COMBINABILITY OF AVIDIN AND STREPTAVIDIN WITH ANALOGS OF BIOTIN

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Avidin, a heat labile protein found in raw egg white, specifically binds biotin into a stable non-digestible complex preventing utilization of the vitamin by animals and microorganisms. It has been used often to induce biotin deficiencies and to bind biotin in isolated enzyme systems. Also, combinability with avidin has been employed to characterize analogs of biotin (Burk and Winzler, 1943; Eisenberg, 1963; Birnbaum and Lichstein, 1965).

Tausig and Wolf (1964) isolated a protein (streptavidin) from fermentation filtrates of streptomycetes which specifically binds biotin. The crystallized material was found to complex 1 μg of biotin per 150 μg of protein. Streptavidin differs from avidin in amino acid and carbohydrate content, UV adsorption, and electrophoretic mobility (Chaiet and Wolf, 1964). The present report is concerned with a comparison of the combinability of avidin and streptavidin with various analogs of biotin.

Materials and Methods

To determine the relative binding of biotin analogs, known amounts of avidin and streptavidin were mixed with excessive amounts of the analogs in distilled water. The mixtures were incubated at room temperature for 1 hour and the remaining unbound analog assayed microbiologically with

Saccharomyces cerevisiae (ATCC 9896) employing the procedure of Hertz (1943). The level of residual free analog was subtracted from the concentration of the control tubes which contained no avidin or streptavidin. The final results are expressed as millimicromoles of biotin analog bound to 1 unit of protein.

Studied also was the ability of avidin and streptavidin to bind biotin and desthiobiotin during the growth of a biotinless mutant of Escherichia coli (ATCC M8178). The basal medium contained the following per liter: 4 g $(NH_4)_2 SO_4$, 1 g NaCl, 0.5 g sodium citrate, 0.7 g $MgSO_4 \cdot 7H_2O$, 1 g KH_2PO_4, 1 g K_2HPO_4 and 5 g charcoal purified acid hydrolyzed casein. Biotin or desthiobiotin was added at a final concentration of 5×10^{-4} µg/ml and 1×10^{-3} µg/ml, respectively. Five ml of sterile media contained in 50 ml screw cap tubes were inoculated with one drop of a light suspension of the organism. Avidin and streptavidin were sterilized by filtration and added aseptically to the basal medium prior to inoculation. The tubes were incubated at 37 C for 18 hours in a shaking water bath. Growth was determined turbidimetrically with a Klett-Summerson photoelectric colorimeter (420 mµ).

Results and Discussion

The values of Tausig and Wolf (1964) for the activity of streptavidin were confirmed, with the finding that 150 µg of protein binds 1 µg of biotin (4.1 mµmoles).

Avidin complexed with biocytin, desthiobiotin, and oxybiotin nearly as well as with biotin (Table 1). This suggests that the carboxyl group and the sulfur atom are not important in the binding of biotin to avidin, a finding which is in agreement with other reports (du Vigneaud et al., 1942;

TABLE 1. Binding of biotin and biotin analogs to avidin and streptavidin

Vitamin Analog	Avidin		Streptavidin	
	bound to 1 unit of protein*	amount bound as compared to biotin	bound to 1 unit of protein*	amount bound as compared to biotin
	mμmoles	%	mμmoles	%
d-Biotin	4.10	100	4.10	100
dl-Biocytin	3.83	93.4	3.82	93.4
dl-Desthiobiotin	3.50	85.4	0.0	0.0
dl-Oxybiotin	3.90	95.0	2.68	65.4
Biotin diamine sulfate	0.0	0.0	0.0	0.0

*Avidin (General Biochemicals, Inc.) had an activity of 2.16 units/mg. Streptavidin was a gift from Merck Sharp and Dohme. A unit is defined (for either material) as the amount of protein that binds 1 μg of d-biotin.

Sebrell and Harris, 1954). The combinability of streptavidin with biocytin further suggests that the carboxyl group is not necessary for binding to either protein. The inability of biotin diamine sulfate to bind to avidin or streptavidin confirms directly the need for an intact urea ring to promote avidin and streptavidin combinability. The most striking difference between the two proteins was the inability of streptavidin to bind desthiobiotin, suggesting that unlike avidin successful binding of a biotin analog to streptavidin would occur only if the sulfur containing ring was intact. Sulfur *per se* is not necessary since a substantial though lower level of binding was observed with oxybiotin.

Qualitative data were obtained suggesting that the addition of an oxygen atom to the sulfur as in d-biotin-d-sulfoxide also prevents the binding to streptavidin but has no effect on combinability with avidin. The d-biotin-d-sulfoxide formed by the oxidation of d-biotin during paper chromatography (Wright *et al.*, 1954; Birnbaum and Lichstein, 1965), was eluted from the paper and tested for combinability in the presence of excess amounts of

each protein. Thus, it may be concluded that the two biotin binding proteins differ in binding selectivity, in that avidin requires only an intact urea ring, whereas streptavidin requires both intact urea and sulfur rings.

Further evidence for the uncombinability of desthiobiotin with streptavidin was provided by a study of the growth of a biotinless mutant of E. coli in the presence of biotin or desthiobiotin (Table 2). It is clear

TABLE 2. Effect of avidin and streptavidin on growth of a biotinless mutant of Escherichia coli

Additions to basal medium	Turbidity at 18 hr Klett units (420 mμ)
None	9
d-Biotin (5×10^{-4} μg/ml)	364
+ avidin (1×10^{-3} units/ml)*	4
+ streptavidin (1×10^{-3} units/ml)*	4
dl-Desthiobiotin (1×10^{-3} μg/ml)	338
+ avidin (2×10^{-3} units/ml)	6
+ streptavidin (2×10^{-3} units/ml)	341
Avidin (2×10^{-3} units/ml)	6
Streptavidin (2×10^{-3} units/ml)	6

* Avidin and streptavidin were supplied at twice the concentration necessary to bind all the vitamin.

that avidin and streptavidin complexed with biotin making the vitamin inaccessible for growth. In cultures containing desthiobiotin and streptavidin the same level of growth was obtained as in the cultures having desthiobiotin alone. Thus, desthiobiotin remained free in the presence of the streptavidin and was utilized for growth. No growth was observed with desthiobiotin or biotin when excess avidin was present.

The employment of both avidin and streptavidin could be an impor-

tant tool in helping to elucidate the structure of unknown analogs of biotin that are biologically active.

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