BULL SEMINAL VESICLE HEMOLYTIC FACTOR. A NEW PHOSPHOLIPID BINDING PROTEIN

Čeněk KYSILKA

Czechoslovak Academy of Sciences, Institute of Physiology and Genetics of Animals, CS - 277 21, Liběchov, Czechoslovakia

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1. Introduction

The hemolytic activity of bull seminal plasma has been described by a number of authors. The first of them appears to be Goetze [1]. Matoušek and Staněk have demonstrated the protein character of hemolytic factor using already the seminal vesicle fluid as the source of the factor [2]. Kysilka has isolated the factor, published the criteria of purity, a number of chemical and biochemical properties and the amino acid composition of the factor [3,4]. The results of Gladigau et al. have had to do with the temperature dependence of hemolytic activity and with binding of the factor on the cell surface [5].

The ability of hemolytic factor to bind membrane phospholipids, sphingomyelins especially, is described in this paper.

2. Material and methods

Bull seminal vesicle hemolytic factor was isolated according to Kysilka [3]. The pure hemolytic factor (fractions 'C' in the paper cited) was used in these experiments; for brevity 'the factor' in this paper.

Bovine red cell ghosts were prepared using the method described by Kabat and Mayer [6]. The chloroform–methanol (2:1, v/v) extract of freeze-dried ghosts served for the isolation of sphingomyelins and phosphatidylethanolamine in chromatographical purity. The extract was fractionated and processed by the methods already cited and used by Souček et al. [7].

Paper chromatography of phospholipids was carried out on the paper Whatman No.3 impregnated according to Hamilton et al. [8]. Solvent system chloroform – methanol – water (60:9:1, by vol.) and the detection of spots with the usual techniques were used [9].

Lipid phosphorus was determined by the method of Chen et al. [10] after ashing procedure according to Ames and Dubin [11].

Interaction of the factor with ghosts was studied in 0.2% suspension of ghosts in 0.1 M citrate – 0.02% sodium azide buffer, pH 6.0 (our pH-optimum). The suspension was incubated with and without the factor at 37°C for 16 hr. Thereafter, the incubation mixture were extracted with chloroform–methanol (2:1, v/v) and the phases were separated by centrifugation. The phosphorus determination and paper chromatography followed.

Interaction of the factor with isolated ghost phospholipids was studied similarly. Sphingomyelins were sonicated in the buffer above and incubated with and without the factor at 37°C for 16 hr. After the incubation, 3-fold repeated extraction with chloroform – methanol was provided. The phosphorus determination and paper chromatography then followed in all three chloroform extracts and residual aqueous phase.

The same procedure was used for 0.6 μmol of phosphatidylethanolamine and for the mixture of this phospholipid with sphingomyelins (0.3 μmol of each).

3. Results

3.1. *Action of the factor on ghost suspension*

The results are summarized in fig.1. There is shown that 84% of total phosphorus is extractable into the chloroform phase in the absence of the factor. The addition of 5 mg (10 mg resp.) of the factor affects

