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Avidin-induced lysis of biotinylated erythrocytes by homologous complement via the alternative pathway depends on avidin's ability of multipoint binding with biotinylated membrane

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It was reported that avidin and streptavidin induce lysis of prebiotinylated red blood cells via the alternative pathway of both homologous and heterologous complement. Both of these proteins have four biotin-binding sites, providing a polyvalent interaction with biotinylated components of the erythrocyte membrane. We have compared the effects of mono- and multipoint avidin attachment on the sensitivity of biotinylated erythrocytes to lysis by the complement system. In the presence of anti-avidin antibody, avidin-bearing biotinylated erythrocytes were rapidly lysed by heterologous serum. This lysis was independent from the mode of avidin attachment, implying that complement activation by the classical pathway triggered by interaction between C1 and avidin-bound antibody on the erythrocyte surface is independent from the avidin's ability of polyvalent (multipoint) binding with biotinylated membrane components. In the absence of anti-avidin antibody, biotinylated erythrocytes bearing polyvalently attached avidin were lysed by homologous complement better than cells bearing avidin, which possesses reduced ability for multipoint binding with biotinylated erythrocyte. Two independent approaches to reduce avidin's ability of multipoint binding were used: decrease in surface density of biotin on the erythrocyte membrane and blockage of biotin-binding sites of avidin. Both methods result in reduced lysis of avidin-bearing erythrocytes as compared with erythrocytes bearing an equal amount of polyvalent-bound avidin. Thus the activation of homologous complement via the alternative pathway depends on avidin's ability to 'cross-link' to the biotinylated components of the erythrocyte membrane.

Introduction

Non-covalent attachment of avidin to the membrane of prebiotinylated erythrocytes induces their lysis in the presence of freshly prepared serum [1]. It was shown [2] that (a) lysis depends on the surface density of avidin; (b) both hetero- and homologous sera exhibit the lytic activity towards avidin-bearing biotinylated erythrocytes; (c) the cells were lysed via the alternative pathway of complement.

In a separate set of experiments we have shown that avidin acylation with succinic anhydride prevents hemolysis [3]. Since this modification reduces the p*I* of avidin (p*I* of native avidin is about 11 [4]), it was suggested that the strong positive charge of the avidin molecule plays an important role in complement activation. However, streptavidin, a bacterial analogue of the avidin, also induced lysis of biotinylated erythrocytes; both streptavidin- and avidin-induced lysis were efficiently inhibited by antiserum to C3, the crucial component of the alternative pathway [5]. This fact contravenes the hypothesis that the positive charge of avidin plays a role in the induction of hemolysis, since streptavidin is electrically neutral [6].

It was shown that hemolysis is dependent on the mode of avidin attachment to the erythrocyte membrane. We have compared two *N*-hydroxysuccinimide biotin esters, *N*-hydroxysuccinimide biotin ester and *N*-hydroxysuccinimide ester of 6-biotinylamidocaproic acid. Both esters bind to the amino groups of the erythrocyte membrane by formation of amide bonds and provide equal numbers of attached avidin molecules per cell. However, with the 6-biotinylamidocaproic acid ester better lysis was achieved, and the critical avidin surface density was 3-fold lower than with the other ester [2].

The biotinylamidocaproic acid ester has an additional 7 Å spacer as compared with the biotin ester, which considerably increases the probability of a multipoint attachment of avidin to biotinylated membrane

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components. This is confirmed by greater agglutination activity of avidin towards the erythrocytes biotinylated by the 'longer' ester as compared with those modified by the 'shorter' ester [2]. On the other hand, acylation not only reduces the positive charge of avidin, but also decreases its ability to 'cross-link' biotinylated proteins [3]. Taken together, these findings indicate that a multipoint attachment of avidin to biotinylated membrane components may play an important role in the complement activation and hemolysis induction.

Both avidin and streptavidin are tetrameric proteins with a molecular weight of about 66 kDa [4,6]. They have four high-affinity ($K_d = 10^{-15}$ M) biotin-binding sites. Therefore, avidin can cross-link biotinylated molecules, e.g., biotinylated components of the erythrocyte membrane. Such a 'cross-linking' may produce dramatic changes in the cell membrane organization and thus reduce the cell resistance to the lysis by complement.

