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THE SUSCEPTIBILITY OF CHOLESTEROL-DEPLETED ERYTHROCYTES TO SAPONIN AND SAPOGENIN HEMOLYSIS

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Summary

The assumption that complex formation between erythrocyte membrane cholesterol and saponins or sapogenins is the cause for their hemolytic activity, was tested by measuring the susceptibility of cholesterol-depleted erythrocytes towards these hemolysins. For some of the hemolysins cholesterol depletion caused inhibition of hemolysis, for others an augmentation. The results suggest that cholesterol does not serve as a specific binding site for these hemolysins.

Saponins are well known for their hemolytic and fungicidic activities [1]. Since they have a high affinity for cholesterol and form stable complexes with it [2] these properties are generally attributed to their interaction with membrane cholesterol causing its extraction from the membrane [3—5]. The fungicidic activity of saponins was shown to be closely related to the relative amount of cholesterol in the membrane [6]. Species whose membranes are depleted of cholesterol are resistant to saponins [7]. There exists, however, some uncertainty about whether cholesterol is the only membrane component responsible for hemolysis. Tschesche and Wulff [2] found that there is no quantitative correlation between the hemolytic activities of saponins and the stability of their cholesterol complex. Furthermore Segal et al. [8] suggested that cholesterol itself probably acts as a hemolysin.

No attempts have as yet been made to test the function ascribed to cholesterol in the hemolytic process on the same lines used for the fungicidic activity. This became possible after it was demonstrated that cholesterol can be removed from erythrocyte membranes by means of aqueous dispersions of egg lecithin [9]. This method was applied in order to test the extent to which cholesterol-depleted erythrocytes are susceptible to the hemolytic effect of saponins. Experiments were carried out with sapogenins as well since they have been shown to produce the same type of hemolysis as the saponins [10,

11] and to form stable complexes with cholesterol [12].

Fresh citrated blood drawn from albino rats (males weighing about 200 g) was used for all experiments. The red blood cells were freed of plasma by three washings in cold isotonic saline. The erythrocytes were depleted of cholesterol by incubation for 1—2 h (37°C hematocrit 20%) with lecithin vesicle suspensions (40 mg lecithin in 5 ml saline per ml cells), according to the technique used by Shinitzky and Inbar [13]. Controls were incubated under the same conditions in vesicle-free media. Incubation periods could not exceed 2 h since erythrocytes incubated for longer periods with lecithin vesicles tended to give spontaneous hemolysis when diluted with isotonic buffer ($Na_2HPO_4 \cdot 2H_2O$, 3.95 g; KH_2PO_4, 0.76 g; NaCl, 7.2 g; aqua dist. ad 1000 ml; pH adjusted to 7.4). The red blood cells were separated by centrifugation at 1000 × *g*, and washed three times to ensure complete removal of the lecithin vesicles. The lipids were extracted from the erythrocytes with chloroform/isopropanol [14] and the cholesterol content was determined according to Zlatkis et al. [15]. The hemolytic activity of saponins and sapogenins was measured on normal and on cholesterol-depleted erythrocytes by determining the hemolysin concentration inducing 50% hemolysis (H_{50}) as described previously [16]. These experiments were performed either in isotonic buffer or in 20% dimethyl sulfoxide (Me_2SO) in buffer. The Me_2SO concentration could not exceed 20% since at higher concentrations the cholesterol-depleted cells tended to undergo spontaneous hemolysis.

The results of the experiments are summarised in Table I. The three water-

TABLE I

EFFECT OF CHOLESTEROL DEPLETION ON ERYTHROCYTE SUSCEPTIBILITY TOWARDS SAPONINS AND SAPOGENINS

Blood sample	Hemolysin	Time of incubation (min)	Cholesterol (μg/ml packed erythrocytes)	Cholesterol depletion (%)	Medium for hemolysis test	H_{50}(M)	H_{50} change (%)
1	Digitonin	—	537		Isotonic buffer	$3.2 \cdot 10^{-6}$	
1	"	60	475	13	"	$3.3 \cdot 10^{-6}$	+ 3
1	"	—	537		"	$3.2 \cdot 10^{-6}$	
1	"	120	350	34	"	$3.6 \cdot 10^{-6}$	+12
2	"	—	487		"	$3.2 \cdot 10^{-6}$	
2	"	60	410	16	"	$3.3 \cdot 10^{-6}$	+ 3
2	"	—	487		"	$3.2 \cdot 10^{-6}$	
2	"	120	300	38	"	$3.6 \cdot 10^{-6}$	+12
3	"	—	500		Me_2SO 20% in	$3.1 \cdot 10^{-6}$	
3	"	120	390	22	isotonic buffer	$3.4 \cdot 10^{-6}$	+10
4	"	—	550		"	$3.0 \cdot 10^{-6}$	
4	"	120	325	42	"	$3.9 \cdot 10^{-6}$	+30
5	Styrax saponin A [17]	—	525		Isotonic buffer	$2.6 \cdot 10^{-7}$	
5	"	60	500	5	"	$2.6 \cdot 10^{-7}$	0
5	"	—	525		"	$2.6 \cdot 10^{-7}$	
5	"	120	400	24	"	$2.95 \cdot 10^{-7}$	+13
6	"	—	437		"	$2.5 \cdot 10^{-7}$	
6	"	120	300	30	"	$3.2 \cdot 10^{-7}$	+27
7	"	—	500		"	$2.6 \cdot 10^{-7}$	
7	"	120	375	26	"	$2.9 \cdot 10^{-7}$	+11
8	"	—	487		"	$2.6 \cdot 10^{-7}$	
8	"	120	287	40	"	$3.15 \cdot 10^{-7}$	+21

