EFFECT OF GLYCYRRHIZIN ON HEPATIC LYSOSOMAL SYSTEMS

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Glycyrrhizin (glycyrrhizic acid, β,β' -glucuronido-glucuronide of 18β -glycyrrhetic acid), occurring naturally in the root of *glycyrrhiza glabra* L., has been used either as a medicament for its desoxycorticosterone-like (1) and cortisol-like (2) effects or as a food additive for its sweetness. Glycyrrhizin, which is classified as a saponin, has a protective effect against hemolysis induced by other saponins or a cationic surfactant (3), though saponins are generally characterized by hemolytic action. Thus, it is probable that glycyrrhizin induces alteration in membrane fluidity by reacting with the components of membrane or intercalating its moiety into the membrane structure.

In this report, we tested biological actions of glycyrrhizin on lysosomes, chosen as a tentative membrane system, by 1) examining whether or not glycyrrhizin affects the hepatic lysosomal enzyme activity itself, and 2) measuring its *in vitro* release of lysosomal enzymes from the lysosome granules.

Glycyrrhizin was purchased from Nakarai Chemicals, LTD., and 18α - or 18β -glycyrrhetic acid was obtained from Sigma Chemical Company. These compounds were used after dissolution in 5 μ l of dimethylsulfoxide.

Hepatic lysosomes were prepared from male mice (ddY strain, 25–30 g of body weight) by centrifugation at $13,000\times g$ for 10 min, according to the method of Wang et al. (4). When we determined direct effects of a test compound on the enzyme activity, we prepared crude enzyme samples

by dissolving lysosomal particles ($13,000\times g$ pellet) with 0.1% Triton X-100.

Lysosomal enzyme activities were measured by the procedures described by Barret and Heath (5, 6). Acid phosphatase (APh) activity was measured by incubating 0.3 ml of enzyme sample with 1.2 ml of 0.2M acetate buffer (pH 5.0) and 0.5 ml of 32 mM 4-nitrophenylphosphate for 20 min at 37°C, and successively adding 2.0 ml of ice-cold 1 M Tris-0.4 M phosphate buffer (pH 8.5) to terminate the reaction. The assay of β -N-acetylglucosaminidase (NAcG) activity was carried out under the following conditions. The incubation mixture contained 0.5 ml of enzyme sample, 0.5 ml of 0.3 M citrate-0.3 M NaCl buffer (pH 4.3), and 0.5 ml of 15 mM 4-nitrophenyl- β -N-acetylglucosaminide. After incubation for 20 min at 37°C, the reaction was stopped by adding 1.5 ml of 0.5 M bicarbonate buffer (pH 10.0). In either case, the presence of the liberated 4-nitrophenol was determined spectrophotometrically at 420 nm.

Integrity of lysosome membrane was ascertained by measuring the release of lysosomal marker enzymes. The release of lysosomal enzymes was induced by incubating for 15 min at 37°C 1.5 ml of lysosome suspension containing 10 mM Tris-HCl (pH 7.1) in 0.44 M sucrose-0.177 M KCl with a test compound in the presence or absence of 0.1% Triton X-100 in the final volume of 2.0 ml. In control experiments, the test compound was replaced by a vehicle. After cooling in ice-water, lysosomes were

centrifuged at $13,000\times g$ for 10 min. A portion of the supernatant was removed for the assay of enzyme activity. We regarded the enzyme activity in the supernatant of the sample treated with Triton X-100 as total recovered activity because sedimentary enzyme activity was negligible. Therefore, alteration in total recovered activity may reflect changes in activity of the enzyme itself.

Table 1 shows the effect of glycyrrhizin and its constituents on the activity of lysosomal enzymes which were prepared by solubilizing lysosome fraction with 0.1% Triton X-100. Both glycyrrhizin and 18β -glycyrrhetic acid, the aglycone of glycyrrhizin, attenuated APh activity significantly, but 18α -glycyrrhetic acid and glucuronic acid showed no effects. On the other hand, NAcG activity remained unchanged when adding of any the compounds listed above, in a dose of 3×10^{-4} M.

Figure 1 shows that the release of APh was enhanced by the addition of glycyrrhizin or 18β -glycyrrhetic acid, but was reduced by 18α -glycyrrhetic acid. Such was evident when the magnitude of the enzyme release was assessed by calculating the ratio of the enzyme activity in the supernatant without Triton X-100 (shaded area) to total recovered activity. On the other hand, a remarkable

release of NAcG was seen with 18β -glycyrrhetic acid. However, glycyrrhizin induced conflicting effects, that is, an increase at 3×10^{-4} M and a decrease at 10^{-3} M.

It has been reported that glycyrrhizin is hydrolyzed to glucuronate and glycyrrhetic acid at a low concentration by β -glucuronidase, a lysosomal enzyme, but inhibits β -glucuronidase activity non-competitively at a high concentration (7). In addition, we have found that glucuronate had no effect on the lysosomal release of these enzymes (data are not shown). Accordingly, it is probable that the increased release of enzyme in the presence of a low concentration of glycyrrhizin may be due to 18β -aglycone as a result of the hydrolysis of glycyrrhizin, while the split of the saponin may be negligible at 10^{-3} M. Segal and Milo-Goldzweig (8) reported that aldono-lactones, which are specific glucosidase inhibitors, inhibited saponin-induced hemolysis, but showed no protective effect on the hemolysis induced by the sapogenins. The action of glycyrrhizin on the release of NAcG in our report resembles their findings in that a glycoside may stabilize the membrane, while its aglycone may not do so.

The present results indicate that the configuration of 18-H of glycyrrhetic acid

Table 1. Effect of glycyrrhizin and its constituents on lysosomal enzyme activity

Compounds (3×10^{-4} M)	Enzyme Activity (nmoles/mg prot./min) $\pm$ S.E.M.	
	APh	NAcG
Control	54 ± 1.9	52 ± 1.2
Glycyrrhizin	31 ± 1.0^a	52 ± 1.2
18α -Glycyrrhetic acid	53 ± 5.9	60 ± 2.8
18β -Glycyrrhetic acid	49 ± 1.3^b	48 ± 2.4
Glucuronic acid	57 ± 1.8	52 ± 1.2

Crude acid phosphatase (APh) and β -N-acetylglucosaminidase (NAcG) were prepared by dissolving the lysosomal fraction ($13,000\times g$ pellet) with 0.1% Triton X-100. Each figure represents the mean $\pm$ S.E.M. of four separate experiments. Significance: a, $p<0.001$; b, $p<0.01$.

Fig. 1. Each bar represents total recovered activity which appeared in the supernatant after incubating lysosomal particles with the drug in the presence of 0.1% Triton X-100 and centrifuging as described in the text. The total recovered activity is a percentage of the control. The shaded area indicates the enzyme activity which appeared in the supernatant after incubating lysosomal particles with the drug in the absence of 0.1% Triton X-100 and centrifuging (free activity). The magnitude of free activity was represented as a percentage of total recovered activity of control. The mean $\pm$ S.E.M. of 3 to 4 separate experiments is shown. Drug concentration: A, 10^{-5} M; B, 10^{-4} M; C, 3×10^{-4} M; D, 10^{-3} M.

membrane. It is also likely that modes of existence of enzymes within lysosomes restrict the action of these drugs because APH and NAcG gave different responses to the same drug.

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plays an important role in the interaction with components of membrane. Furthermore, the hydrophilic moiety of glucuronate can modify the affinity of the aglycone for lipid layer of membrane by conferring an amphipathic property, and thereby producing alteration in structure and/or fluidity of the