

Natural antibodies to avidin in human serum

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1. Summary

Human serum was found to contain natural antibodies to the egg-white glycoprotein avidin. Of 270 samples tested, all contained antibodies to different extents, mainly of the IgG and IgM classes. Anti-avidin antibodies could be isolated by affinity chromatography.

2. Introduction

The avidin-biotin system has evolved into a very useful tool for a broad range of applications in research, industry and medicine [1,2]. With its increased use for in vivo diagnostics [3] and drug delivery [4,5], some unusual properties of avidin were observed. These include rapid blood clearance and relatively low organ retention [6], induction of complement-mediated lysis of biotinylated human erythrocytes [7,8], and inconsistent results when human serum is used in immunoassays. All these observations suggest that anti-avidin antibodies occur naturally in human serum.

Key words: Anti-avidin antibody; Avidin-biotin system; Natural human antibody

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Abbreviations: ELISA, enzyme-linked immunosorbent assay; PBS, phosphate-buffered saline pH 7.4; TST, Tris-saline buffer pH 8.0, containing 0.05% Tween 20.

In the current study, we tested this premise by examining 270 samples of donor sera for the presence of anti-avidin antibodies using an ELISA technique [9]. In addition, avidin-specific antibodies were isolated from human serum by affinity chromatography either on a protein A column or on an avidin-Sepharose column.

3. Materials and Methods

3.1. Materials

Avidin, Lite avidin, and NeutraLite avidin were products of Belovo Chemicals (Bastogne, Belgium). Streptavidin was prepared from spent growth medium of *Streptomyces avidinii* ATTC 27419 by affinity chromatography on iminobiotin-Sepharose [10].

Anti-human IgG was conjugated to horseradish peroxidase according to Wilson and Nakane [11], essentially as described in an earlier report [9]. Protein A was obtained from Bio Processing (Durham, UK).

3.2. Detection of anti-avidin antibodies

An ELISA procedure was used to detect the presence of anti-avidin antibody, as detailed previously [9]. Samples (100 µl per well) of avidin, Lite avidin, NeutraLite avidin and streptavidin (1 µg/ml, dissolved in 0.05 M carbonate buffer pH 9.6) were coated on 96-well microtiter plates (Dynatech 129B plates, Zollikon, Switzerland). Donor sera (50 µl) were added to the corresponding

wells on each of the three types of plates. The samples were incubated for 30 min at 45°C and washed with TST (Tris-saline buffer pH 8.0, containing 0.05% Tween 20) using the 'washing shower' procedure [12]. Anti-human IgG-conjugated horseradish peroxidase was diluted optimally in 0.5 M Tris-0.1 M NaCl buffer pH 8.0, containing 2% bovine serum and 2% sheep serum (to give the maximal positive/negative ratio as determined in preliminary experiments), and 50- μ l samples were added per well. The plates were incubated and washed with TST as above. *o*-Phenylenediamine-containing chromogenic substrate solution (50 μ l, [9]) was added, the color was allowed to develop for 30 min at room temperature, and the reaction was stopped with 1 M H₂SO₄ (100 μ l). Absorbance (492 nm) was determined using a Dynatech MR700 Microelisa Auto-reader.

3.3. Avidin-induced hemolysis

The following procedure is a modification of that described by Muzykantov et al. [7,8]. Biotin and a long-chained derivative (biotinyl aminocaproic acid) were coupled via the corresponding *N*-hydroxysuccinimide ester to human erythrocytes. Briefly, a solution (0.1 ml) of 0.1 M sodium tetraborate was added to a 10% suspension (1 ml) of PBS-washed human erythrocytes. A 3- μ l aliquot of a 0.1 M solution of the desired biotin reagent in dimethyl formamide was then added. After a 20-min incubation period at room temperature, the cells were washed five times with PBS. Avidin, at a concentration of 1 mg in 1 ml PBS, was then added to 1 ml of a stirred 10% suspension of the biotinylated erythrocytes. The incubation was carried out for 60 min at 4°C, and the erythrocytes were washed three times with PBS.

