Research Report

Effects of Detergents on Avidin-Biotin Interaction

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ABSTRACT

The effects of several detergents (anionic, non-ionic, and zwitterionic) on the association of biotin with avidin are described. Even in the presence of 1% SDS, ¹⁴C-avidin became bound to biotinyl-agarose in tris-buffered saline, pH 7.6. Dissociation of this complex required heating and treatment with urea. ¹⁴C-avidin also complexed with biotinyl-agarose in Tween 20, Tween 40, Triton X-100 and zwitterionic detergents 693017 and 693019. The stability of the complex to dissociation by 6 M urea or heat varied among these detergents, but dissociation of ¹⁴C-avidin was always less than that obtained using SDS, heat and urea. In the converse experiment, biotinylated transferrin bound to avidin-agarose in the presence of 1% SDS, but this complex was less stable and could be dissociated by heating to 95.6°C independent of treatment with urea. Biotinylated transferrin was specifically bound to avidin in the presence of non-biotinylated ovalbumin and 1% SDS. The biotinylated protein was isolated after washing the avidin-matrix with SDS-containing buffer, followed by heating in the presence of SDS.

INTRODUCTION

The extremely strong binding between avidin and biotin has led to broad usage of this interaction for detecting or isolating biotinylated molecules. Biotinylated molecules have been used as probes in the labeling and localization of specific proteins and nucleic acids with avidin serving as a specific detection agent (1-4, 13, 14). The stability of the avidin-biotin complex during extensive washing procedures permits removal of non-specifically bound material, thus providing sharper resolution of specifically tagged molecules in cells, in subcellular fractions isolated from cells, and in the isolation of specific fragments of proteins or nucleic acid polymers.

Some of the properties of avidin and biotin and their interactions have been the subjects of previous work (6-8, 11). Free avidin is susceptible to denaturation (and loss of biotin binding capacity) by heat, 0.1 M SDS and 6 M guanidine hydrochloride, whereas the avidin-biotin complex is considerably more stable (7, 15). Heating to 100°C dissociates only a fraction of avidin-biotin complexes with low ionic strength favoring dissociation (15). The complex can be destroyed by some oxidizing agents, such as N-bromosuccinimide which oxidizes tryptophan residues in avidin (5). Not all of the characteristics reported for avidin-biotin interactions, however, are conserved when the biotin is presented in covalent attachments to proteins which have been chemically modified with biotin derivatives. In fact, avidin-binding properties can be altered by varying the length of the linkers used to attach biotin to the modified protein (9). Since biotin binding may be as far as 9Å below the avidin surface (8), it is reasonable that increased linker length increases the avidin-biotinyl protein stability (9).

Because studies on the proteins and on the protein-protein interactions in cellular membranes and in the cytoskeletal matrix have required the use of detergent extracts, it is important to know the effects of these detergents on the complex formed by avidin with biotin and with biotinylated proteins in order to utilize the biotin-avidin system to probe these interactions. This report demonstrates the effects of the detergents SDS, Tween 40, Tween 20, Triton X-100 and zwitterionic detergents 693017 (N-tetradecyl-N,N-dimethyl-3-ammonio-l-propanesulfonate) and 693019 (N-octyl-N,N-dimethyl-3-ammonio-l-propanesulfonate) on avidin-biotin complexes, and shows the specific interaction of avidin with biotinylated transferrin in the presence of SDS.

MATERIALS AND METHODS

Avidin-Biotin Binding

Avidin (obtained from Pierce

Chemical Co., Rockford, IL) was labeled with ^{14}C-formaldehyde (New England Nuclear Co., Boston, MA, 40-60 mCi/micromole) via reductive methylation (10). The methylated avidin contained 8.85 x 10^6 cpm/mg. Sodium cyanoborohydride and avidin-agarose (egg avidin attached to epoxy-activated agarose) were purchased from Sigma Chemical Co., St. Louis, MO. Immobilized-D-biotin (biotin attached to agarose via a diaminodipropylamine spacer) was obtained from Pierce Chemical Co.

Fifty microliters of 2% w/v solutions of each detergent (SDS, Tween 20, Tween 40 (Sigma), Triton X-100 (New England Nuclear), zwitterionic detergent 693017, zwitterionic detergent 693019 (CalBiochem)), was added to 25 μl tris-saline (0.1 M NaCl, 0.05 M tris, 0.01% NaN_3, pH 7.6), 3 μl 150 mg/ml bovine serum albumin (BSA) in tris-saline, 20 μl immobilized-D-biotin (50% slurry) and 20 μg of ^{14}C-avidin in 5 μl. Each mixture was incubated for 30 min at 24°C in a 1.5 ml microcentrifuge tube with periodic mixing on a vortex mixer. The samples were heated in a boiling water bath (95.6°C) for 5 min and then cooled to 24°C. Some samples were then made 6 M in urea by adding 36 mg of urea. After 5 min with mixing, the samples were centrifuged at 11,600 x *g* for 5 min in a Beckman Model 11 microcentrifuge to separate agarose-associated material from solution. The supernatant was removed and 10 μl aliquots, in 4 ml of Aquasol, were counted in a Beckman scintillation counter Model LS 7500. The biotin-agarose pellet was washed 4 times with 100 μl tris-saline, suspended in 4 ml Aquasol, and counted.

