ELSEVIER

Comparative Biochemistry and Physiology, Part C 140 (2005) 79–86

CBP

www.elsevier.com/locate/cbpc

Exposure of tilapia pituitary cells to saponins: Insight into their mechanism of action

Berta Levavi-Sivan[a,*], Rachel Hedvat[b], Tamir Kanias[a], George Francis[c], Klaus Becker[c], Zohar Kerem[b]

[a]*Department of Animal Sciences, Faculty of Agricultural, Food and Environmental Quality Sciences, The Hebrew University, P.O. Box 12, Rehovot 76100, Israel*

[b]*Institute of Biochemistry, Food Science and Nutrition, Faculty of Agricultural, Food and Environmental Quality Sciences, The Hebrew University, P.O. Box 12, Rehovot 76100, Israel*

[c]*Department of Animal Nutrition and Aquaculture, Institute for Animal Production in the Tropics and Subtropics, University of Hohenheim (480), D 70593 Stuttgart, Germany*

Received 2 September 2004; received in revised form 3 January 2005; accepted 4 January 2005

Abstract

Cell permeation and durable effects of triterpenoidal saponin preparations from soybean (SbS), *Quillaja saponaria* Molina (QsS) and *Gypsophila paniculata* (GypS), were studied. A concentration-dependent change in hemolysis rates was observed when cells were incubated with QsS or GypS, but not with SbS. Dose dependence was also observed for the leakage of lactate dehydrogenase (LDH; MW 142,000) and of Luteinizing Hormone (LH; MW 35,000) from tilapia pituitary dispersed cells. Exposure of pituitary fragments to a combination of GnRH and GypS or QsS, resulted in a significantly high release of LH. GypS were shown to be more potent in inducing hemolysis of human RBC's and LH release from tilapia pituitary fragments. Interestingly, tilapia pituitary fragments treated with QsS were able to secrete LH in a characteristic manner, in response to a second Gonadotropin Releasing Hormone (GnRH) pulse, while fragments exposed to GypS did not respond to the second hormone pulse. The rapid recovery of pituitary fragments after the removal of QsS, may suggest a rearrangement of membranes rather than pore formation as the mechanism of action of QsS. Understanding the structural features underlying the reversible rearrangement of membranes and the lack of hemolysing activity by specific saponins may lead to the development of novel bioactive drugs.

Keywords: Membrane; Rearrangement; Pituitary; Pores; Saponin; Tilapia; *Quillaja*; Soybean

1. Introduction

Saponins are widely distributed naturally occurring glycosides, consisting of a non-sugar aglycone coupled to sugar-chain units. *Quillaja saponaria* (Qs) bark and *Gypsophila paniculata* (Gyp; 'baby's breath') are two of the four major commercially available sources of saponins (Fulcheri et al., 1998; Rao and Gurfinkel, 2000; Francis et al., 2002a,b,c). In Qs, saponins may represent 20–25% of the extractable material (Guo and Kenne, 2000). They were recently found to have the potential to improve growth and body-composition parameters in tilapia fry and to stimulate growth and inhibit egg production by female Nile tilapia (Francis et al., 2001a,b; 2002a,b,c). Qs saponins (QsS) have a five-ringed (triterpenoid) quillaic acid (1 in Fig. 1) backbone with small carbohydrate chains consisting of two to five sugar units, attached at the 3 and 28 carbons of the aglycone and are frequently branched (Bomford et al., 1992). Attached to the first fucose sugar unit at the 28 position of the carbohydrate chain is an 18-carbon acyl chain with a small carbohydrate chain, consisting of one or two sugar units at its terminal end.

* Corresponding author. Tel.: +972 8 9489988; fax: +972 8 9465763.
E-mail address: sivan@agri.huji.ac.il (B. Levavi-Sivan).