In the present study we attempted to examine the role of multipoint (cross-linking) avidin attachment to biotinylated erythrocytes in the complement activation and hemolysis induction. Two approaches were employed to reduce the probability of multipoint attachment: the decrease in the biotin surface density on the erythrocytes and the blockage of the avidin biotin-binding sites by free biotin. In both cases decrease in the probability of multipoint avidin attachment to biotinylated cells results in less efficient lysis by homologous serum via alternative pathway of complement. In contrast to homologous lysis via alternative pathway, lysis of avidin-bearing biotinylated erythrocytes by heterologous serum induced by anti-avidin antibody (activation of classical pathway) does not depend on the valency of avidin attachment to biotinylated erythrocyte. It can be suggested from these data that multipoint attachment of avidin to biotinylated erythrocytes induces functional alterations in the membrane inhibitors of alternative pathway and the restriction of homologous hemolysis is abolished as a result of the multipoint binding of avidin to biotinylated membrane.

Materials and Methods

Avidin was purified from hen egg white [7]. Sheep erythrocytes were preprared by defibrinating the blood with glass beads. Lyophilized guinea pig serum or fresh autologous sheep serum was used as a source of complement.

Biotin immobilization on the erythrocyte surface with subsequent attachment of avidin were performed as described previously [8]. Briefly, 0.1 ml of 0.1 M sodium tetraborate and 3 μl of 0.1 M of biotin *N*-hydroxysuccinimide ester were sequentially added to 1 ml of 10% PBS-washed erythrocyte suspension (PBS, phosphate-buffered saline, pH 7.4). After a 20-min incubation at 20°C cells were washed with PBS (5 × 15 ml).

Avidin (0.14 mg in 0.1 ml PBS) was added to 0.9 ml of erythrocyte suspension at constant stirring. After 20–60 min of incubation at 4°C with periodical shaking cells were washed with PBS (3 × 15 ml). To quantitate avidin binding to the erythrocyte surface the avidin was labeled with ^{125}I-Bolton-Hunter reagent (Amersham) accordingly to the manufacturer recommendations. Radioactivity was measured in a RackGamma counter (LKB).

Anti-avidin antiserum was prepared from the blood collected from rabbits 2 weeks after a second immunization with purified avidin (200 μg per animal in complete Freund's adjuvant). By indirect immunoenzyme analysis, the antibody binding to plastic-immobilized avidin occurred after incubation with immune rabbit antiserum, but not with non-immune serum. Serum titer in ELISA was equal to $1:10^4$. There was no antibody binding to plastic-immobilized albumin after the addition of either immune or non-immune serum.

Hemolytic assay was performed in microtitration plates [9] using 4 mM veronal-buffered saline (VBS, 3 mM diethylbarbituric acid, 1 mM sodium salt of diethylbarbituric acid, 145 mM NaCl, 1.8 mM $MgCl_2$, 0.25 mM $CaCl_2$, pH 7.2). The erythrocytes examined (50 μl of 2% suspension) were added to 50 μl of complement (2-fold serial dilution in VBS). Reference point of 100% hemolysis was attained by replacing VBS with distilled water. Plates were incubated at 37°C for 60 min and the hemolysis degree was measured as absorbance at 630 nm in an MR-580 Micro Elisa Auto Reader (Dinatech, USA). Wells with complement-free VBS and erythrocytes were used as a reference point of 0% hemolysis.

Sheep erythrocytes were routinely sensibilized with rabbit hemolytic antibody [9]. Lysis of anti-erythrocyte antibody-sensitized native sheep erythrocytes or anti-avidin antibody-treated avidin-bearing sheep erythrocytes by heterologous complement via the classical pathway was performed with lyophilized quinea-pig serum diluted in VBS. Lysis was examined after 60-min incubation at 37°C of a reaction mixture containing avidin-carrying sheep erythrocytes, heterologous guinea pig serum and heat-inactivated rabbit anti-avidin antiserum (final dilution 1:80).

The agglutination of biotinylated erythrocytes in avidin solution was assayed as described previously [2]. The standard agglutination titer was equal to 1 μg/ml of avidin.

