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Alteration of Na^+ Permeability in Human Erythrocytes as Studied by ^{23}Na-NMR and Inhibition of the Kidney Na^+,K^+-ATPase Activities with Saponins : Interaction of *Gleditsia* Saponins with Human Erythrocyte Membranes

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Abstract: Interaction of *Gleditsia* saponin C **1**, bis-monoterpenyl echinocystic acid 3,28-O-bisglycoside, with human erythrocyte membranes and artificial membranes was investigated by means of the hemolytic activities, alteration of Na^+ permeability by ^{23}Na-NMR spectroscopy and inhibition of Na^+,K^+-ATPase activities.

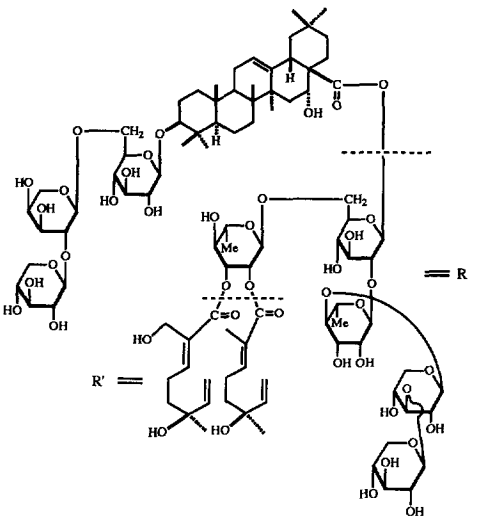
The saponins that are the glycoside components widespread in plants and invertebrate animals interact with cholesterol in biological membranes altering membrane fluidity and permeability characteristics.[1] Saponins are known to have various physiological activities, depend on their chemical structures.[2] Steroid and triterpene saponins with a single sugar chain at C-3 have a strong hemolytic activity, whereas those with two sugar chains at C-3 and C-28, respectively, show less activity.[3] In spite of *gleditsia* saponin (*g.* saponin) C **1**, isolated from *G. japonica* Miquel[4] has two sugar chains at C-3 and C-28 positions in its structure, the strong hemolytic activity was observed.[5] Arichi et al[6] also reported that no hemolytic activity was observed in soyasaponin-I, nevertheless in which has a single sugar chain at C-3.

We have studied the direct action of *g.* saponins, *g.* saponin C **1** and *g.* saponin C 3-O-glycosides methyl ester **3**, on human erythrocyte membranes, and the relationships between alteration of Na^+ permeability and the structure of *g.* saponins were studied. The present paper reports the effects of *g.* saponins on the osmotic behavior of human erythrocytes using the sodium-23 nuclear magnetic resonance (^{23}Na-NMR) along with the interaction of artificial membranes with **1** by ^1H-NMR technique, and inhibition of the rat kidney Na^+,K^+-ATPase activities.

Alteration of intra- and extracellular Na^+ in human erythrocytes was evaluated with ^{23}Na-NMR by $Dy(PPPi)_3^{7-}$ and $Dy(TTHA)^{3-}$ as shift reagents[7] according to previous paper.[8] From the analytical point of view, this approach implies a major advantage of the shift reagents technique whereby physical separation of intra-

and extracellular compartments is, in principle, not required. Furthermore, it begs the quotation of the extent of NMR invisibility of the intra- or extracellular cation.

The time course of intracellular Na^+ concentration in NMR buffer (145mM NaCl, 5mM KCl, 1mM Mg_2SO_4, 10mM glucose, 10mM Hepes at pH 7.4; 5mM $Dy(PPPi)_3^{7-}$; 43% hematocrit; 37°C) with **1** (0.1mM) is shown Figure 1. Saponin C **1** caused remarkable increase of intracellular Na^+ concentration than that of ouabain, the Na^+, K^+-ATPase inhibitor, whereas the 3-O-glycosides methyl ester **3** has no increase for 7h. These data should seem at a glance that **1** effected the Na^+ permeability of human erythrocyte membranes but **3** not affected to that. The modulation of these saponins towards Na^+, K^+-ATPase activity was then examined with ouabain as an indication of their reliability.



Gleditsia saponin C (**1**)
G. saponin C desmonoterpenyl derivative : R' = each H (**2**)
G. saponin C 3-O-glycoside methyl ester : R = Me (**3**)

The rat kidney Na^+,K^+-ATPase[9] inhibitory activities of saponins **1** and **3** were examined by Fiske-SubbaRow method.[10] As shown in Figure 2, the IC_{50} value (0.025mM) of **3** was approximately ten-fold compared with that (0.2mM) of ouabain, whereas that (0.4mM) of **1** was only half, respectively.

From above two mutually competing effects of Na^+ permeability of erythrocyte membranes and inhibitory activities towards Na^+,K^+-ATPase as membrane-embedded enzymes imply that saponins **1** and **3** interacted to the different parts of biomembranes. Interaction of saponin **1** with lecithin and lecithin-cholesterol

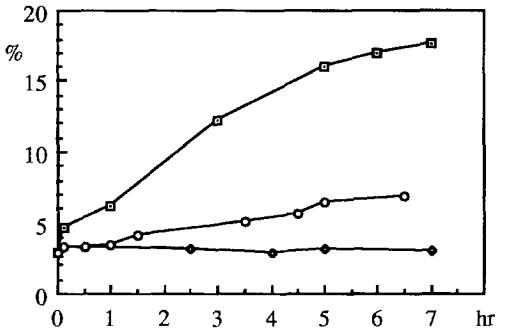


Fig. 1. Changes of intracellular Na^+ concentration of human erythrocytes by ^{23}Na-NMR with saponins. **1** (5μM), **3** (25μM), and ouabain (1mM).