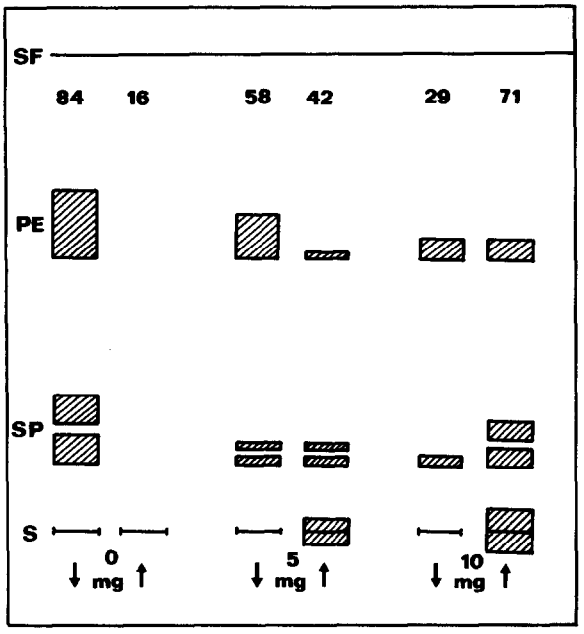

Fig.1. Interaction of the factor with 0.2% ghost suspension. The suspension was incubated with given quantities of the factor (pH 6.0, 37°C, 16 hr). Thereafter, the mixtures were extracted with chloroform–methanol, the phases were chromatographed and phosphorus was determined in each of them. The arrows indicate upper (aqueous) and lower (chloroform) phases. Values in miligrams, quantity of the factor in incubation mixture; the numbers on the top, percents of total phosphorus; S, start; SP, sphingomyelins; PE, phosphatidylethanolamine; SF, solvent front. Detection of spots: acid fuchsin. No detectable spot occurred in the aqueous phase after the incubation without the factor.

the rapid shift of phosphorus distribution: the chloroform phase contained only 58% of total phosphorus after the incubation with 5 mg of the factor, 29% with 10 mg, resp. The residual phosphorus was found in aqueous phase.

Paper chromatography has shown only the spots of original phospholipids both in chloroform and aqueous phases and the additional spot on the start in aqueous phase. There is the factor (as a protein) under these conditions on the start. The intensity of phospholipid spots from aqueous phase seems not to be adequate to the phosphorus content of this phase, they are much weaker, while the spots detectable on the star are intensively coloured. No product of phospholipid degradation was detected.

We can suppose that the phospholipids are bound on the protein molecule.

3.2. *Binding of sphingomyelins on the factor and their liberation*

Red cell ghost sphingomyelins were bound similarly as described above. The experiment to liberate unchanged sphingomyelins from the molecule of the factor was successful by repeated extraction. The qualitative and quantitative results of this experiment are given in fig.2. The first extraction liberated only 12% of total sphingomyelins into the chloroform phase, the second and third ones 22, 29% resp. Residual 37% of total sphingomyelins left to bind under these conditions. Neither in this case, any degradation product of sphingomyelins was observed.

3.3. *Further interactions of the factor with phospholipids*

The absolute negative results were obtained in the experiment with isolated phosphatidylethanolamine under the above conditions. This phospholipid was

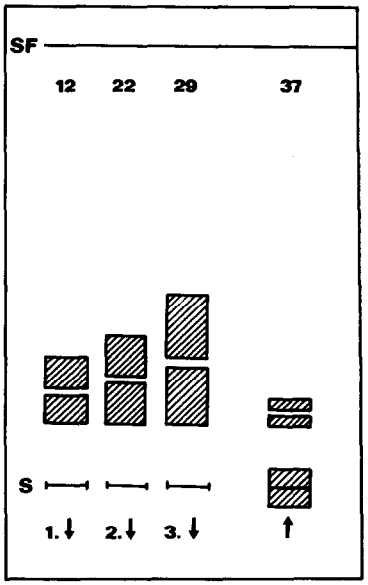

Fig.2. Liberation of bound sphingomyelins. Red cell ghost sphingomyelins (0.6 μmol) were bound with 1.5 mg of the factor (pH 6.0, 37°C, 16 hr incubation). After the incubation, 3-fold repeated extraction with chloroform–methanol followed. The lower phases obtained and the residual aqueous phase were chromatographed and the phosphorus was determined. The numbers on the top, percents of total phosphorus. S, start; SF, solvent front; the numbered arrows, chloroform phases after each extraction; the alone arrow, residual aqueous phase after all three extractions. Detection of spots: acid fuchsin.

not bound and was fully extracted into the chloroform phase during the first extraction.

As opposed to this result, both sphingomyelins and phosphatidylethanolamine were bound on the factor in the experiment with the mixture of these phospholipids.

4. Discussion

Bull seminal vesicle hemolytic factor is demonstrated as a new phospholipid binding protein. The main phospholipid components of bovine red cell ghosts are not bound equivalently. Sphingomyelins can be bound independently, phosphatidylethanolamine only in the presence of sphingomyelins. This result could explain possible mechanism of lysis in good agreement with the fact of asymmetrical arrangement of phospholipids in membranes [12].

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