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Lack of correlation between surface and interfacial activities of saponins and their hemolytic properties

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For a series of model compounds (digitonin, aescine, tomatine, stevioside and ginsenoside Rg1) it was demonstrated that neither the surface nor the interfacial tension (n-decane/water) lowering properties of saponins can be correlated with their ability to induce hemolysis. Furthermore, no correlation was observed between the surface and interfacial activities of saponins and their hemolytic properties.

1. Introduction

Among the numerous pharmacological effects ascribed to saponins, their ability to promote absorption and diuresis, to stimulate the immune system and to prevent oedema has led to their widespread therapeutic applications. Further interest in this diverse group of compounds has been stimulated by their pronounced hemolytic properties [1].

The precise nature of the mechanism(s) by which saponins bring about membrane hemolysis is still unclear and a matter of some controversy [2–8]. The formation of complexes with components of the erythrocyte membrane is thought to be key to the hemolysis process. For example, the spirostanol glycoside digitonin (**1**) is known to form a stable complex with cholesterol [9–10], one of the main components of the erythrocyte membrane. The structure of this complex is the focus of current nmr studies. Earlier investigations suggested that not the saponins themselves, but rather their lipophilic aglycones produce hemolysis [11]. In addition, it has been postulated that surface activity is the determining factor in the hemolytic effect of saponins [1]. Whether surface activity plays a role in hemolysis-producing mechanisms and if so, what degree of correlation exists between surface and hemolytic activity for sponins remains an open question due to the lack of systematic investigations in this urea.

2. Investigations, results and discussion

In order to investigate the relationship between saponin surface properties and hemolytic activity, the concentration dependency of the surface tension of aqueous solutions of a series of saponins with widely varying hemolytic properties was determined. Four monodesmosidic saponins with strong hemolytic activity [digitonin (**1**), aescine (**2**), tomatine (**4**) and the commercially available glycoside mixture digitonin (**6**)] and two bisdesmosidic saponins, stevioside (**3**) and ginsenoside Rg1 (**5**), with virtually no hemolytic activity, were studied.

Fig. 1a shows that ginsenoside Rg1 (**5**) was the least effective in reducing surface tension. The dierpenoid glycoside stevioside (**3**), the steroidalkaloid saponin tomatine (**4**) and the saponin mixture aescine (**2**) all resulted in similar reductions in surface tension, which were greater than those observed for **5**. The greatest reduction in surface tension was seen with digitonin (**1**). From these results it appears that there is neither a quantitative nor a qualitative correlation between the ability to reduce surface tension and hemolytic activity for the compounds studied.

Because the disruption of the erythrocyte membrane by saponins is primarily an interfacial phenomenon, the surface activities of **1**–**6** as a function of $-d\sigma/dc$ (Gibbs equation) were also measured. From these calculations it was evident that the hemolytically active saponins **1**, **2**, **4** and **6** have a higher level of surface activity than the ineffective saponins **3** and **5** (see Table 1). The lack of a rank order correlation among the surface activities of the hemolytic effective saponins together with the small difference in values observed between the hemolysis-inducing **1** and ineffective saponin **3** led, however, to the conclusion that no general correlation exists here either.

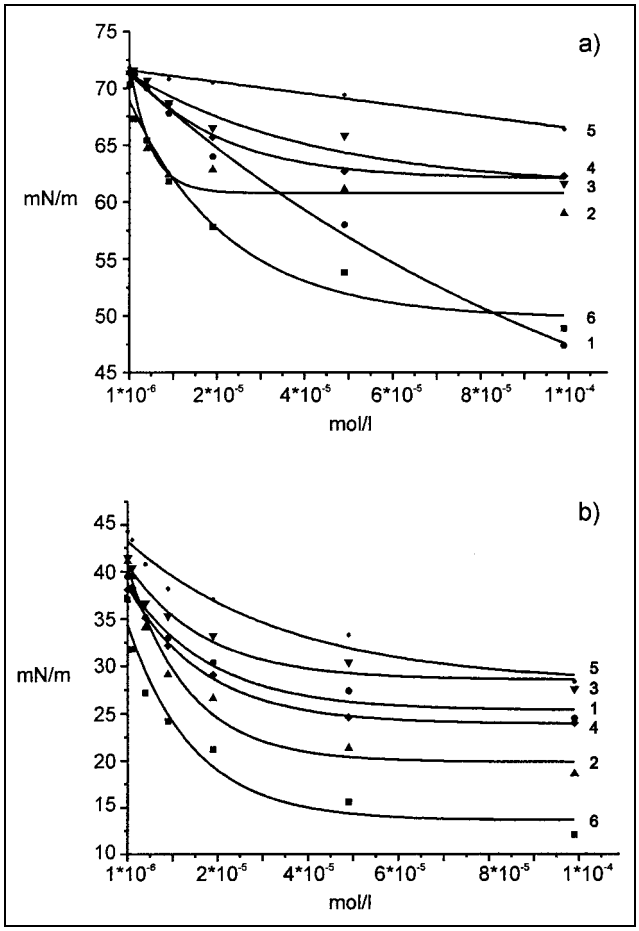

Fig. 1: a) Dependency of surface tension (air/water system; 20.0 °C) on saponin **1**–**6** concentration
b) Dependency of interfacial tension (n-decane/water system; 20.0 °C) on saponin **1**–**6** concentration

