α-Amylase and Protein Tyrosine Phosphatase 1B Inhibitory of Some Vietnamese Medicinal Plants Used to Treat Diabetes

Tran Manh Hung1, Hoang Duc Manh2, Pham Thi Hong Minh3, Uj Joung Youn1, MinKyun Na2, Won Keun Oh2, Byung Sun Min4, and KiHwan Bae1,*

1College of Pharmacy, Chungnam National University, Daejeon 305-764, Korea
2Korea Research Institute of Bioscience and Biotechnology, Daejeon 305-333, Korea
3Institute of Chemistry, Vietnamese Academy of Science and Technology, 18 Hoang Quoc Viet, Ha Noi, Viet Nam
4College of Pharmacy, Catholic University of Daegu, Gyeongsan 712-702, Korea

Abstract – In this study, the twenty-four ethyl acetate extracts of twenty-two medicinal plants, traditionally used in Vietnam as anti-diabetes agents, were investigated for α-amylase and protein tyrosine phosphatase 1B (PTP1B) enzymes inhibitory activity in vitro. The results indicated that, twelve materials (50.0%) showed moderate to strong inhibitory activity in α-amylase inhibitory activity with IC50 values ranging from 2.5 to 48.8 µg/mL; meanwhile, ten extracts (41.6%) could demonstrate PTP1B activity with IC50 values less than 30.5 µg/mL. Some plants presented interesting activities against both of α-amylase and PTP1B enzymes such as Catharanthus roseus, Carthamus tinctorius, Momordica charantia, Gynostemma pentaphyllum, Glycyrrhiza glabra, Smilax glabra, Psidium guajava (leave), and Rehmannia glutinosa. The study may provide a proof, at least in a part, for the ethno-medical use in diabetes disease of these plants.

Keywords – Vietnamese medicinal plants, diabetes, α-amylase, protein tyrosine phosphatase 1B

Introduction

Diabetes mellitus is only major diseases that is becoming more common, in part because of the ageing population, a lifestyle that promotes obesity, a growing Hispanic community that has a particularly prevalence of diabetes, and more poor people than the national average (King et al., 1998). The real problem of diabetes is that the condition brings with it a train of chronic complications including accelerated arteriosclerosis, and disease of the eye, foot, and kidney, each of which is costly to manage and can be devastating from the patient. More than 80% of people with type 2 diabetes will live in developing countries (White and Rafique, 2002). Those of underdeveloped countries including Vietnam cannot afford the increasing burden of chronic renal failure and blindness. The large poor populations has lower prevalence rates than the rich, but have higher rates of complications because of later diagnosis, inaction on risk factors, and poor management. In 1990s, about 1-1.5% Vietnamese people were affected by diabetes which would increased to 4% by 2001 but at least 70% patients were clearly not excavated and had right treatment (Vietnam National Diabetes Federation Conference, June 2003). Therefore, there is an urgent need to develop necessary therapies for these diseases.

One of the therapeutic approaches for treating diabetes is to decrease the postprandial hyperglycemia. This is done by retaining the absorption of glucose through the inhibition of the carbohydrate-hydrolyzing enzymes, α-amylase and α-glucosidase, in the digestive tract (Ali et al., 2006). Inhibitors of these enzymes delay carbohydrate digestion and prolong overall carbohydrate digestion time, causing a reduction in the rate of glucose absorption and consequently blunting the post-prandial plasma glucose rise (Defronzo et al., 2004). Recently, protein tyrosine phosphatase 1B (PTP1B) has been shown to be a negative regulator of the insulin signaling pathway, suggesting that inhibitors of this enzyme may be a promising therapeutic target in effective treatment of type 2 diabetes. PTP1B is a major nontransmembrane phosphotyrosine phosphatase in human tissues and was one of the earliest PTP identified. PTP1B inhibitors would increase insulin sensitivity by blocking the PTP1B-mediated negative insulin signaling pathway and might be an attractive target in type 2 diabetes mellitus and obesity.

*Author for correspondence
Fax: +82-42-823-6566; E-mail: baekh@cnu.ac.kr
Research on plants used in traditional medicine as anti-diabetes offers an alternative to the development of new drugs and/or validation of their use in folk medicine (Grover et al., 2002). In Vietnam, no less than 2500 species have been used in ethno medicine and approximately 30 species were used for treatment of diabetes (Loi, 2001). Although these have been used as folk medicines for a long time, they are still untapped source for potential bioactive agent. Therefore, it is expected that medicinal plants not only continue to be used traditionally but also be a useful source of drug discovery. In this study, 22 Vietnamese anti-diabetes plants belong to 16 families were investigated on the basis of their used in traditional medicine with the aim of characterizing on inhibition of α-amylase and PTP1B.