Pooled whole donor serum (50 μ l) was tested in duplicate against the avidin-coupled erythrocytes (50 μ l). The cells were incubated for 2 h at 37°C with no detectable hemolysis. The cells were incubated subsequently in the presence of fresh human complement (100 μ l) and 0.25 mM calcium ions, either with or without the addition of 5 mM EDTA. Untreated red cells from the same source were also included as a control to eliminate the

possibility that either erythrocyte agglutination or complement activation was a product of antibody other than anti-avidin. Complete hemolysis of the red cells was observed after a 4-h incubation period.

4. Results and Discussion

In recent attempts to use the avidin-biotin system for *in vivo* drug targeting [4,5] and cancer localization [3,13], we observed that avidin exhibited an extremely rapid clearance from the blood and relatively low organ retention [6]. This led us to suspect that antibodies against avidin are present in human serum. Our suspicions were enhanced by the inconsistent results achieved when using avidin-based detection systems on whole human serum (unpublished data). In order to examine this possibility, we developed an ELISA system for detecting anti-avidin antibodies in human serum. For this purpose, coating efficiency curves were determined for avidin, Lite avidin, NeutraLite avidin and streptavidin (data not shown); separate 96-well ELISA plates were then coated at the optimal concentrations of the latter avidins (1 μ g/ml). The resultant plates were employed to detect antibodies in serum samples, using an anti-human IgG-peroxidase conjugate as described in Materials and Methods.

Using this procedure, we could not detect any antibodies to streptavidin in human serum. On the other hand, all 270 serum samples reacted with the other three types of plates, giving varying levels of response in each case. In fact, we could not find any sample which failed to show antibodies to all three avidin derivatives, i.e., the native glycoprotein, Lite avidin (a deglycosylated form of avidin) and NeutraLite avidin (a neutrally charged, deglycosylated form of avidin). Overall, there was no genuine preference in the individual serum samples for any particular derivative of avidin. Interestingly, in some samples the signals achieved for the deglycosylated derivatives were higher than that for the native avidin, whereas in others the signal for the native glycoprotein was higher than those for Lite and NeutraLite avidin. This finding suggests that the sugar residues of avidin are not an absolute requirement for interaction with natural anti-avidin anti-

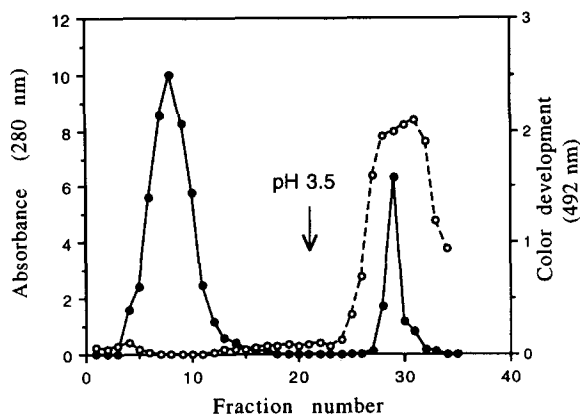


Fig. 1. Purification of anti-avidin antibodies from normal human serum on protein A-Sepharose. A sample of undiluted serum (4 ml) was applied to the affinity column (1.5 × 5 cm). Equilibrating buffer: 0.05 M Tris-0.1 M NaCl pH 8.0. Eluting buffer: 0.1 M acetate pH 3.5. Samples were neutralized with base, when necessary, and examined by ELISA using avidin-coated microplates for the presence of anti-avidin antibodies. See text for details. Solid line, protein (A₂₈₀). Dashed line, antibody (A₄₉₂).

bodies and that different individuals bear antibodies which express different specificity patterns.

It was also of interest to examine whether different individuals tend to express different classes of anti-avidin antibody. We examined this possibility by performing ELISAs using peroxidase-conjugated anti-human IgG, IgM and IgA. Using this approach, most of the samples showed antibodies of the IgG and/or IgM classes; only one out of 50 samples tested showed any reaction with anti-IgA antibody.