Results

In the first series of experiments we analyzed the relationship between avidin-induced hemolysis and the

degree of the erythrocyte membrane modification by biotin *N*-hydroxysuccinimide ester. It can be assumed that the probability of a polyvalent (multipoint) interaction between avidin and biotinylated erythrocyte membrane decreases concomitantly with the decrease of biotin surface density. At the same time, the reduced number of accessible biotin groups may cause a decrease in the amount of attached avidin. Earlier we have shown that the lysis of erythrocytes biotinylated under standard conditions is strongly dependent on the surface density of native avidin [2]. Therefore, first of all we have examined the relationships between the avidin attachment and (a) its concentration in the medium at standard degree of erythrocyte biotinylation and (b) the concentration of biotin ester during the biotinylation procedure at standard avidin concentration during subsequent avidin attachment. It can be seen from Fig. 1. that $(0.5–7)\cdot 10^5$ of avidin molecules per cell has been attached upon the biotin ester and avidin concentration ranges 3–300 μM and $(1.6–15)\cdot 10^5$ molecules/RBC, respectively. It can be assumed that upon the decrease in avidin concentration in the reaction mixture (Fig. 1B) the ability of each attached molecule to a polyvalent (multipoint) interaction would not be decreased. By contrast, if the degree of the membrane biotinylation is reduced (Fig. 1A), the possibility of avidin molecules to multipoint attachment can be assumed to decrease.

We have compared lysis of biotinylated erythrocytes carrying equal amounts of attached poly-avidin (poly-AV) * and mono-avidin (mono-AV) by homologous serum (antibody-independent activation of the complement by the alternative pathway). At equal surface density of poly-AV and mono-AV $(1.3\cdot 10^5$ molecules per cell), erythrocytes bearing poly-AV were lysed better than mono-AV cells (Fig. 2).

We have also compared lysis of mono-AV- and poly-AV-bearing biotinylated erythrocytes by heterologous serum in the presence of anti-avidin antibody (antibody-dependent activation of the heterologous complement via the classical pathway). Lyophilized guinea pig serum was used as a source of complement, since this serum did not lyse both native and biotinylated sheep erythrocytes (in contrast to human and rat serum). This serum exhibits a high hemolytic activity towards sheep eryhtrocytes sensitized by rabbit anti-erythrocyte antibody, however, it is practically inactive

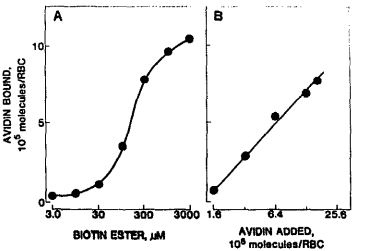

Fig. 1. Attachment of avidin to biotinylated erythrocytes. (A) Dependence of the avidin attachment on the concentration of biotin *N*-hydroxysuccinimide ester. The concentration of avidin in reaction mixture is 0.14 mg/ml $(1.5\cdot 10^6$ molecules per cell). (B) Dependence of the avidin attachment on the concentration of avidin. The concentration of biotin ester in reaction mixture upon biotinylation is 300 μM.

towards avidin-bearing biotinylated sheep erythrocytes (Fig. 3). This feature may be caused by inactivation of the alternative pathway components during lyophilization [10]. Thus the data shown in Fig. 3 indicate that lyophilized guinea pig serum can be used to study antibody-induced lysis of avidin-bearing sheep erythrocytes by heterologous serum via classical pathway without lysis by alternative pathway (there was no lysis of avidin-bearing cells in the absence of antibody).

Fig. 4 illustrates virtually the same antibody-dependent heterologous lysis of biotinylated sheep erythrocytes bearing equal amounts of poly-AV and mono-AV $(1.3\cdot 10^5$ molecules per cell). Without avidin, biotinylated cells were not lysed by heterologous serum irrespective of the biotin ester concentration employed to

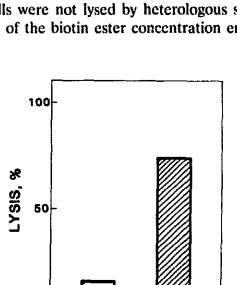

Fig. 2. Lysis of biotinylated erythrocytes bearing poly-AV (shaded bar) or mono-AV (open bar) by homologous serum. In both cases erythrocyte bears $1.3\cdot 10^5$ molecules of avidin. Serum dilution 1:16.