Experimental

Plant material – Most of the medicinal plants were collected in the North of Vietnam in spring, 2006. Some of them were purchased at Dongxuan oriental herbarium market in Hanoi, Vietnam at the same time. The plants were botanically identified by Professor Pham Thanh Ky, Department of Pharmacognosy, Hanoi College of Pharmacy, Vietnam where the voucher specimens were deposited.

Preparation of plant extracts – The collected plants were dried and powdered. Twenty gram of each material was extracted with boiled ethyl acetate for 2 hours under reflux two times. The obtained extracts were filtered through Whatman No. 2 filter paper, and then freeze-dried at 40°C. Both extracts were stored at –4°C until used. The ethyl acetate extract was selected and tested for inhibitory effects because of the containing negligible amount of sugar compounds, which would cause complication in the maltose detection.

α-amylase inhibitory activity – The α-amylase assay was performed using the chromogenic method adopted from Sigma-Aldrich with slight modification. A potato starch solution (0.5% w/v) was obtained by stirring potato starch in 20 mM phosphate buffer with 6.7 mM sodium chloride, pH 6.9 at 65°C for 15 min (R1). The enzyme solution was prepared by mixing α-amylase in ice-cold distilled water to give a concentration of 4 unit/mL (R2). Those extract of collected plants were dissolved in DMSO to give various concentrations. The colorimetric reagent (DNS) solution was prepared mixing 96 mM 3,5-dinitrosalicylic acid and 5.31 M sodium potassium tartrate in 2 M NaOH (R3). In the experiment, 40 µL of extract, 160 µL distilled water, 400 µL R1 and 200 µL R2 were mixed and incubated at 25°C for 3 min. After incubation, 200 µL of mixture was removed and added into other separate tube which containing 100 µL R3. The mixture was boiled at 85°C for 15 min, and then diluted with 900 µL distilled water. The reaction was detectable at 540 nm.

Experimental incubations were conducted with the same method but replacing extract samples with 40 µL DMSO. The blank incubation tubes were carried out as above but replacing extract samples by 40 µL DMSO and R2 was replaced by distilled water. Ursolic acid was used as positive control (Ali et al., 2006).

Protein tyrosine phosphatase 1B (PTP1B) inhibitory activity – PTP1B (human, recombinant) was purchased from BIOMOL® International LP (USA) and the enzyme activity were measured using p-nitrophenyl phosphate (p-NPP) as a substrate (Na et al., 2006). To each 96-well (final volume: 200 µL) were added 2 mM p-NPP and PTP1B (0.05 - 0.1 µg) in a buffer containing 50 mM citrate (pH 6.0), 0.1 M NaCl, 1 mM EDTA, and 1 mM dithiothreitol (DTT) with or without test compounds. Following incubation at 37°C for 30 min, the reaction was terminated with 10 M NaOH. The amount of produced p-nitro phenol was estimated by measuring the absorbance at 405 nm. The nonenzymatic hydrolysis of 2 mM p-NPP was corrected by measuring the increase in absorbance at 405 nm obtained in the absence of PTP1B enzyme. RK-682 used as a positive control (Hamaguchi et al., 1995).

Statistics analysis – The results were expressed as mean ± S.D. of three determination at each concentration for each sample. The inhibitory concentration 50% (IC50) was calculated using Microsoft Excel program. Statistical significance was calculated by one-way analysis of variance (ANOVA), followed by Dunnett’s test.

Results and discussion

The plants are listed in alphabetical order of their family name, followed by the scientific name, vernacular name, as well as part used (Table 1). In the present study, twenty-two plant species which belonging to sixteen families were selected, and total of twenty-four extracts were investigated based on their ethno-medical use for the treatment of diabetes and other diseases by the natives of Vietnamese folk medicinal systems (Loi, 2001). The inhibitory effect of twenty-four extracts on α-amylase and PTP1B activities are summarized (Table 2).

About α-amylase inhibitory activity, the results indicated that, the ethyl acetate extract of 12 materials (50.0%)