BILE SALTS-, LYSOLECITHIN- AND SAPONIN-INDUCED POTENTIATION OF PHOSPHOLIPASE A HEMOLYSIS

P.G. LANKISCH*, C. JACOBI and K. SCHONER
Max-Planck-Institut für experimentelle Medizin, Department of Biochemical Pharmacology, 34 Göttingen, Germany

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1. Introduction

Phospholipase A (Ph-ase A) hardly attacks the cellular membrane of washed red cells in the absence of exogenous lecithin. However, in the presence of the direct lytic factor of cobra venom (DLF) and other basic disulfide peptides, Ph-ase A lyses red cells completely, while these compounds themselves have only a weak or no direct lytic activity [1–3]. As the same effect is produced by *N*-ethylmaleimide and *p*-chloromercuribenzoate, it is supposed that these peptides, being attracted to the proper sites of the membrane by their basic charge, lead to an increase of permeability and to osmotic swelling thus giving free access of Ph-ase A to otherwise hidden membrane phospholipids [2,4,5]. Recent investigations in this department have shown that DLF leads to a prolytic sodium gain of red cells, while potassium loss roughly parallels hemolysis.

Other lysins with a quite different mode of action show prolytic potassium loss accompanied by a sodium gain [6]. This prompted us to study whether their induced change of permeability also leads to membrane changes which enhance Ph-ase A hemolysis. We therefore chose cholic acid and its derivatives, taurocholic acid and deoxycholic acid, all in their neutralised Na-solution, and also other surface-active agents such as lysolecithin and saponin.

* Supported by Deutsche Forschungsgemeinschaft, Bad Godesberg; Present address: Dept. of Medicine, Div. of Gastroenterology and Metabolism, University of Göttingen, Germany.

2. Materials and methods

2.1. *Compounds*

Ph-ase A was separated from bee venom according to the procedure of Habermann and Reiz [7]. Other preparations were used as supplied: Na-cholate, Na-taurocholate, Na-deoxycholate (all Serva), saponin (Merck). Lysolecithin was prepared according to Vogt [8].

2.2. *Hemolysis*

Heparinized guinea-pig or human blood was centrifuged and packed red cells were washed 3 times with 1% NaCl solution. The red cells were finally suspended in 0.01 M phosphate buffer, pH 7.3, 20 times the original volume of blood, containing 0.9% NaCl. Ca^{2+} was added as chloride to give a concentration of 0.45 mM. Hemolysis was measured by photometric estimation of hemoglobin released after conversion to cyanomethemoglobin [2].

2.3. *Surface tension*

The tension was measured with a glass thread of 0.2 mm Ø hanging on a torsion balance and dipping in the test solution, according to Lenard et al. [9].

3. Results

3.1. *Hemolytic activity of Ph-ase A alone and combined with bile salts*

Na-deoxycholate has much more lytic effect of its own on guinea-pig red cells than the other bile salts. At a conc. of 4 mM it lysed the red cells completely

within a few minutes. A conc. of 0.25 mM had no lytic effect (fig. 1). Na-cholate and Na-taurocholate at 4 mM lysed between 10–40% of the red cell suspension within 320 min (curves 5 and 6), whereas at lower concentrations they had no lytic effect (curve 7). Ph-ase A alone was also practically non-haemolytic (curve 6). However, in the presence of Na-deoxycholate at 0.25 mM or together with Na-cholate or Na-taurocholate both at a concentration of 4 mM hemolysis occurred within 80 to 120 min (curves 2 and 1). The latter two bile salts also showed potentiation of Ph-ase A at lower concentrations, although the time course of hemolysis was much more prolonged (curves 3 and 4).

Similar results were obtained with human erythrocytes.

3.2. *Hemolytic activity of Ph-ase A alone and in combination with saponin and lysolecithin*

At higher concentrations, saponin and lysolecithin lysed guinea-pig red cells directly and completely. In sublytic concentrations, however, (fig. 2, curves 3 and 4) they strongly enhanced Ph-ase A hemolysis (curves

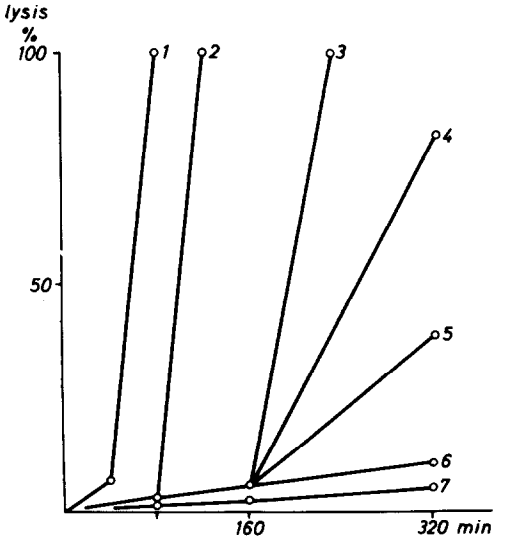

Fig. 1. Hemolytic activity of Ph-ase A alone and combined with bile salts. 1) Ph-ase A + Na-cholate 4 mM; Ph-ase A + Na-taurocholate 4 mM; 2) Ph-ase A + Na-deoxycholate 0.25 mM; 3) Ph-ase A + Na-taurocholate 1 mM; 4) Ph-ase A + Na-cholate 1 mM; 5) Na-cholate 4 mM; 6) Na-taurocholate 4 mM: Ph-ase A; 7) Na-cholate 1 mM; Na-chlate 1 mM; Na-taurocholate 1 mM; Na-deoxycholate 0.25 mM; control without hemolysins; Ph-ase A concentration: 10^{-6} g/ml.