* Here and below in the text poly-AV and mono-AV will be used as indication of the mode of avidin attachment to erythrocyte membrane irrespectively of the methods for decreasing of probability of multipoint interaction. Therefore, term mono-AV will be applied both for the description of the attachment of intact avidin to the membrane with decreased biotin surface density and attachment of avidin with partially blocked biotin-binding sites to the membrane with standard biotin surface density.

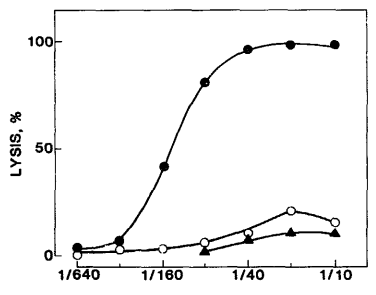

Fig. 3. Activation of heterologous complement via the classical pathway. Lyophilized guinea pig serum (GPS) was used as a source of complement. Sheep erythrocytes sensitized by rabbit hemolytic antibody are easily lysed (●). Biotinylated sheep erythrocytes bearing $4 \cdot 10^5$ avidin molecules are not lysed (○), as well as avidin-free biotinylated sheep erythrocytes (▲). The native sheep erythrocytes also are not lysed by guinea pig serum (not shown).

prepare mono-AV (30 μM) or poly-AV (300 μM) bearing erythrocytes.

Thus the mode of avidin attachment to biotinylated erythrocytes has no effect on their antibody-induced lysis by heterologous serum via the classical pathway,

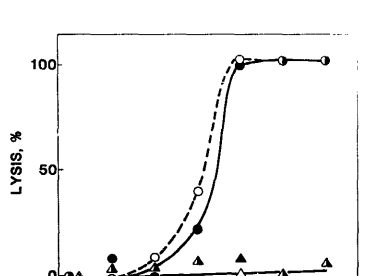

Fig. 4. Antibody-induced lysis of poly-AV (●)- and mono-AV (○)-bearing biotinylated erythrocytes by heterologous serum via the classical pathway. Lyophilized guinea pig serum (GPS) was used as a source of complement which was triggered by rabbit polyclonal anti-avidin antibody. In both cases erythrocyte beared $1.3 \cdot 10^5$ avidin molecules. Avidin-free biotinylated erythrocytes (triangles) were not lysed. The biotin ester concentration employed for biotinylation was 300 μM (▲) and 30 μM (△). Dilution of anti-avidin rabbit antiserum was equal to 1:80.

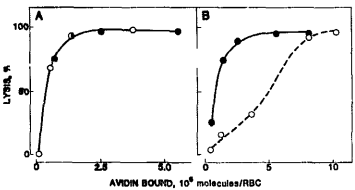

Fig. 5. Dependence of lysis of avidin-bearing biotinylated erythrocytes on the amount of attached mono-AV (○) and poly-AV (●). The valency of the avidin–biotin interaction was lowered by decreasing the biotin ester concentration upon treatment of erythrocytes. (A) Activation of the heterologous complement by the classical pathway in the presence of anti-avidin antibodies (1:80) and guinea pig serum (1:20). (B) Activation of the complement by the alternative pathway in the presence of homologous serum (1:8).

but influences considerably their resistance to antibody-independent lysis by homologous serum via the alternative pathway. This is confirmed by the dependence of the degree of lysis of biotinylated sheep erythrocytes on the amount of 'polyvalently' and 'monovalently' attached avidin (Fig. 5). It can be seen (Fig. 5A) that in the presence of anti-avidin antibody and guinea pig serum lysis of poly-AV- and mono-AV-bearing biotinylated erythrocytes is the same throughout the range of avidin surface densities examined. By contrast, upon incubation with homologous serum without antibody mono-AV induces considerably lower lysis in a range of $(0.5–7) \cdot 10^5$ avidin molecules per cell (Fig. 5B). Thus the reduced probability of a polyvalent (cross-linking) interaction between native avidin and biotinylated erythrocyte membrane as a result of decreased surface density of biotin groups diminishes the hemolysis by homologous complement activated via the alternative pathway.

