素活性。這是首例將糖接至天然物 polyketide 之糖基轉移酵素異源表現，並直接證明酵素活性與功能，且製造純化出被該糖修飾的天然有機分子。(二)成功的將微生物中的鼠李糖二磷酸核糖？生物合成途徑，在生物體外應用三個酵素合成，並能大量製造且純化。更重要的，我們能應用合成途徑的個別酵素或其組合，來獲得鼠李糖合成途徑中的每個中間產物。這些中間產物亦可被用來作為鼠李糖轉移酵素的受質，進而人工合成新奇的含糖天然物。

總之，本計畫成果已達成預期之酵素活性表現，為目前首例將天然物合成中糖接 polyketide 之糖基轉移酵素於異源微生物中大量表現且純化，並於試管中觀察獲得酵素活性。這個突破使科學家能進一步在將來應用此酵素用各類的糖來修飾重要天然物的糖？，進而達到藥物研發的重要里程碑。

關鍵詞：糖基轉移酵素，鼠李糖，天然物生物合成，去氧糖二磷酸核糖？

Abstract

In biosynthesis of bioactive glycosylated secondary metabolites, glycosyltransferases play a key role in the attachment of diverse unusual deoxysugars to their aglycones. Many of these important natural glycosides require the presence of the deoxysugars for their reported biological activities. Thus, it has become a very important issue to study and understand functions and mechanisms of these biocatalysts. This project chose a rhamnosyltransferase involved in the biosynthetic pathway of spinosyns, and aimed to provide biochemical insights into the first ever overexpressed sugar-to-polyketide glycosyltransferase in a heterologous host, *E. coli*. On the other hand, like many deoxysugar nucleotide diphosphates (NDP-sugars), NDP-rhamnose is difficult to be synthesized by traditional organic synthesis. Along our study in rhamnosyltransferase, three enzymes involved in the biosynthesis of NDP-rhamnose was also studied so as to provide the substrates for glycosylation of the spinosyn aglycone.

This research has succeeded in the followings: (1) The glycosylation of spinosyn aglycone by the rhamnosyltransferase has been successfully observed, providing the first example ever demonstrated in the enzymatic sugar-to-polyketide glycosylation *in vitro*. The active enzyme has allowed us to generate pure and a large quantity of the glycosylated polyketide aglycone. (2) We have successfully cloned and overexpressed three biosynthetic enzymes of NDP-rhamnose and are capable of generating

the essential substrate for the glycosylation *in vitro*. In particular, we have demonstrated the ability to isolate and purify the enzymatic intermediates of the pathway starting from NDP-glucose. The isolated intermediates are valuable in serving as alternative substrates for many glycosyltransferases in nature.

In summary, the first sugar-to-polyketide glycosyltransferase has been “functionally” expressed in this research. The enzyme may serve as an important tool to modify various polyketide aglycones with various NDP-sugars, which may allow us to generate novel polyketide glycosides for drug discovery.

Keywords: Glycosyltransferase, Spinosyn, Biosynthesis, Polyketide, NDP-sugar, Enzyme

二、緣由與目的

Glycosyltransferases (Gtfs) catalyze the attachment of specific sugar residues to structurally diverse molecules, constituting one of most important and abundant reactions in nature. Recent genome sequencing and cloning strategies have revealed more than 3000 open reading frames related to Gtfs. However, little is known about a special group of the Gtf superfamily, Family 1, which is responsible for transfers of a wide spectrum of sugar residues into varieties of bioactive secondary metabolites. And yet, it has been shown that biological potency and selectivity of these glycosylated organic molecules must rely on the presence and kinds of the sugars. Therefore, Gtfs have become key targets to be engineered for transferring selected and various sugars, leading to a generation of novel glycosides of important natural products. Such subjects are critical in the discovery of new drugs and compounds of pharmaceutical and industrial importance. To reach the goals, one must, however, gain biochemical, mechanistic and structural information this special group of Gtfs. Many important bioactive secondary metabolites belong to a family of polyketides. Among this group of natural

products are erythromycin A, nystatin and enediynes, etc. To date, none of the sugar-to-polyketide glycosyltransferases involved in the biosynthesis of polyketide glycosides has ever been functionally expressed and characterized in *E. coli* as a heterologous host, especially with the active enzymes purified and assayed.