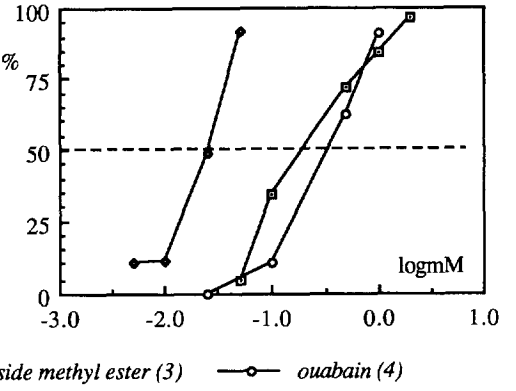


Fig. 2. Inhibition of rat kidney Na^+,K^+-ATPase by **1**, **3** and ouabain.

(molar ratio 1/1) bilayers then was examined respectively by $PrCl_3$[11] as shift reagents in ^{1}H-NMR spectroscopy. The chemical shifts of internal and external choline methyl signals ($-N^+Me_3$) of egg yolk lecithin bilayers were not affected by saponin **1** as shown in Figure 3, but those of lecithin-cholesterol bilayers were changed remarkably at the spectral position from inside to outside by the addition of **1** in ^{1}H-NMR spectra. Moreover the line width ($W_{1/2}$ 200Hz) of the methylene regions of lecithin bilayers fell out nearly double than that of original lecithin-cholesterol bilayers. These observations indicated that the complex micelle was formed by saponin **1** and cholesterol, drew out from lecithin-cholesterol bilayer, followed by fusing to give the large lecithin-cholesterol bilayer such as action of alamethicin.[12]

Thus, interaction of these saponins with human erythrocyte membranes was confirmed in all our experiments as follows. *G.* saponin **1** formed the complex with cholesterol, drew out from outerface of erythrocyte membranes. Such a loss of cholesterol on erythrocytes caused the inhibition of Na^+,K^+-ATPase activities as results of change of membrane fluidity. The erythrocytes were fused with the resulting micelle to give the large biomembranes accompanying by hemolysis, while *g.* saponin C 3-O-glycosides methyl ester **3** was incorporated into the erythrocytes to form the complex with cholesterol, followed by the inhibition of Na^+,K^+-ATPase activities of erythrocytes.

Thus the combination of ^{23}Na-NMR measurements, inhibitory activities of Na^+,K^+-ATPase and hemolytic activities is useful method for understanding the targets of biomembranes with saponins.

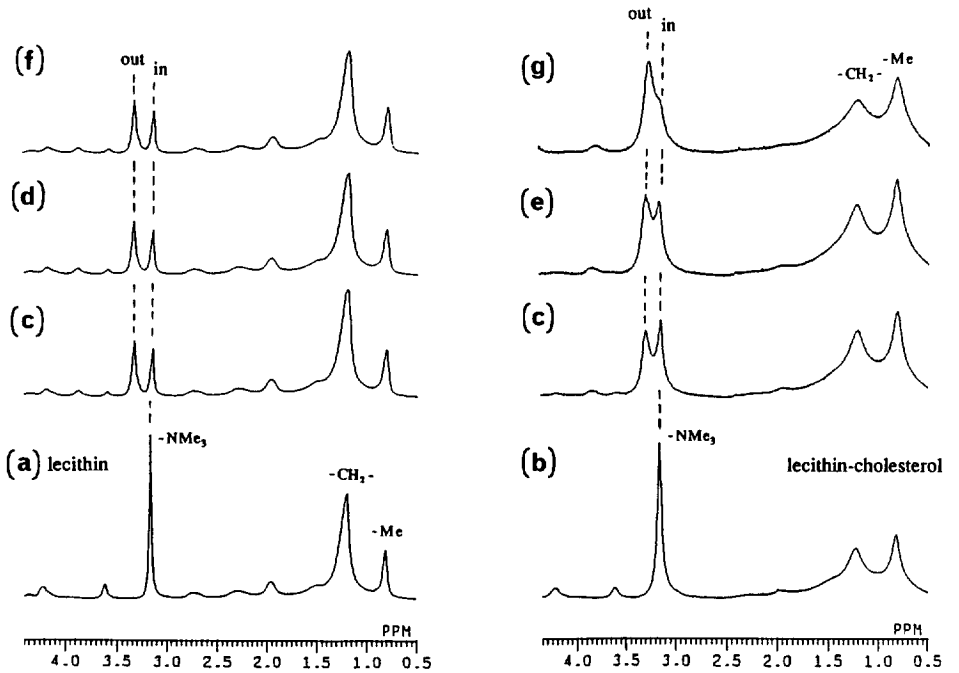


Fig. 3 The proton magnetic resonance signal of a 5% (w/v) aqueous dispersion of egg yolk lecithin[13]: (a) Sonicated in D_2O. (b) Sonicated with cholesterol (1/1) in D_2O. (c) On addition of 5mM $PrCl_3$. (d,e,f and g) On addition of saponin **1** (d: 0.2mM; e: 0.1mM; f: 1.0mM; and g: 0.4mM).

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13. Lecithin, purified by silicic acid column chromatography, was sonicated in D_2O under nitogen atmosphere using Heat System sonicator (W-225) with Ti microtip. To maintain the solution below 40°C during sonication, it was cooled externally by ice-water bath.

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