

Streptavidin-induced lysis of homologous biotinylated erythrocytes

Evidence against the key role of the avidin charge in complement activation via the alternative pathway

Vladimir R. Muzykantov, Michael D. Smirnov and Gennady P. Samokhin

Institute of Experimental Cardiology, USSR Cardiology Research Center, Moscow, USSR

Received 3 January 1991

It is shown that non-covalent attachment of streptavidin, as well as of avidin, to biotinylated human erythrocytes induces homologous hemolysis by complement. Rabbit antiserum against human C3 is found to inhibit the lysis specifically as compared with non-immune rabbit serum. Efficiency of lysis inhibition is greater for avidin- and streptavidin-induced lysis of biotinylated human erythrocytes than for antibody-sensitized sheep erythrocytes. In contrast to positively charged avidin (pI 11), streptavidin is a neutral protein. Hence, hemolysis of streptavidin-carrying erythrocytes is inconsistent with the suggestion on the crucial role of avidin charge in lysis. Membrane alterations (cross-linking and clusterization of biotinylated components) induced by avidin (streptavidin) seem to be a more plausible explanation for the lysis.

Complement; Avidin-biotin interaction; Membrane modification

1. INTRODUCTION

It was previously shown that non-covalent attachment of avidin to pre-biotinylated erythrocytes induces activation of complement via the alternative pathway (APC) [1,2]. Activation of complement was Mg^{2+} - but not Ca^{2+} -dependent and resulted in complete lysis of avidin-carrying but not avidin-free erythrocytes [2]. Avidin-induced hemolysis both by autologous (homologous) and heterologous complement was observed for all mammalian species studied. Free avidin in solution had no effect on complement activity. It was proposed that membrane-bound avidin induces some alterations in cell membrane, which leads to the conversion of 'APC-non-activator' cell into 'APC-activator'. Avidin acylation by succinic anhydride prevents lysis [3,4]. Since acylation was accompanied by a decrease in avidin charge, it was suggested that interaction of avidin with the charged components of the erythrocyte membrane may be involved in the mechanism of conversion. To prove this, in the present work we have substituted avidin by streptavidin, an avidin-like protein of bacterial origin [5]. Both avidin and streptavidin molecules have a similar size and biotin-binding proper-

ties, but in contrast to positively charged avidin, streptavidin is a neutral protein [6]. The results obtained show that streptavidin also induces homologous hemolysis via the alternative pathway. This suggests that not the avidin charge but some other properties are involved in conversion of the cell into an 'APC-activator'.

2. MATERIALS AND METHODS

Avidin was purified from hen egg white [7] and its activity was determined by spectrophotometric titration with biotin [8]. Biotin *N*-hydroxysuccinimide ester (BOSu), rabbit antiserum against human C3 and buffer components were from Sigma (USA). Streptavidin was from Boehringer. Blood from healthy volunteers and from animals was collected in citrate-dextrose anticoagulant.

2.1. Avidin (streptavidin) immobilization on erythrocyte surface

Procedures were performed as described previously [9]. Briefly, 0.1 ml of 0.1 M sodium tetraborate and 3 μ l of 0.1 M BOSu in dimethylformamide were sequentially added to 1 ml of 10% PBS-washed erythrocyte suspension. Reaction mixture was incubated at 20°C for 20 min and cells were washed with PBS (5 \times 15 ml). Then 0.01-1 mg of avidin (streptavidin) in 1 ml PBS was added to 1 ml of 10% suspension of biotin-carrying erythrocytes at constant stirring. After a 20-60-min incubation at 4°C with periodical shaking, erythrocytes were washed 3 times with 15 ml of PBS.

2.2. Analysis of the avidin-induced hemolysis

Hemolytic assay was carried out in microtitration plates as described previously [10]. Veronal-buffered saline was used: 4 mM veronal buffer containing 3 mM diethylbarbituric acid, 1 mM diethylbarbituric acid sodium salt, 145 mM NaCl, 0.25 mM $CaCl_2$, 1.5 mM $MgCl_2$, pH 7.2 (VBS). Serum was 2-fold serially diluted in 50 μ l of VBS and then 50 μ l of the 2% suspension of the erythrocytes being tested was added. Wells with complement-free VBS and erythrocytes were used as a zero reference. 100% hemolysis was attained by the addition of distilled water instead of VBS. The plates were incubated at