To approach above goals, we chose spinosyns as our model system to study the polyketide glycosyltransferases. Spinosyns are a naturally derived group of insect control agents that possess activity against several classes of insects (e.g. flies, worms, beetles, caterpillars and leafminers). The rhamnosyltransferase that attaches rhamnose to the spinosyn aglycone was chosen for the purposes described above. In order to carry out enzymatic assays of glycosyltransferases, the availability of the NDP-sugars as substrates has long been a critical and rate-limiting issue. To solve this problem, we have thus cloned and overexpressed the three enzymes, Gdh, Epi and Kre, from the spinosyn producing organism to make NDP-rhamnose biosynthetically for functional assays of the spinosyn rhamnosyltransferase.

三、結果與討論

The following goals have been achieved during the period of 90/08/01-91/07/31.

(1) In order to conduct enzymatic assays of the spinosyn rhamnosyltransferase, both the spinosyn aglycone and NDP-rhamnose are absolutely required. We have previously synthesized the aglycone by acidic hydrolysis of the spinosyns to yield the aglycone and released sugars. Moreover, the conditions for the HPLC (High Performance Liquid Chromatography) analysis of the enzymatic reaction products were also determined before. The only key material left was to synthesize NDP-rhamnose. To achieve the goal, the three enzymes (Gdh, Epi and Kre) were cloned into a pET expression system containing a terminal His tag using PCR and typical techniques in molecular cloning with the genomic DNA of the

organism as the PCR template. The enzymes were then individually overexpressed in *E. coli* under the control of a T7 promoter and IPTG induction. The resulting proteins were purified by a Ni-affinity chromatography, yielding more than 95% pure proteins, as determined by a protein SDS-page, for the NDP-rhamnose biosynthesis. The enzymatic product, NDP-rhamnose, was observed by HPLC when NDP-glucose was incubated with the enzymes and the NADH cofactor. The products of Gdh and Epi were also independently isolated by a C18 HPLC method.

(2) With both substrates (the spoinosyn aglycone and NDP-rhamnose) in hands, we proceeded to carry out the enzymatic glycosylation of the spoinosyn aglycone by rhamnosyltransferase. The results showed that the enzymatic glycosylation of the aglycone by the enzyme was observed and exhibited a time-dependent kinetic manner as well as substrate- and enzyme-dependent behaviors. With sufficient time, the aglycone can be fully glycosylated with rhamnose as determined by HPLC analysis. Furthermore, when all the enzymes (Gdh, Ei, Kre and rhamnosyltransferase) were mixed together *in vitro*, we observed time-dependent consumption of the NDP-rhamnose biosynthetic intermediates and production of the glycosylated aglycone. This is the first time that the on-line biosynthesis of the glycosylated polyketide was demonstrated *in vitro* using enzymes expressed in *E. coli*. We have also tested the ability of the rhamnosyltransferase to utilize other NDP-sugars as substrates to glycosylate the polyketide aglycone. The preliminary result showed that the enzyme indeed possessed a relaxed substrate specificity towards other NDP-sugars. The products of the glycosylation with other NDP-sugars are being characterized by NMR (Nuclear Magnetic Resonance) and MASS spectrometers.

demonstrated the *in vitro* enzymatic activity of a sugar-to-polyketide glycosyltransferase, the spoinosyn rhamnosyltransferase. The recent results also indicate that the possibility of using the enzyme to incorporate other deoxysugars to the polyketide aglycone. The result strongly support our original idea that one may use a polyketide glycosyltransferase to modify natural polyketide with various sugars, thereby generating various polyketide glycosides that may present novel or modified biological activities. In another aspect, we have also demonstrated our ability to utilize *E. coli*-expressing microbial enzymes to synthesize NDP-sugars *in vitro*. This has facilitated our future studies in making various polyketide glycosides with biosynthetically synthesized NDP-sugars from many different unusual NDP-sugar producing microorganisms.

Overall speaking, we have used a combination of chemical and biological methods to study and develop a general analytical and preparative system to assay glycosyltransferases, the key plays in the biosynthesis of a huge group of bioactive polyketide glycosides in nature. The conclusion and results of this research may lead to the methodology useful in applications of glycosyltransferases in drug discovery.

四、計畫成果自評

In summary, we have, for the first time,