Another approach employed to reduce the probability of multipoint avidin attachment was the blocking of its biotin-binding sites by biotin. Erythrocytes were modified at a standard concentration of biotin ester (300 μM) providing conditions for multipoint attachment of intact avidin.

Incubation of avidin with free biotin in solution diminishes its capability of multipoint (cross-linking) attachment to biotinylated erythrocytes which results in the decrease of agglutination activity of avidin towards the biotinylated erythrocytes (Fig. 6A). At the same time, the avidin attachment to erythrocytes biotinylated under standard conditions also decreases (Fig. 6B). At a molar biotin/avidin ratio of 4:1 agglutination activity of avidin is lost (blockage of at least 3 out of 4 biotin-binding sites). At molar ratios ranging from 3.5:1 to 1:1 attachment of $(2–4) \cdot 10^5$ avidin molecules per

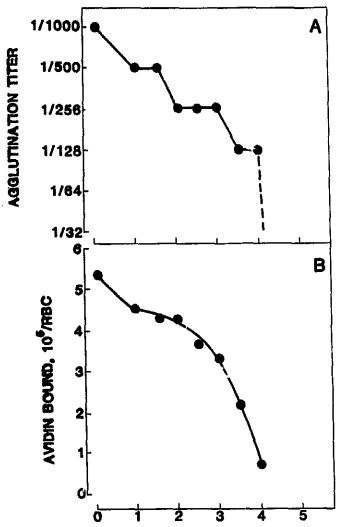

Fig. 6. Agglutination activity of avidin (A) and its attachment to biotinylated erythrocytes (B) after blockage of biotin-binding sites with biotin.

cell is attained at reduced agglutination activity of avidin. These values are comparable to attachment of native avidin at a concentration range of $(1.6–6.4) \cdot 10^5$

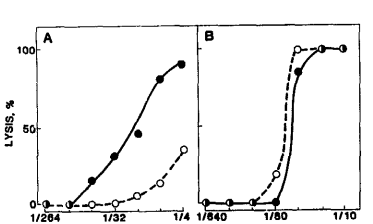

Fig. 7. Lysis of poly-AV (●) and mono-AV (○) biotinylated erythrocytes upon activation of homologous complement via the alternative pathway (A) or activation of heterologous complement via the classical pathway (B). Erythrocytes biotinylated under standard conditions bear $1.8 \cdot 10^5$ (poly-AV) or $2.2 \cdot 10^5$ (mono-AV) avidin molecules per cell.

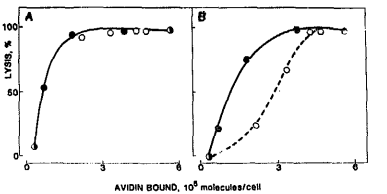

Fig. 8. Effect of the amount of attached mono-AV and poly-AV on the lysis via the classical (A) and alternative (B) pathways. The valency of the avidin–biotin interaction was lowered by blockage of the biotin-binding sites by preincubation with biotin. All symbols, indications and dilutions are the same as in Figs. 5, A and B, respectively.

molecules per cell (Fig. 1B). However, despite the equal amount of avidin attached the mode of attachment will be quite different. In the case of avidin with partially blocked biotin-binding sites the probability of multipoint (cross-linking) avidin attachment to the membrane will be lower as compared to that of native avidin.