In order to confirm further that the results sup-

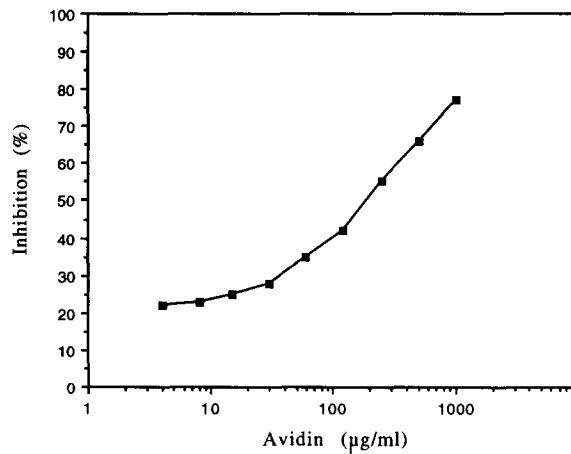


Fig. 2. Percentage inhibition of anti-avidin binding activity by avidin in solution. Protein A-purified anti-avidin antibodies were introduced into avidin-coated wells in the presence of varying concentrations of free avidin. The wells were washed, and the level of bound antibody was determined by ELISA, using enzyme-conjugated anti-human IgG. The results are expressed as the percent of inhibition displayed by a given sample, compared with the color development in the absence of soluble avidin.

port the presence of anti-avidin antibodies and not the mere nonspecific binding of serum immunoglobulins to avidin, we passed the serum through a protein A-Sepharose affinity column. As can be seen in Fig. 1, the binding of avidin is associated with the γ -globulin fraction (i.e., the antibody fraction), as determined by ELISA. Free avidin inhibited this binding, as shown in Fig. 2. We have thus established that there are endogenous anti-avidin antibodies in human serum.

To study the properties of these antibodies fur-

TABLE 1

Serum-dependent hemolysis of avidin-bound, biotinylated erythrocytes.^a

Avidin-sensitized erythrocyte preparation	Additives		
	Complement	Complement + EDTA	None
Long-chain biotin-derivatized cells ^b	100	7.4	60.9
Short-chain biotin-derivatized cells ^c	100	0	57.5
Underivatized cells	11.2	0	0

^aValues represent the percentage of hemolysis (determined according to [8]) observed for the indicated cell preparation.

^bCells derivatized with biotin ϵ -aminocaproyl *N*-hydroxysuccinimide ester.

^cCells derivatized with biotin *N*-hydroxysuccinimide ester.

ther, we examined whether they can activate the complement system and cause lysis of avidin-sensitized, biotinylated erythrocytes. We modified red blood cells with both long and short chain *N*-hydroxysuccinimide derivatives of biotin, as described by Muzykantov et al. [7,8], and avidin was coupled to the biotinylated erythrocytes. The cells were then checked for lysis in the presence either of serum samples or of the purified antibody preparation. The results (Table 1) indicate that natural human antibodies against avidin are capable of activating the complement system. It has recently been claimed that lysis of such avidin-bearing erythrocytes may be induced via the alternative complement pathway, without the involvement of immune complexes [7,8]. This proposal should be reinterpreted in view of our findings presented herein. The only difference between our data and those of Muzykantov et al. [7,8] is that the latter researchers observed lysis after only a 1-h incubation period, whereas in our studies a 4-h incubation was required. This apparent discrepancy may be a function of the density of the avidin which is bound to the respective biotinylated erythrocyte preparation.

The present findings open the way for new studies which may help us understand how and why antibodies against avidin are being elicited in humans. Is their formation a result of the consumption of eggs? Indeed, preliminary results (not shown) indicate that individuals who maintain an egg-free diet (although the subjects tested have consumed egg products) exhibit only very low levels of natural anti-avidin antibodies. In this regard, the avidin-biotin complex is a very stable protein and is known to withstand the hydrolytic conditions which exist in the stomach [14]; perhaps avidin is capable of penetrating the blood system, and natural antibodies are thus produced. Alternatively, the results may reflect the inclusion of egg proteins in the preparation of vaccines for the immunization of children and adults [15]. At any rate, it seems that humans can develop a tolerance to avidin. We are currently checking the nature of the immunogenic epitopes on the avidin molecule and to what extent the profile of anti-avidin antibodies varies in sera from different individuals.

The current communication was written to make scientists aware of the natural anti-avidin antibodies in human serum. This is especially important when applying the avidin-biotin system for in vivo studies [4,16] or when whole human serum is used in conjunction with avidin-biotin technology [1,17].

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