Affinity of Avidin for Biocytin. (17875)

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The isolation of a crystalline complex of biotin, termed biocytin, from yeast extract recently has been announced(1). Pursuant to studies on the metabolic significance of biocytin the affinity of avidin for biocytin has been investigated. In these studies a quantitative method(2,3), whereby the affinity of avidin for biocytin is compared with the affinity of the protein for biotin, was employed. The procedures used involved the addition of avidin to mixtures of biotin and biocytin. The biotin and avidin were used in approximately stoichiometric amounts while the amount of biocytin was varied. Under these conditions the amount of biotin available for the growth of biotin-requiring species is equivalent to the amount of biocytin taken up by the avidin. The relative affinity then is expressed arbitrarily as the ratio of the concentration of biocytin to biotin at which one-half of the biotin remains free and available for the growth of the test organism. The ratio will be low for a compound for which avidin has considerable affinity and high for a compound that does not combine readily with avidin. For these studies a biotin-requiring microorganism that is incapable of responding to biocytin as such must be employed.

Experimental. The methods employed in this study were those commonly used in microbiological assays with lactic acid bacteria. *Lactobacillus arabinosus* was used as the assay organism in conjunction with a previously described medium(4). The extent of bacterial growth was determined turbidimetrically after 24-36 hours of incubation at 30°C.

Three different sources of avidin were employed in this study, (a) sterile egg white, (b) a commercial concentrate containing 50 units of activity per gram (SMACO), and (c) a highly purified preparation (kindly supplied by Dr. Fraenkel-Conrat of the Western Regional Research Laboratory.) Sterilization of avidin solutions by filtration through fritted glass filters resulted in unpredictable adsorption of activity. Finally it was found satisfactory to weigh or to pipette out each avidin preparation into sterile water and to use with aseptic technic aliquots of each solution.

Combinability studies were carried out in the microbiological assay medium after autoclaving and just prior to seeding by the addition of avidin to tubes containing biotin or a combination of biotin and biocytin (Table I).

Separate studies have shown that the sample of crystalline biocytin(1) used in these studies had no demonstrable biotin activity either alone (up to 0.5 γ/tube) or in combination with an amount of biotin (0.001 γ/tube) promoting one-half maximal growth of *Lactobacillus arabinosus.*

Results and discussion. Typical data required for the calculation of the affinity ratio of avidin for biocytin are summarized in Table I. These data were obtained using the commercial concentrate as the avidin source.

It is not always possible to use in the combinability phase of an experiment (tubes 12-19) an amount of avidin that combines exactly with the level of biotin employed. Indeed for all practical purposes it is not essential that the two be used in exactly stoichiometric amounts. In this experiment biotin was in slight excess. Prior to further consideration of the data the biotin added in slight excess, that is the biotin with which

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2. Wright, L. D., and Skeggs, H. R., *Arch. Biochem.*, 1947, v12, 27.

3. Wright, L. D., Skeggs, H. R., and Cresson, E. L., PROC. SOC. EXP. BIOL. AND MED., 1947, v64, 150.

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TABLE I.
Protocol for Avidin Combinability Studies.

Tube No.	Biotin, γ/tube	Biocytin, γ/tube	Avidin, γ/tube	Turbidity
1	0			52
2	.00025			115
3	.00050			170
4	.00075			204
5	.00100			248
6	.00150			294
7	.00250			370
8	.0025		10	330
9	.0025		25	250
10	.0025		50	70
11	.0025		100	67
12	.0025	.0025	50	197
13	.0025	.0050	50	254
14	.0025	.0075	50	272
15	.0025	.0125	50	300
16	.0025	.025	50	325
17	.0025	.050	50	345
18	.0025	.075	50	360
19	.0025	.125	50	360

50γ of avidin concentrate did not quite combine (tube 10) is subtracted from that found present in the various mixtures of avidin, biotin and biocytin (tubes 12-19). After subtraction of the biotin in excess of the avidin equivalence, the percentage of added biotin available for growth of *Lactobacillus arabinosus* (equivalent to the biocytin combined in the avidin) is plotted as a function of added biocytin. A curve illustrated by Fig. 1 thus is obtained. By definition the affinity ratio is the ratio of the concentration of biocytin to biotin at which one-half (50%) of the biotin remains free and available for the growth of the organism. Thus in this experiment the level of biocytin required to attain this condition is approximately 0.0070γ/tube (abscissa scale). The total concentration of biotin is approximately 0.00245γ/tube (ordinate scale) after correction for the biotin used in excess of that combining with 50γ of the avidin concentrate employed. The affinity ratio in this experiment is about 3. The affinity ratios obtained were independent of the avidin source used. In 5 experiments employing avidin from 3 sources described an average affinity ratio of 4.1, range 2.9-5.0, was obtained. Thus avidin has a greater affinity for biotin than for biocytin.

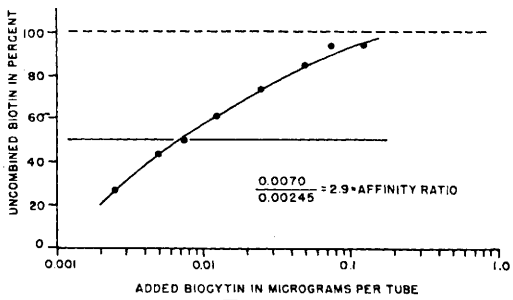

FIG. 1.
The relative affinity of avidin for biotin and biocytin.

Summary. Avidin has a greater affinity for biotin than for biocytin. The affinity ratio for biocytin, as previously defined, is about 4.

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