1532-0456/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.cca.2005.01.002

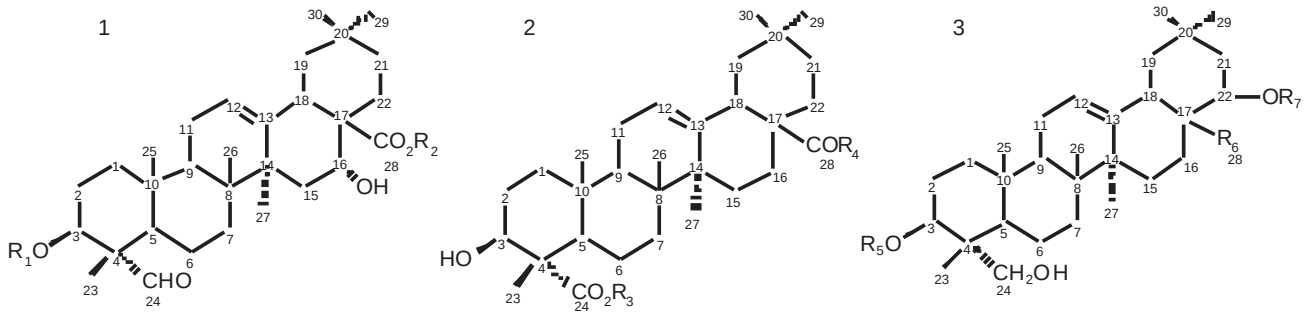

Fig. 1. Chemical structure of soybean saponin with a 2,3-dihydro-2,5-dihydroxy-6-methyl-4H-pyran-4-one moiety (SbS-DDMP; 1), *Quillaja saponaria* saponin (QsS; 2) or *Gypsophila paniculata* saponin (GypS; 3).

The five-ringed (triterpenoid) gypsogenic acid (2 in Fig. 1) is the aglycone present in saponins from Gyp, that are of interest to the pharmaceutical industry (Fulcheri et al., 1998). Soybean saponins (SbS) are found in many legumes and are the major dietary source for saponins (Hu et al., 2002). They are triterpenoid saponins of the oleanane-glucuronide type, i.e., olean-12-ene triterpenes with a C-28 methyl group and a glucuronic acid moiety linked at the C-3. They are divided into two groups, A and B, of which group B (3 in Fig. 1) has been found to contain the primary saponins present in soybeans (*Glycine max*) (Hu et al., 2002). The genuine group B saponins are conjugated with 2,3-dihydro-2,5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP) (Kudou et al., 1994).

It is usually suggested that the biological effects of saponins result from their potent membrane-permeabilizing activities. Common modern uses of saponins in biology include the permeation of cell membranes and active adjuvant components, in their pure form or as part of immuno-stimulating complexes (ISCOMs) (Ronnberg et al., 1995). Using saponins for experimental permeation allows small molecules or macromolecules to cross the cell membrane (Mick et al., 1988; Humbel et al., 1998; Baumann et al., 2000). Although saponins are broadly used as permeabilizing agents, the mechanism by which they allow the entrance of molecules into the cell, and the extent of the reversibility of this process, have not been adequately addressed (Melzig et al., 2001).

Gonadotropin releasing hormone (GnRH) is the most universal and important stimulator of teleost gonadotropins (GtH) such as Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH; reviewed by Yaron et al. (Yaron et al., 2003)). Exposure of pituitary fragments or dispersed cells of tilapia to GnRH results in the dose- and time-dependent release of LH (Levavi-Sivan and Yaron, 1993; Levavi-Sivan et al., 1995).

This study was aimed at exploring the long-lasting effect that triterpenoid saponins may exert on cells. To accomplish this objective, triterpenoid saponin preparations from three different plant sources (Qs, Gyp and Sb) were used, and changes in membrane permeability were measured as LDH and LH release from tilapia pituitary, and hemolysis of human RBC.

2. Materials and methods

2.1. Source of saponins

Preparation and isolation of the SbS (100 gr) were performed as described previously (Hu et al., 2002). Dried, finely ground soybean (*Glycine max*) powder was extracted with 1 L of 70% aqueous ethanol with stirring for 3 h at room temperature. The extract was condensed to 100 mL with a rotary evaporator (Büchner, Brinkman, R-114, Switzerland) at <30 °C, and loaded on a C-18 extractclean™ column (High Capacity C18, Alltech, IL) equilibrated with 10:90 (v/v) methanol/water and then fractionated with a linear gradient of aqueous methanol from 30 to 100%. Saponin-rich fractions were visualized on TLC plates (Z. Karem et al., 2005) and pooled. The fractions containing the SbS were collected and evaporated to dryness at <30 °C. The residue was redissolved in water to obtain a crude SbS solution. HPLC showing the characteristic absorbance of the DDMP moiety at 292 and 205 nm (Hu et al., 2002) served to ascertain the presence of the DDMP saponin as the major compound in these preparations (Fig. 2). HPLC was also used to ensure the DDMP stability throughout the experiments.

Quillaja saponaria Molina saponin was obtained from Sigma Chemical (St. Louis, MO). *Gypsophila paniculata* saponin was obtained from Merck KgaA (Darmstadt, Germany). Commercial powders were dissolved in aqueous methanol (50%) and fractionated on a C-18 extractclean™ column (High Capacity C18, Alltech) with a linear gradient of aqueous methanol from 50 to 100%. Fractions containing saponins were identified using TLC and staining with anisaldehyde reagent (Oleszek, 2002), collected, dried and redissolved as above.

2.2. Fish

The fish used in this study were tilapia hybrids (*Oreochromis niloticus x O. aureus*). They were collected from the fish farms of local kibbutzim and then housed at the university's fish facility, in 500-L tanks under a natural photoperiod and 26±2 °C. For the experiments, the fish (weighing 50–150 g) were sexually mature.