Table 1. ^{14}C-avidin released from D-biotin-agarose in the presence of various detergents.

Treatment			
Detergent	Heated	Urea	Avidin Released (%)*
None	−	−	0
None	−	+	14.5
None	+	+	12
SDS	−	+	11.5
SDS	+	−	18
SDS	+	+	65
Tween 20	+	−	2.5
Tween 20	+	+	18
Tween 40	+	−	0
Tween 40	+	+	11
Triton X-100	+	−	0
Triton X-100	+	+	11.5
693017	+	−	30.5
693017	+	+	39
693019	+	−	0
693019	+	+	11

*Percent ^{14}C-avidin release was determined as:

$$\% \text{ release} = 100 \times \frac{\text{supernatant-n (supernatant + pellet)}}{\text{(supernatant + pellet) -n (supernatant + pellet)}}$$

where n = proportion of radioactivity not bound to biotin-agarose in tris-saline, so n (supernatant + pellet) is the total radioactivity which could not be bound to biotin-agarose. All experiments were done twice and the value reported is the mean of both results.

Binding of Biotinylated Transferrin to Avidin-Agarose

Transferrin (Sigma) was dissolved to a concentration of 1 mg/ml in 0.1 M sodium borate, pH 8.0. NHS-X-biotin (Biotin-ε-aminocaproic acid, N-hydroxysuccinimide ester.) (CalBiochem) was dissolved to 20 mg/ml in dimethylformamide and 100 μl of the NHS-X-biotin solution was added to 2.5 ml of the transferrin. The mixture was incubated in the dark for 2 h at 24°C with mixing, and then dialyzed against 2 l

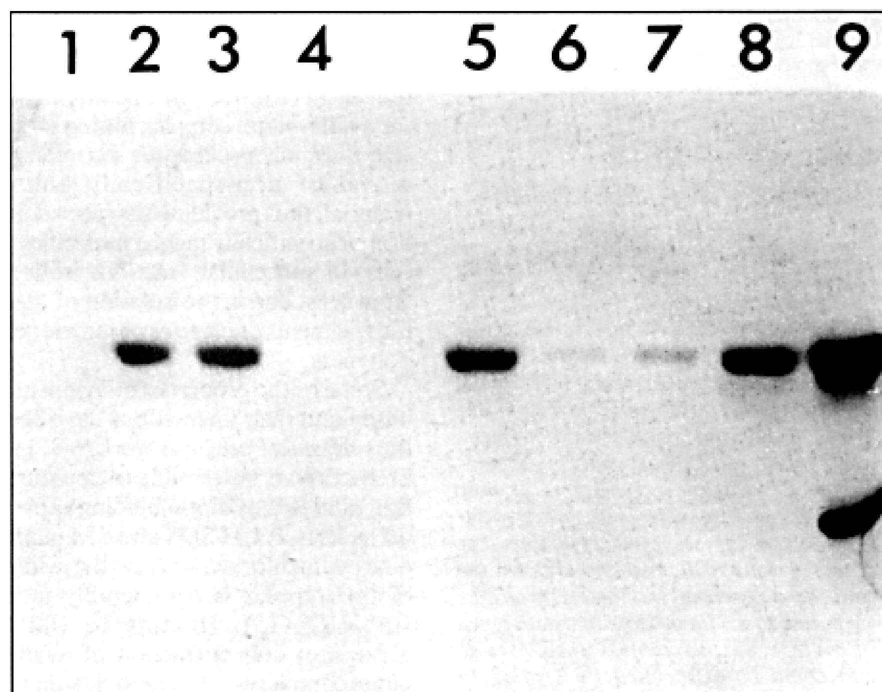

Figure 1. Polyacrylamide gel analysis of biotinylated transferrin adsorbed to avidin-agarose in the presence of ovalbumin. Aliquots (25 μl) were analyzed on a 12.5% polyacrylamide gel in the presence of SDS. Lanes 1-4: supernatant fractions. Elution conditions were (lane 1) 1% SDS + 6 M urea; (lane 2) 1% SDS + 95.6°C; (lane 3) 1% SDS + 95.6°C + 6 M urea; and (lane 4) 1% SDS. Lanes 5-8: residual biotinylated transferrin remaining in pellet fractions after elution with (lane 5) 1% SDS + 6 M urea; (lane 6) 1% SDS + 95.6°C; (lane 7) 1% SDS + 95.6°C + 6 M urea; and (lane 8) 1% SDS. Lane 9: biotinylated transferrin (M_r = 75,600) and ovalbumin (M_r = 43,000) mixture prior to experiment.

tris-saline at 4 °C for 72 h in a light-shielded vessel.