We have compared the lysis of biotinylated erythrocytes bearing native (poly-AV) and biotin-blocked (mono-AV) avidin. It can be seen from Fig. 7A that at essentially similar values of avidin attachment in both cases $(2.2 \cdot 10^5$ molecules of mono-AV and $1.8 \cdot 10^5$ molecules of poly-AV per cell) much more stronger lysis by homologous serum via the alternative pathway was observed with poly-AV. At the same time, practically equal lysis occurred in the case of antibody-induced activation of heterologous complement via the classical pathway irrespective of the mode of avidin attachment (Fig. 7B). Fig. 8 shows the dependence of degree of lysis on the surface density of mono-AV and poly-AV. In the entire range of surface densities both poly-AV and mono-AV induces equal lysis of biotinylated erythrocytes by heterologous serum via the classical pathway (Fig. 8A). By contrast, poly-AV induces more efficient lysis by homologous complement via the alternative pathway in the range of $(0.5–5.0) \cdot 10^5$ avidin molecules per cell. Thus, both ways of reduction of the probability of multipoint avidin attachment to biotinylated erythrocyte membrane provide similar attenuation of the lysis by homologous complement via the alternative pathway, but do not influence the lysis by heterologous complement via the classical pathway.

Discussion

As we have found in previous work [2], homologous lysis via the alternative pathway of the avidin-bearing biotinylated erythrocytes depends on the mode of the

avidin attachment: attachment through the 'spaced biotin' provides greater lysis as compared with the native biotin at equal surface density of avidin. Since the avidin molecule has four biotin-binding sites, it can be attached to biotinylated erythrocyte multivalently, i.e., one avidin molecule can bind more than one biotin group inserted in the erythrocyte membrane. Therefore, multipoint avidin attachment to biotinylated erythrocyte may provide cross-linking of biotinylated components of the membrane. In the present study we have tried to evaluate the role of multipoint avidin attachment to biotinylated erythrocytes in the lysis by the homologous complement via the alternative pathway.

While avidin possesses four biotin-binding sites, upon interaction with biotinylated molecules and cells the degree of steric freedom is much less than upon interaction with a small biotin molecule [11]. When avidin reacted with biotinylated erythrocytes, part of biotin-binding sites probably are not involved in the binding. It can be suggested that a large portion of cell-bound avidin molecules is attached to the membrane not tetravalently, but rather bivalently. However, since bivalent interaction is sufficient to 'cross-link' biotinylated molecules, in this consideration bivalent interaction will be referred to as polyvalent. On the other hand, the statistical character of the distribution of poly- and monovalent attachment should be taken into account. This is exemplified by incubation of avidin in a biotin solution at molar ratios 1:1, 1:2, etc. (Figs. 6–8). Each population of biotin-substituted avidin molecules contains particles on which all biotin-binding sites are occupied and biotin-free particles as well. However, it seems reasonable to suggest that the degree of the biotin-binding sites blockage in a large part of molecules is essentially proportional to the biotin/avidin molar ratio. Certainly, it would be desirable to obtain a 'true' monovalent avidin population of avidin molecules possessing only one biotin-binding site and free from contaminating populations of bi-, three- and tetravalent avidin molecules. However, it is too hard to obtain such a contamination-free population of the monovalent tetrameric avidin molecule [11].

Bearing in mind the above-mentioned consideration that avidin with partially occupied biotin-binding sites is not 'true monovalent avidin', we used two independent approaches to reduce probability of multipoint avidin binding to biotinylated erythrocytes. Both the blockage of the biotin-binding sites on avidin molecules (decrease in avidin valency) and reduced concentration of biotin ester upon erythrocyte treatment (decrease in surface density of the biotin groups on the erythrocyte membrane) attenuated activation of the homologous complement by the alternative pathway. Since in both cases the lysis of erythrocytes bearing equal amounts of attached mono-AV and poly-AV is compared, one can suggest that the mode of avidin attachment is fundamental to the erythrocyte resistance to homologous lysis via the alternative pathway. Summarizing the presented data with our previous observations on the loss of avidin ability to cross-link biotinylated structures upon acylation (procedure preventing avidin-induced lysis, [3]) and on the enhanced lysis by the 'spaced' biotin ester [2], we can suggest that multipoint avidin binding to biotinylated membrane plays a key role in lysis by homologous complement via the alternative pathway.

In contrast, the mode of avidin attachment had no effect on the resistance of avidin-bearing erythrocytes to antibody-induced lysis by heterologous complement via the classical pathway. Therefore, the mode of avidin attachment is critical in activation of homologous complement mediated by avidin-induced rearrangement of erythrocyte membrane, but has no effect when avidin serves as an antigen for antibody-mediated activation of heterologous complement via the classical pathway.