Correspondence address: V.R. Muzykantov, Institute of Experimental Cardiology, USSR Cardiology Research Center, 3rd Cherepkovskaya Str. 15a, Moscow 121552, USSR

Abbreviations: APC, alternative pathway of complement; PBS, phosphate-buffered saline; VBS, veronal-buffered saline; DAF, decay accelerating factor; CRI, complement receptor type 1; HRF, homologous restriction factor; EA, sheep erythrocytes sensitized by hemolytic antibody

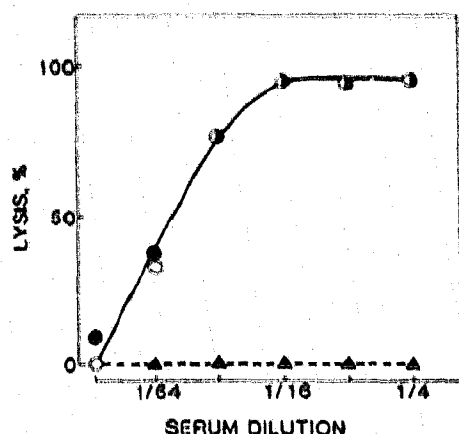


Fig. 1. Lysis of avidin-carrying (open circles) and streptavidin-carrying (closed circles) biotinylated human erythrocytes in homologous serum. Both avidin-free biotinylated erythrocytes and native cells (closed triangles) are not lysed. The typical result from the three experiments is shown.

37°C for 60 min and the degree of hemolysis was determined as absorbance at 630 nm, using an MR-580 Micro Elisa Auto Reader (Dynatech, USA). Percentage of lysis was calculated as follows:

$$\frac{A_{0\%} - A}{A_{0\%} - A_{100\%}} \times 100$$

where $A_{100\%}$ = the absorbance corresponding to 100% hemolysis, $A_{0\%}$ = the absorbance corresponding to 0% hemolysis, and A = the absorbance at the intermediate experimental point. $CH_{50\%}$ was calculated as serum dilution providing 50% lysis.

For inhibition of hemolysis fresh human serum at 1/5 dilution was mixed with an equal volume of heated rabbit antiserum against human C3 or with non-immune rabbit serum. An equal volume of VBS was added instead of rabbit serum as a control. After a 1-h incubation at room temperature, the mixtures were centrifuged and used as sources of complement. Percentage of inhibition was calculated by subtracting of the lysis percentage in experimental point from that in control point.

3. RESULTS AND DISCUSSION

As shown in Fig. 1, both avidin- and streptavidin-carrying pre-biotinylated human erythrocytes are lysed by homologous serum. Efficiency of lysis is similar in both cases and $CH_{50\%}$ is about 1/40. Hemolysis also proceeds in fresh heterologous sera (guinea pig and rabbit sera, data not shown). Both avidin- and streptavidin-carrying cells were equally stable upon storage in complement-free buffers. As it was demonstrated previously, biotinylated erythrocytes were not lysed in any case [1,2].

As shown in Fig. 2, antiserum to human C3 efficiently inhibits homologous lysis of both avidin- and streptavidin-carrying human erythrocytes as well as heterologous lysis of antibody-sensitized sheep erythrocytes (EA). Non-immune rabbit serum used as a control produced no effects in all cases. It should be noted that inhibitory efficiency of anti-C3 towards avidin (streptavidin)-carrying erythrocytes lysis was

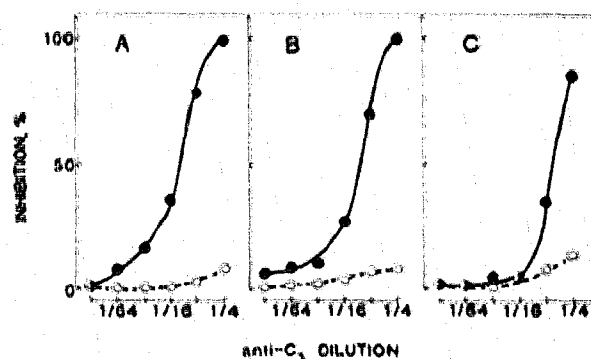


Fig. 2. Inhibition of hemolysis by anti-C3 antiserum (closed circles). Non-immune rabbit serum is presented as open circles. (A) Avidin-carrying biotinylated erythrocytes. (B) Streptavidin-carrying biotinylated erythrocytes. (C) Sheep erythrocytes sensitized by rabbit hemolytic antiserum. The typical results from the two experiments is shown.