Reaction mixtures containing 200 μl of 2% SDS in tris-saline, 65 μl biotinylated transferrin, 65 μl ovalbumin (1 mg/ml in tris-saline) and 70 μl of avidin-agarose (50% slurry) were incubated for 30 min with mixing. The mixtures were centrifuged at 11,600 x *g*. The supernatants were discarded and the avidin-agarose pellets were washed 5 times with 200 μl of 1% SDS in tris-saline. The pellets were suspended in 65 μl 1% SDS in tris-saline, and heated to 95.6 °C (boiling water) for 5 min. The tubes were cooled to 24 °C and a subset was made 6 M with respect to urea. The tubes were mixed 5 min, centrifuged to separate the agarose and its associated proteins from solution and the supernatants were removed. The supernatants were diluted 2:1 in SDS-gel sample buffer (4.1 M glycerol, 0.21 M SDS, 0.04 mM bromphenol blue, 0.188 M Tris base, pH 6.8), mixed, heated to 95.6 °C for 5 min and aliquots were analyzed on a 12.5% polyacrylamide gel in the presence of SDS (12). The avidin-agarose pellets were washed 3 times with 200 μl 1% SDS in tris-saline. To determine residual releasable material, the pellets were resuspended in 65 μl 1% SDS in tris-saline, mixed, heated and cooled as above. The supernatants were removed after centrifugation and diluted 2:1 in SDS-gel sample buffer. Aliquots were analyzed by polyacrylamide gel electrophoresis in the presence of SDS (12).

RESULTS AND DISCUSSION

The results of the interaction of ^{14}C-avidin with immobilized biotin in the presence of detergents are presented in Table 1. ^{14}C-avidin binds to biotinyl-agarose even in the presence of 1% SDS, and this binding is not disrupted by heating or by treatment with urea. The interaction between ^{14}C-avidin and biotinyl-agarose is, however, markedly decreased by treatment with urea subsequent to heating in the presence of SDS.

Complex formation also occurred between ^{14}C-avidin and biotinyl-agarose in the presence of Tween 20, Tween 40, Triton X-100, and the zwitterionic detergents 693017 and 693019. With the exception of zwitterionic detergent 693017 which contains a tetradecyl side chain, the avidin-biotin complexes form in these detergents to the same extent as in the absence of detergent, are stable to heat, and are only partially dissociated by subsequent urea treatment.

In order to test whether a biotinylated protein would specifically bind to avidin-agarose in the presence of 1% SDS, transferrin modified with NHS-X-biotin was mixed with ovalbumin and then incubated with avidin-agarose. Fig. 1, lane 9, shows the electrophoretic pattern of the mixture of biotin-X-transferrin (75,600 daltons) and ovalbumin (43,000 daltons) prior to incubation with the avidin-agarose. The avidin-agarose pellets were washed 3 times with 200 μl 1% SDS in tris-saline. Specific treatments were performed as indicated in the figure legend and the material released from the avidin-agarose into the supernatant was examined by polyacrylamide gel electrophoresis (Fig. 1, lanes 1-4). Lane 1 shows that biotinylated transferrin was not observed in the supernatant after 1% SDS and 6 M urea treatment. Lanes 2 and 3 show that 1% SDS and heating or 1% SDS, heating, and 6 M urea released biotinyl-transferrin from the avidin-agarose, while lane 4 shows that 1% SDS without heating did not release any protein. The proteins remaining associated with the avidin-agarose after the above treatments were eluted from the avidin-agarose pellets by heating with SDS in tris-saline (lanes 5-8). Lanes 6 and 7 demonstrate that the avidin-agarose pellets previously treated by exposure to 1% SDS and heating had released virtually all bound biotinylated transferrin that could be dissociated by this treatment. Lanes 5 and 8 show that biotinylated transferrin had not been released from SDS-treated avidin-agarose without heat even in the presence of urea. In the presence of SDS no ovalbumin was bound to the avidin-agarose, confirming that the specificity of the avidin-biotin interaction was maintained.

The binding of ^{14}C-avidin to biotinyl-agarose was clearly different than the binding of biotinylated protein to avidin-agarose. SDS and heat treatment dissociated the complex between the biotinylated transferrin and avidin-agarose, while SDS, heat, and urea treatment were necessary to dissociate the complex between avidin and biotinyl-agarose. This difference is consistent with previous work (9) which demonstrated that the half-life for biotin dissociation from avidin or succinoyl-avidin is measured in hundreds of days whereas biotinylated insulin dissociated from avidin with a half-life measured in hours. It is also consistent with the slightly longer spacer between the biotin and agarose than between the biotin and protein. The greater stability of the avidin-biotin compared to biotinylated protein-avidin might explain the different requirements for urea in achieving dissociation of the complexes.

The work reported here demonstrates that, even though the binding of biotinyl – proteins to avidin-agarose may be weaker than binding of biotin to avidin alone, the association occurs and remains stable in a variety of commonly used detergents. Therefore, the

interaction of avidin with biotin provides a useful approach for the isolation of cellular components dissolved in detergents, and may be especially useful for those dissolved in SDS where other affinity protocols are not successful. □

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