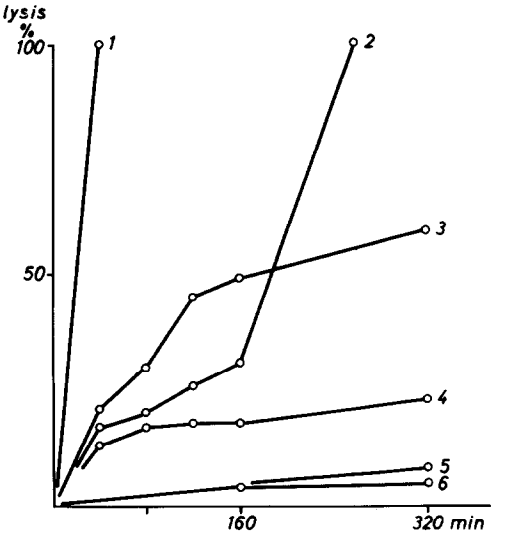

Fig. 2. Hemolytic activity of Ph-ase A alone and in combination with saponin and lysolecithin. 1) Ph-ase A + saponin; 2) Ph-ase A + lysolecithin; 3) saponin; 4) lysolecithin; 5) Ph-ase A; 6) control without hemolysins. Concentrations: Ph-ase A: 10^{-6} g/ml; saponin: 2×10^{-5} g/ml; lysolecithin: 10^{-5} g/ml.

1 and 2). The course of hemolysis was less rapid when damage to cells by the compounds alone was less extensive (curves 1 and 3, curves 2 and 4).

3.3. *Influence of the chosen compounds on surface tension*

The lowering of surface tension by all compounds investigated for potentiation of Ph-ase A was measured. Results are shown in table 1.

4. Discussion

As shown above, bile salts in fact facilitated the Ph-ase A hemolysis at sublytic concentrations. Na-cholate and its derivative Na-taurocholate potentiated Ph-ase A at similar concentrations, whereas the other derivative, Na-deoxycholate, was much more active. Lytic activity and degree of potentiation showed a dependance on their degree of lowering of the surface tension of solutions. Lysolecithin and saponin, both surface-active and surface tension lowering agents, also potentiated Ph-ase A hemolysis (the latter in

Table 1
Lowering of surface tension by bile salts, saponin and lysolecithin (in dyn/cm).

Solute	Solvent 0.01 M phosphate buffer pH 7.3
Without	62.4
Na-cholate 1 mM	52.0
Na-cholate 4 mM	48.7
Na-taurocholate 1 mM	53.4
Na-taurocholate 4 mM	45.9
Na-deoxycholate 0.25 mM	42.3
Na-deoxycholate 4 mM	41.3
Saponin 2×10^{-5} g/ml	50.0
Lysolecithin 10^{-5} g/ml	43.8

contradiction to earlier findings [2]). Other mechanism of such potentiation have been described before. Klibansky et al. [10] found that synthetic basic peptides which were strongly lytic themselves potentiated Ph-ase A. The potentiation mechanism was supposed to be due to the basic charge and lipophilic side chains of these peptides. Vogt et al [2] were able to show a synergism between basic disulfide peptides and Ph-ase A. These peptides had no or only little lytic effect, and their facilitating action was dependent on intact disulfide bonds and basic charge. The red cell surface was supposed to be rendered susceptible to Ph-ase A not by changing surface tension, but by reacting with membrane SH-groups.

Our results show potentiation of Ph-ase A by different compounds. They all have in common the ability to lower the surface tension of cells, thus influencing membrane permeability and membrane structure [6, 11]. In contrast to other such potentiating agents reported earlier [2, 10], they are neither peptides, nor basic, nor dependent in their lytic action on their chemical structure.

Apparently several possibilities of facilitating hemolysis and phospholipid cleavage of Ph-ase A have to be envisaged [2], but again all have one general principle in common: by altering the outer membrane of red cells, they give free access of Ph-ase A to the otherwise hidden phospholipid containing layer.

In this connection it is of interest to note that the described synergism between bile salts and Ph-ase A on red cells *in vitro* has also been found for *in vivo* investigations on pancreas. Simultaneous action of bile acids and Ph-ase A led to parenchymal necrosis [12, 13] and Ph-ase A therefore seems to be of decisive importance in the pathogenesis of acute pancreatitis [14].

Our results further agree to some extent with the most recent findings of Roelofson et al. [15]. Among other studies, they found a synergism between Ph-ase C of *Bacillus cereus* and human pancreatic Ph-ase A2 with sublytic concentrations of sodium deoxycholate but not with Triton X-100. As the latter compound is surface-active, too, this result is at variance with our findings. We therefore used Triton X-100 as well, at sublytic concentrations (between 0.1 to 0.05 mM) and indeed found potentiation of Ph-ase A on guinea-pig erythrocytes. This difference may be due to the different origins of Ph-ase A used (bee venom instead of human pancreas) or to species differences between guinea-pig and human red cell structure.

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