Table 1: Surface tension, hemolytic index and surface activity of saponins 1–6

$\Delta\sigma$[a] (mn/m)	Digitonin (**1**)	Digitonin (**6**)	Aescine (**2**)	Tomatine (**4**)	Stevioside (**3**)	Ginsenoside Rg1 (**5**)
	24.8	22.4	11.6	10.3	10.2	5.8
H.I.[b]	Tomatine (**4**)	Aescine (**2**)	Digitonin (**1**)	Digitonin (**6**)	Stevioside (**3**)[c]	Ginsenoside Rg1 (**3**)[c]
	170000	98000	88000	79000		
$-d\sigma/dc\ (c = 0)$	Aescine (**2**)	Digitonin (**6**)	Tomatine (**4**)	Digitonin (**1**)	Stevioside (**3**)	Ginsenoside Rg1 (**5**)
	2.16×10^6	0.82×10^6	0.46×10^6	0.35×10^6	0.24×10^6	0.051×10^6

[a] Differences in surface tension between air/water (72.4 mN/m) and saponins **1–6** at a concentration of $c = 10^{-4}$ M
[b] Hemolytic Index according Lit. [9]
[c] H.I. < 500 [1]

Table 2: Surface tension and surface activity of saponins 1–6

$\Delta\sigma$[a] (mn/m)	Digitonin (**6**)	Aescine (**2**)	Tomatine (**4**)	Digitonin (**1**)	Stevioside (**3**)	Ginsenoside Rg1 (**5**)
	32.1	25.9	21.8	20.4	17.2	16.7
$-d\sigma/dc\ (c = 0)$	Aescine (**2**)	Digitonin (**6**)	Tomatine (**4**)	Digitonin (**1**)	Stevioside (**3**)	Ginsenoside Rg1 (**5**)
	1.48×10^6	1.36×10^6	0.83×10^6	0.71×10^6	0.69×10^6	0.42×10^6

[a] Differences in interfacial tension between n-decane/water (45.8 mN/m) and saponins **1–6** at a concentration of $c = 10^{-4}$ M

In a further series of experiments we sought to improve the simulation of the interface between the lipid double layer of erythrocytes and the surrounding plasma using an n-decane/water model instead of the air/water interface. Decreases in interfacial tension with increasing saponin concentration is shown in Fig. 1b. A comparison with Fig. 1a indicates that the concentration-dependency of the interfacial tension (n-decane/water) does not correlate with the concentration dependency of the surface tension. As in the case of the surface tension, there is no clear correlation between the effects on interfacial tension and hemolytic activity.

In contrast to observations with surface and interfacial tension, the interfacial activities were markedly higher than the corresponding values for surface activities and there was a strong correlation between the two ($r = 0.98$ for **1–5** and $r = 0.87$ for **1–6**) Although the interfacial activity of the highly hemolytic digitonin (**1**) was not as different from the hemolytically ineffective stevioside (**3**) as their surface activities, the rank order of the interfacial activities corresponded to that of the surface activities (see Table 2).

Characteristics of the saponins investigated in this study are their high surface and interfacial activities. Moreover, compounds with the greatest ability to induce hemolysis also demonstrate the greatest surface and interfacial activities. Despite these observations, no rank order correlation exists between interfacial activity and the ability to induce hemolysis. For example, the hemolytically ineffective stevioside (**3**) has an interfacial activity comparable to that of tomatine (**4**) and digitonin (**1**). Although it is highly probably that interfacial activity is a precondition for saponin enrichment at the erythrocyte membrane, other factors must also be important in the subsequent genesis of hemolysis.

3. Experimental

3.1. Materials and methodology

Surface and interface tensions were measured with a TE 1C tensiometer (Lauda), in Duran-Schott crystal vessel ($\varnothing = 80$ mm, height = 45 mm) at 20.0 ± 0.2 °C (ring method according to Du Noüy method [12] with a Zuideman/Waters correction procedure [13]). Before each set of measurements, the tensiometer was calibrated as specified for the TE 1C model. All glassware (crystal vessels, pipettes, volumetric flasks etc.) were scrupulously cleaned.

3.2. Reagents

Digitonin (**6**) Merck; aescine (**2**) Fluka; tomatine (**4**), Roth, puriss.; digitonin (**1**), p.a. [14]; stevioside (**3**) and ginsenoside Rg1 (**5**) were kindly provided in p.a. quality by Prof. Dr. Bruno Danieli, University of Milan; n-decane, Merck, for synthesis, water, nanopure quality. Aqueous stock solutions (10^{-4} M) were prepared from the saponins investigated and were then diluted correspondingly for each measurement (5×10^{-5}, 2×10^{-5}, 10^{-5}, 5×10^{-6}, 2×10^{-6}, 10^{-6} M).

3.3. Measurements

In order to measure surface tension, 60 ml of each of the diluted solutions was pipetted into a crystal beaker, which was then placed on the sample platform of the tensiometer by means of crucible tongs. The adsorption balance was achieved following a waiting time of approx. one hour. A measuring cycle was then carried out as many times as necessary to achieve five individual measurements that fell within a predetermined standard deviation (± 0.15 mN/m). A mean value of four measuring cycles was taken for each of the measuring points (relative standard deviation ± 0.2 to $\pm 1.5\%$). Interfacial tension was investigated using the water/n-decane system 60 ml of each concentration was pipetted into a crystal beaker, covered with 60 ml n-decane and then measured as described above (density difference water/n-decane: 0.268 g/cm^3).

3.4. Data analysis and presentation

Figs. 1a and 1b show the effect of concentration of the saponin solutions investigated on their surface/interfacial tensions, respectively. The individual measurements were computer fitted to the following function,

$$\sigma_0 + A \cdot e^{(-(c-c_0)/t)} \quad (1)$$

and the coefficients A and t derived for the individual data sets.

To calculate the surface and interfacial activities at infinite dilution, the curves for surface/interfacial tension vs. concentration were derived to the function $d\sigma/dc = -A/t \cdot e^{c-c_0/t}$ and the slope at zero concentration calculated for each individual saponin.

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