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Prodigiosin synthesis in *Serratia marcescens*: isolation of a pyrrole-containing precursor*

Serratia marcescens produces a distinctive red pigment, prodigiosin, which has been characterized[1] as a tripyrrylmethene. Evidence has recently been obtained by RIZKI[2] and by WILLIAMS AND GREEN[3] that some stable prodigiosin-deficient mutants of this species accumulate substances capable of causing the appearance of pigment in certain other mutants impaired in prodigiosin synthesis. A pair of such mutants, not previously reported to exhibit cross-feeding, has been examined in some detail, since one of the two mutants (9-3-3) was found to excrete a relatively stable substance that permitted pigment formation in the other (W-1). The excreted substance has now been isolated in pure crystalline form; the progress of the isolation was followed by bioassay with strain W-1. This assay detects about 0.1 μg of the pure material.

For the isolation of the excreted substance, strain 9-3-3 was grown at 30° for four days in aerated peptone-glycerol medium[4]. The culture was centrifuged, and the supernatant liquid was extracted with chloroform. The chloroform layer was washed successively with 1 *N* hydrochloric acid, 1 *N* sodium hydroxide, and distilled water, and was then taken to dryness *in vacuo*. The residue was taken up in hexane (in which the active substance is sparingly soluble) and transferred to an alumina (Fisher Scientific Co., for chromatographic analysis) column. The latter was washed successively with benzene, and benzene-ether, and the active substance was then eluted with ether and ether-chloroform. After recrystallization from ethanol, the substance was obtained as colorless needles that do not melt at 250°, but decompose on further heating. The yield was about 1 mg per liter of culture. Elementary analysis agrees with the formula $C_{10}H_{10}O_2N_2$ (calculated: C, 63.2; H, 5.3; O, 16.8; N, 14.7; CH_3O, 16.3; found: C, 63.0; H, 5.3; O, 17.0; N, 14.7; CH_3O, 16.6)**. The isolated substance has neither pronounced basic nor pronounced acidic properties. It is sparingly soluble in such solvents as ethanol, chloroform, ethyl ether, benzene, or tetrahydrofuran; it is very sparingly soluble in water. The substance absorbs strongly in the ultra-violet; in ethanol solution it gives absorption maxima at 363 and 254 mμ with molar extinction coefficients of about $3.5 \cdot 10^4$ and $1.3 \cdot 10^4$, respectively. It rapidly forms a red color at 25° with *p*-dimethylaminobenzaldehyde in ethanolic hydrochloric acid; therefore, it presumably contains a pyrrole ring with a free alpha-position.

The excretion of the isolated material by one mutant strain and its utilization by another indicate that this compound is an intermediate in the synthesis of prodigiosin. This indication was supported by tracer experiments. Strain 9-3-3 was cultivated in the usual medium supplemented with glycine-2-^{14}C (*cf.* HUBBARD AND RIMINGTON[5]). Crystalline material was isolated as

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** The value for oxygen was obtained by direct analysis. Molecular weight determinations are complicated by the low solubility of the substance in common solvents; the formula given agrees with one methoxyl group per formula weight. This formula weight was used in the computation of the molar extinction coefficients and the specific activity of the substance. Microanalyses were performed by the Schwarzkopf Microanalytical Laboratory, by the Microchemical Laboratory of New York University, and by Mr. STANLEY MILLS.

described above and found to be radioactive. It was supplied to strain W-1, and the red pigment consequently formed by the organisms was extracted by a modification of the method of WREDE AND HETTCHE[6] and was then chromatographed on paper. The paper was pretreated with propylene glycol, and the pigment was applied in toluene solution; chloroform was used as the mobile phase (*cf.* ZAFFARONI AND BURTON[7]). The pigment, which had the same R_F as authentic prodigiosin, was eluted with 50% aqueous methanol and rechromatographed on paper with hexane-glacial acetic acid (9:1). Upon elution, the red pigment showed the same absorption spectrum in the visible region as did authentic prodigiosin. These results indicate that the major pigment produced by strain W-1 in the presence of the isolated substance is indeed prodigiosin. Moreover, the prodigiosin thus formed was found to be labeled, its specific activity being equal, within experimental error, to that of the labeled material supplied to strain W-1. It is therefore concluded that the substance isolated from cultures of strain 9-3-3 is in fact a precursor of prodigiosin.

Strain 9-3-3 was generously supplied by Dr. M. I. BUNTING and strain W-1 by Dr. M. T. M. RIZKI. A sample of authentic prodigiosin was kindly provided by Mr. J. E. MCKEON. Valuable discussions with Drs. D. M. BONNER, M. I. BUNTING, and M. T. M. RIZKI are gratefully acknowledged.

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The adenosine deaminase of crustaceans*

DUCHATEAU, FLORKIN AND FRAPPEZ[1–4] have reported that invertebrates contain adenase but no adenosine deaminase and that vertebrates contain adenosine deaminase but no adenase. FLORKIN[4] has concluded "The appearance of an enzymatic system for the deamination of nucleosides is a biochemical characteristic of vertebrates in contrast to invertebrates". On the other hand, WAGNER AND MITCHELL[5] have reported adenosine deaminase in the larva of *Drosophila melanogaster* and LENNOX[6] has found adenosine deaminase and guanase in blowfly larva. The present report deals with the demonstration of adenosine deaminase in two crustaceans, a lobster (*Homarus americanus*) and a local fresh water crayfish (unidentified species). We have been unable to detect adenase in extracts of the hepatopancreases of either of these crustaceans. The lobster hepatopancreas is a convenient source of adenosine deaminase that is relatively free of other enzymes of purine metabolism.

The animals were killed by decapitation, the hepatopancreases removed immediately and homogenized with five volume of 0.1 ionic strength pH 7.0 phosphate buffer in a glass homogenizer. The extraction and all subsequent steps were carried out near 0° C. The homogenates were centrifuged for an hour at 20,000 × *g* and the clear supernatants were used in tests for the various enzyme activities. An extract of lobster hepatopancreas was fractionated with ammonium sulfate and most of the adenosine deaminase precipitated between 0.5 and 0.7 saturation. Acetone powders of the hepatopancreases were prepared; these and the fresh tissues were extracted with pH 9.5 borate buffer for 20 min at 40° C[7] to determine if uricase could be removed. Enzyme activities were measured spectrophotometrically with the following substrates and by methods indicated in the references: adenosine deaminase, adenine deoxyriboside deaminase, adenosine-3′-phosphate and adenosine-5′-phosphate deaminases, xanthine oxidase, nucleoside phosphorylase, and uricase[8]; adenase[9]; guanase, guanosine and guanine deoxyriboside deaminases[10]; cytosine deaminase[11]; purine-purine transribosidase and purine-purine transdeoxyribosidase[12].

The positive findings are listed in Table I. Adenosine deaminase and a very weak guanase activity were found in the crayfish hepatopancreas extracts; adenosine deaminase, guanase, and

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