TABLE I (continued)

Blood sample	Hemolysin	Time of incubation (min)	Cholesterol (μg/ml packed erythrocytes)	Cholesterol depletion (%)	Medium for hemolysis test	H_{50} (M)	H_{50} change (%)
9	Styrax sapo-	—	527		Isotonic buffer	$2.6 \cdot 10^{-7}$	
9	-nin-A [17]	120	410	26	"	$3.0 \cdot 10^{-7}$	+15
10	"	—	500		Me_2SO 20% in	$2.3 \cdot 10^{-7}$	
10	"	120	375	25	isotonic buffer	$3.4 \cdot 10^{-7}$	+48
11	"	—	537		"	$2.1 \cdot 10^{-7}$	
11	"	120	400	25	"	$2.8 \cdot 10^{-7}$	+37
12	"	—	487		"	$2.3 \cdot 10^{-7}$	
12	"	120	287	40	"	$3.0 \cdot 10^{-7}$	+30
13	Aescin	—	450		Isotonic buffer	$1.8 \cdot 10^{-5}$	
13	"	60	375	18	"	$2.1 \cdot 10^{-5}$	+17
13	"	—	450		"	$1.75 \cdot 10^{-5}$	
13	"	120	340	25	"	$2.4 \cdot 10^{-5}$	+37
14	"	—	537		Me_2SO 20% in	$1.5 \cdot 10^{-5}$	
14	"	120	325	40	isotonic buffer	$1.8 \cdot 10^{-5}$	+20
15	"	—	587		"	$1.5 \cdot 10^{-5}$	
15	"	120	450	23	"	$1.75 \cdot 10^{-5}$	+17
16	Smilagenyl-	—	450		"	$1.1 \cdot 10^{-5}$	
16	β-maltoside [8]	120	365	19	"	$1.1 \cdot 10^{-5}$	0
16	Tigogenyl-β-	—	450		"	$1.0 \cdot 10^{-5}$	
16	maltoside [8]	120	365	19	"	$9.0 \cdot 10^{-6}$	—10
17	"	—	487		"	$1.0 \cdot 10^{-5}$	
17	"	120	400	18	"	$8.8 \cdot 10^{-6}$	—12
18	Styrax sapo-	—	475		"	$2.7 \cdot 10^{-7}$	
18	genin-A [17]	60	400	16	"	$4.6 \cdot 10^{-7}$	+76
18	"	—	475		Me_2SO 20% in	$2.7 \cdot 10^{-7}$	
18	"	120	350	26	isotonic buffer	$4.9 \cdot 10^{-7}$	+80
19	"	—	537		"	$2.9 \cdot 10^{-7}$	
19	"	60	437	19	"	$4.8 \cdot 10^{-7}$	+66
19	"	—	537		"	$2.9 \cdot 10^{-7}$	
19	"	120	350	25	"	$5.2 \cdot 10^{-7}$	+80
20	"	—	487		"	$2.8 \cdot 10^{-7}$	
20	"	120	400	18	"	$4.6 \cdot 10^{-7}$	+64
21	Lithocholic	—	487		"	$6.0 \cdot 10^{-7}$	
21	acid methyl ester	120	425	13	"	$5.6 \cdot 10^{-7}$	—7
22	"	—	480		"	$6.0 \cdot 10^{-7}$	
22	"	120	370	23	"	$4.8 \cdot 10^{-7}$	—20
23	Diosgenin	—	500		"	$2.7 \cdot 10^{-5}$	
23	"	60	460	8	"	$2.6 \cdot 10^{-5}$	—4
23	"	—	500		"	$2.7 \cdot 10^{-5}$	
23	"	120	340	32	"	$2.2 \cdot 10^{-5}$	—18

soluble saponins digitonin, aescin and styrax saponin A [17], were tested both in buffer and in Me_2SO buffer in order to ensure that the presence of Me_2SO did not significantly affect the results.

It should be emphasized that although the cholesterol level differed in the various blood samples, the H_{50} values in the untreated erythrocytes were constant for each saponin. This conforms to previous findings that the extent of the hemolytic activity of a saponin (at standard conditions) depends on the species from which the blood is taken [18].

In all hemolysins tested, with the exception of smilagenyl-β-maltoside, there was always a difference in the H_{50} values of the cholesterol-depleted erythrocytes as compared to the control samples. Moreover, this divergence generally tended to become more pronounced with the decrease in the cholesterol level. However, the trend of this divergence and its extent were not uniform. While for digitonin, aescin, styrax saponin A and styrax sapogenin A an increase in the H_{50} was observed in the cholesterol-depleted erythrocytes, in tigogenyl-β-maltoside, diosgenin and lithocholic acid methyl ester, depletion caused a decrease in the H_{50}. An increase in the H_{50} indicates inhibition of the hemolysis, and a decrease, a higher susceptibility to the hemolysin.

The results of our experiments clearly indicate that the cholesterol level in a particular blood sample markedly influences saponin- and sapogenin-induced hemolysis. The inconsistency in the trend of this effect suggests that the cholesterol does not serve as the specific binding site for these hemolysins, but a decrease in its level affects the susceptibility through structural changes in the membrane.

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