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Yeast-Growth-Promoting Effect of Diaminocarboxylic Acid Derived from Biotin.*

VINCENT DU VIGNEAUD, KARL DITTMER, KLAUS HOFMANN AND DONALD B. MELVILLE.

From the Department of Biochemistry, Cornell University Medical College, New York City.

In a previous report in which it was shown that biotin is a cyclic urea derivative, the question was raised whether the urea structure is of significance in the affinity of biotin for avidin.[1] Some evidence in favor of this possibility is afforded by the results we have obtained in a study of the yeast-growth-stimulating activity, both in the presence and absence of avidin, of the diaminocarboxylic acid derived from biotin. In the formation of the diaminocarboxylic acid the urea ring of biotin is hydrolyzed with strong $Ba(OH)_2$. Under these conditions the rest of the molecule remains intact as demonstrated by the fact that treatment of the diaminocarboxylic acid with phosgene yields biotin identical with the original starting material.[1]

The yeast-growth-stimulating activity of the diaminocarboxylic acid sulfate prepared as previously described[2] was determined by the assay method described by Snell, Eakin and Williams[3] with minor modifications. On a molecular basis the carefully purified diaminocarboxylic acid sulfate was found to have approximately 10% of the activity of biotin. The activities of the two compounds were compared at the levels which produced half-maximum growth. Repeated recrystallization of the diaminocarboxylic acid sulfate did not change its activity.

The activity of the diaminocarboxylic acid may be due either to an intrinsic activity of the molecule or to the conversion of the compound to biotin within the yeast cell. Since the full yeast-growth activity and optical rotation of biotin can be restored by again forming the urea ring by treatment of the diaminocarboxylic acid with phosgene, the lower yeast-growth activity of the diaminocarboxylic

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1 Melville, D. B., Hofmann, K., and du Vigneaud, V., *Science*, 1941, **94**, 308.

2 Hofmann, K., Melville, D. B., and du Vigneaud, V., *J. Biol. Chem.*, 1941, **141**, 207.

3 Snell, E. E., Eakin, R. E., and Williams, R. J., *J. Am. Chem. Soc.*, 1940, **62**, 175.

acid is not due to racemization in the preparation of this compound, but must be due to the absence of the urea ring. This strongly suggests the importance of the urea ring to the high degree of growth-stimulating activity possessed by biotin.

Although avidin can completely inhibit the activity of biotin, it was found that it does not affect the activity of the diaminocarboxylic acid. The purified avidin was prepared by the method of Eakin, Snell and Williams.[4] 0.35 γ of our avidin concentrate was required to completely inhibit the amount of biotin, 7×10^{-4} γ, which produced half-maximum growth of the yeast culture. The same amount of avidin had no effect on the activity of the diaminocarboxylic acid. Increasing amounts up to 100 γ of our avidin preparation did not affect the growth-stimulating properties of the diaminocarboxylic acid.

The lack of inhibition of the diaminocarboxylic acid by avidin demonstrates that the activity of the diaminocarboxylic acid could not be due to the presence of any unchanged biotin. These results lead to the inference that the urea ring is vital for the effect of avidin on biotin; for under the same conditions, as soon as the urea ring is opened, avidin does not affect the growth-stimulating property of the new compound. If for its yeast-growth-stimulating action the diaminocarboxylic acid is converted to biotin, this conversion evidently takes place within the cell where the avidin cannot counteract the biotin as it is formed.

Summary. The diaminocarboxylic acid resulting from the hydrolysis of the urea ring of biotin is capable of stimulating the growth of yeast in a biotin-free medium. The compound possesses about 10% of the activity of biotin. The yeast-growth-promoting activity of the diaminocarboxylic acid is not inhibited by avidin.

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[4] Eakin, R. E., Snell, E. E., and Williams, R. J., *J. Biol. Chem.*, 1941, **140**, 535.