STREPTAVIDIN - A SUBSTANCE WITH AVIDIN-LIKE PROPERTIES PRODUCED BY MICROORGANISMS

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Animal dietary studies demonstrating the toxic effect of raw egg white (Boaz, 1924), and the recognition of the protective action of certain foods and extracts against this egg white injury (Boaz, 1927) led to the work on Vitamin H (Gyorgy, 1931, 1939) and subsequent proof of the latter's identity with the yeast growth factor biotin (Gyorgy et al., 1940). The toxic factor of egg white, a heat labile protein, was first isolated by Eakin and coworkers (Eakin, Snell and Williams, 1940, 1941), and named avidin. It was shown to exert its toxic effect by binding biotin into a firm, non-digestible complex and thus preventing the utilization of the vitamin by both animals and microorganisms. Raw egg white, or purified avidin obtained from this source, has since been used extensively as a highly specific and selective biotin-binding agent to induce biotin deficiencies in animals and man, as well as in biotin requiring microorganisms and isolated enzyme systems.

Despite the numerous subsequent investigations in the field of biotin and biotin antagonists, avidin or avidin-like activity has thus far been reported to occur only in egg white of birds (Hertz and Sebrell, 1942; Jones and Briggs, 1962), the egg jelly of the frog (Hertz and Sebrell, 1942), and in the albumen secreting oviduct tissues of laying hens (Fraps, Hertz and Sebrell, 1943). The oviduct tissues of non-laying hens and mucosal scrapings from the oviducts of pigs, cows and guinea pigs were found devoid of avidin (Hertz, 1946). To date, no other source for avidin

or truly avidin-like materials has been reported. The very slight biotin-binding activity of pure salinine, 0.2 percent that of avidin (Wooley and Longsworth, 1942) and of bull sperm, 0.2 percent that of hen egg white (Jones and Briggs, 1962), is of such a low order that non-specific binding, rather than true avidin activity, may be implicated.

The present paper presents evidence for the elaboration by Streptomycetes of a protein with biotin-binding properties similar to avidin. This material was encountered during a search for new antibiotics in which several different types of Streptomycetes (Stapley et al., 1963) were found to produce antibiotic substances primarily active against gram-negative bacteria. Other, more detailed reports will describe the antibacterial and other aspects of these substances, such as isolation and purification (Chaiet et al., 1963), animal studies (Miller, 1963) and mode of action.

Current Investigations

During the course of purification of the antibiotic activity it was found that the fermentation broths contained two different kinds of components; a dialysable, small component (labelled "S") and a non-dialysable, large component (labelled "L"). Separation of the bioactive mixture of the two components by exhaustive dialysis or gel-filtration on Sephadex G-25 resulted in complete loss of antibacterial activity as determined in the usual complex assay medium. Full activity was restored upon recombination of the two fractions, either one of which was inactive, even at very high levels, when assayed separately (Chaiet et al., 1963).

However, the purified "S" fraction by itself was found to be highly active against the same strain of E. coli when assayed in synthetic medium, whereas the "L" fraction remained devoid of antibacterial activity. It was demonstrated that the difference in activity of "S" in the two media could be attributed to the reversal of the inhibitory properties of "S" by biotin (see Table I). The reversal was evident in both media and indicated that "L" acted as a biotin-binding agent, analogous to avidin.

TABLE I

ACTIVITY OF "S" AGAINST E. COLI IN SYNTHETIC MEDIUM AND ITS REVERSAL BY BIOTIN

"S" (γ/ml.)	Inhibition Zone (mm.)	"S" (γ/ml.)	Biotin (γ/ml.)	Inhibition Zone (mm.)
40	48	10	0	45
8	43	10	.5	0
1.6	38	10	.05	0
.32	33	10	.005	35-45, growth to 35
.064	28	10	.0005	35-45, growth to 25

The medium contained only glucose, citrate, methionine and inorganic salts, plus agar. Paper disks, 13 mm. diameter, were used saturated with the test solutions.

This relationship of "L" to the egg white injury factor was confirmed by the use of purified avidin[1]. The known biotin-binding agent was found to be identical to "L" in its ability to permit "S" to exert its antibacterial properties in natural environments (see Table II). Analogous results were obtained using tube dilution assays.