greater than that for EA lysis (Fig. 2). This difference may be explained in terms of different mechanisms of C3-convertase formation upon activation of classical and alternative pathways: it is known that one molecule of C3b is necessary for triggering in the first case vs two molecules in the second case [11]. Hence, upon APC activation the C3 component may be a limiting factor. Therefore different inhibitory efficiency of anti-C3 for lysis of avidin (streptavidin)-carrying erythrocytes and EA supports the conclusion that avidin (streptavidin)-carrying cells activate complement via the alternative pathway. This corroborates with the character of lysis dependence on the presence of divalent cations which was demonstrated separately: avidin-induced lysis is Mg^{2+} -dependent, but Ca^{2+} -independent [2].

The results obtained are inconsistent with the previous suggestion on the key role of the avidin charge in complement-dependent hemolysis. This suggestion was based on the decrease in the lytic ability of acylated avidin whose charge was reduced as compared with native avidin (pI, 11) [3,4]. Since streptavidin is a neutral protein, its high lytic activity implies that there should be other explanations for transformation of APC-non-activator cell into APC-activator. One of these explanations may be the following one.

As was recently observed, the efficiency of lysis depends on the mode of avidin attachment to the erythrocyte membrane. Thus, at equal amounts of erythrocyte-bound avidin lysins was more efficient if a 7A spacer exists between biotin and surface amino group [2]. Agglutination activity of 'spacer-biotinylated' erythrocytes in avidin solution was also enhanced. This indicates higher probability of multipoint interaction of avidin with biotinylated groups on the erythrocyte surface. Both avidin and streptavidin have four biotin-binding sites and, therefore, are capable of a multi-point binding. As a result of this cooperative interaction with biotinylated membrane components

cross-linking, re-organization and clusterization of these components may occur. Specific APC restrictors, such as DAF, CRI, HRF, etc., may be among these membrane components [12]. It can be suggested that their activities depend on the state in the membrane and that avidin (streptavidin) binding switches the 'active' state into 'non-active'. The final result is elimination of APC restriction followed by lysis in the presence of complement.

In this context one of the 'side' results of our work on avidin acylation should be noted: not only charge, but also the avidin ability to cross-link biotin-containing structures was decreased upon modification of avidin [3]. Therefore, decreased lysis may reflect reduced probability of multi-point avidin-biotin interaction, but not a decrease in the avidin charge. Bearing in mind the above-mentioned results obtained with spacer-biotin, these data suggest that the mode of avidin attachment to the erythrocyte membrane, but not avidin charge, is a more plausible explanation for the conversion of the 'APC-non-activators' cells into the 'APC-activators'.

REFERENCES

- [1] Samokhin, G.P., Muzykantov, V.R., Smirnov, M.D. and Domogatsky, S.P. (1984) *Abstr. 16th FEBS Meeting, Moscow*, J, p. 199.
- [2] Muzykantov, V.R., Smirnov, M.D. and Samokhin, G.P. (1990) *Biol. Membranes* 7, 831-837 (Russian).
- [3] Muzykantov, V.R., Smirnov, M.D. and Samokhin, G.P. (1990) *Biol. Membranes* 7, 274-280 (Russian).
- [4] Muzykantov, V.R., Smirnov, M.D. and Samokhin, G.P. (1990) *Biochem. J.* (in press).
- [5] Chalet, L., Wolf, F.J. (1964) *Arch. Biochem. Biophys.* 106, 1-5.
- [6] Gitlin, G., Bayer, E.A. and Wilchek, M. (1988) *Biochem. J.* 256, 279-282.
- [7] Green, N.M. (1970) *Methods Enzymol.* 18a, 414-417.
- [8] Green, N.M. (1970) *Methods Enzymol.* 18a, 418-424.
- [9] Muzykantov, V.R., Sakharov, D.V., Smirnov, M.D., Samokhin, G.P. and Smirnov, V.N. (1986) *Biochim. Biophys. Acta* 884, 355-362.
- [10] Muzykantov, V.R., Samokhin, G.P., Smirnov, M.D. and Domogatsky, S.P. (1985) *J. Appl. Biochem.* 7, 223-227.
- [11] Dahan, M.R., Fearon, D.T. and Austen, K.F. (1976) *J. Immunol.* 117, 630-634.
- [12] Pangburn, M. (1986) *J. Immunol.* 136, 2216-2221.