The mechanism of elimination of homologous restriction by multipoint avidin binding is not clear yet. One possible suggestion for this mechanism may be the following. The membrane of erythrocytes (and other cell types in mammals) contains specific complement regulators, such as CR1-receptors [12], decay accelerating factor (DAF) [13], homologous restriction factor (HRF) [14], membrane inhibitor of reactive lysis (MIRL) [15] and others. The function of the membrane inhibitors may depend not only on their amounts and individual activities, but also on their microenvironment, topology and interactions in the membrane. It was reported that some complement inhibitors are clustered on the cell membrane [16]. There is evidence to suggest that a cooperative interaction of CR1 with activated C3 is important for the inactivation of membrane-bound C3 [17]. Polyvalent avidin attachment may disrupt native clusters of the membrane complement regulators and create new 'avidin-induced' clusters, which may result in elimination or attenuation of the complement restriction.

An alternative explanation of the obtained results might be based on the assumption that there is a requirement for a critical number of membrane sites to be bound irrespective of monovalent or polyvalent binding to provide effective lysis. According to this model the difference in the lysis at equal surface densities of mono-AV and poly-AV is accounted for by the different number of these sites bound. However, not all the results can be explained in terms of this assumption. Let us consider the possible relationship between densities of mono-AV and poly-AV providing equal efficiencies of lysis. It is clear that theoretically under no circumstances the ratio of these densities can exeed 4 (the value, which can be attained if poly-AV is bound with its four biotin-binding sites and mono-AV

– with only one). Furthermore, the results of Green [11] allow one to consider avidin as a bifunctional cross-linking agent rather than tetrafunctional one, because its four biotin-binding sites are located pairwise on the opposite sites of the molecule so, that the binding of the biotinylated macromolecule to one site of the pair leads to the steric blocking of the neigbouring site. This consideration reduces the maximal ratio of mono-AV to poly-AV surface densities to the value of 2. Moreover, the ability of poly-AV attached to membrane still to bind biotinylated proteins (antibody and enzyme [8]) allows one to conclude that the average number of binding sites anchoring poly-AV to biotinylated membrane is less than maximally attainable. It can be also assumed that part of the mono-AV molecules is bound to the membrane by more than one binding site (Fig. 5), because it is reasonable to suggest that the decrease in the degree of membrane biotinylation leads to an increase of the average distance between randomly distributed sites, and therefore among these distances there will be a part of the total population allowing avidin binding by more than one site. Summing up these considerations one can reasonably state that the ratio of mono-AV to poly-AV surface densities providing equal lysis always must be less than 2. However, this ratio calculated from the obtained results (for instance, presented in Fig. 5B, say, for the degree of lysis equal to 25%) is approximately equal to 6. Thus, the model based on the simple saturation of a critical number of membrane sites seems to be not valid because it can not explain all the features of the observed phenomenon.

The nature of membrane rearrangements which are possibly induced by avidin as yet remains obscure. Since there is no evidence in favour of specific biotinylation of the membrane proteins, the suggestion that avidin attachment may alter the microenvironment of any membrane inhibitor of the complement seems quite relevant. We hope that further studies provide more insight into the mechanisms responsible for the elimination of homologous restriction of the alternative pathway of complement (APC). However, even the data now available allow us to suggest that an APC-non-activating cell may be converted into an APC-activating cell due to rearrangements of the membrane components. Activation of the alternative pathway by anti-erythrocyte monoclonal antibody which does not fix complement is probably accomplished via this mechanism [18].

The investigation into the mechanisms responsible for the elimination of the complement inhibiton by the target cells is closely related to viral transformation of complement-resistant cells into APC-activating cells. Though the data on the possibility of the direct involvement of virus in the complement activation has been reported [19], the possibility of virus-induced rearrangements of the complement inhibitors cannot be excluded. This issue is particularly important in connection with the recent observation that lympoid cells infected by HIV virus, the agent that causes AIDS, are lysed via the alternative pathway [20]. The results obtained also suggest that a monovalent attachment of avidin may be applied to preparation of complement-resistant immunoerythrocytes for targeted drug delivery [3,8].

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