TABLE II

SIMILARITY IN EFFECT OF "L" AND AVIDIN VERSUS BIOTIN

Conc. of each component, in γ/ml.				E. coli Inhibition Zone (mm.)
"S"	"L"	Avidin	Biotin	
200	0	0	0	0
100	500	0	0	19
100	50	0	0	0
100	0	500	0	14
100	0	50	0	13
100	0	5	5	0
66	333	0	33	0
66	333	0	3.3	0
66	333	0	.33	18
66	0	333	33	0
66	0	333	3.3	0
66	0	333	.33	14

The assay medium was made up of Difco nutrient agar plus yeast extract.

[1] Obtained from Nutritional Biochemical Corp. Activity - 2,500 units per gm.

Animal experiments, reported elsewhere in this journal (Miller and Tausig, 1963), not only provided further evidence for the similarity of avidin and this new protein from microorganisms, but also constitute the first demonstration of the rapid binding of free tissue biotin by parenterally administered avidin. Furthermore, this is the first clear-cut use of avidin as an adjunct in chemotherapy.

Purified "L" preparations exhibit many properties closely similar to those of avidin. The biotin-binding capacity is approximately 1γ of biotin per 150γ "L". The non-dialysable protein-biotin complex is destroyed by heating with the liberation of biotin activity. The chemical and physical properties of "L", as well as the methods used for its purification, will be presented elsewhere (Chaiet et al., 1963).

Discussion

The finding that microorganisms produce a protein with avidin-like activity is rather startling, particularly in view of the highly specific type of action and the fact that until now avidin had been thought to occur exclusively in eggs and the oviduct tissues of laying birds and amphibia. No explanation had been found for the presence of this antivitamin in these specific sources. However, it had been suggested that avidin plays a role in the physiology of avian reproduction. This postulate was based not only on the restricted sites of occurrence, but also on the finding that it is possible to induce the production of avidin in the oviducts of immature and adult non-laying hens by the administration of hormones (Hertz, Fraps and Sebrell, 1943, 1944).

Any attempted interpretation of the significance of the production of avidin by microorganisms is, of necessity, highly speculative at this time. One plausible explanation would seem to be that it is part of the "defense mechanism" of these antibiotic producing microbes. As the data show, removal of excess exogenous biotin from the surrounding environ-

ment is essential for the activity of the biotin-reversable antibiotic elaborated by these Streptomycetes.

Another possibility is that biotin-binding proteins are actually the active enzymes involved in fixing biotin prior to transfer of carbon dioxide. As such these proteins would be present in minute quantities in all cells. Excretion in detectable quantities is, however, very unusual.

In view of the close similarity of this microbial protein to avidin and its isolation from Streptomyces, we propose the name STREPTAVIDIN as an appropriate designation for the substance.

References

Boaz, M. A., Biochem. J. 18, 422 (1924).
Boaz, M. A., Biochem. J. 21, 712 (1927).
Chaiet, L., Miller, T. W., Tausig, F. and Wolf, F. J., Antimicrob. Agents & Chemotherapy, in press (1963).
Eakin, R. E., Snell, E. E. and Williams, R. J., J. Biol. Chem. 136, 801 (1940).
Eakin, R. E., Snell, E. E. and Williams, R. J., J. Biol. Chem. 140, 535 (1941).
Fraps, R. M., Hertz, R. and Sebrell, W. H., Proc. Soc. Exptl. Biol. Med., 52, 140 (1943).
Gyorgy, P., Ztschr. f. Arztl. Fortbild. 28, 377 (1931).
Gyorgy, P., J. Biol. Chem. 131, 733 (1939).
Gyorgy, P., Rose, C. S., Hofmann, K., Melville, D. B. and du Vigneaud, V., Science 92, 609 (1940).
Hertz, R., Physiol. Rev. 26, 479 (1946).
Hertz, R. and Sebrell, W. H., Science 96, 257 (1942).
Hertz, R., Fraps, R. M. and Sebrell, W. H., Proc. Soc. Exptl. Biol. Med., 52, 142 (1943).
Hertz, R., Fraps, R. M. and Sebrell, W. H., Science 100, 35 (1944).
Jones, P. D. and Briggs, M. H., Life Sciences 1, 621 (1962).
Miller, A. K., Antimicrob. Agents & Chemotherapy, in press (1963).
Miller, A. K., Tausig, F., this issue.
Woolley, D. W. and Longsworth, L. G., J. Biol. Chem. 142, 285 (1942).