RACEMIZATION OF LYSINE BY A PSEUDOMONAD

PHILIP S. THAYER

Arthur D. Little, Inc., Cambridge, Massachusetts

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The recent paper by Huang and Davisson (J. Bacteriol., 76, 495, 1958) on the distribution of lysine racemase in bacteria reports its occurrence in only a few species of Escherichia and Proteus. Various other bacteria including 22 strains of various genera of the Pseudomonadaeae did not possess such activity. I wish to report evidence for the occurrence of lysine racemase activity in a Pseudomonas sp., which produced the fluorescent pigment typical of many such isolates from soil enrichment cultures. This work was done at the University of California at Los Angeles in 1953 and 1954 but has not been reported previously because circumstances prohibited the completion of the larger planned study on the metabolism of lysine.

The specific point which would seem to explain the occurrence of the enzyme in the strain utilized is that it was isolated, by enrichment techniques, for its ability to grow on DL-lysine as the only source of both carbon and nitrogen. Another bacterium, highly pleomorphic and tentatively identified as a Corynebacterium sp., was isolated during the same sequence of enrichments but did not possess lysine racemase, nor could it metabolize D-lysine oxidatively, as measured in a Warburg apparatus. With this strain, oxygen uptake on DL-lysine was approximately one-half that observed with an equivalent amount of the L-isomer, whereas cells of the pseudomonad oxidized both L- and DL-lysine to an equal extent.

In both bacteria, the ability to oxidize lysine was inducible. Succinate-grown cells showed a typical adaptive lag in oxygen uptake in the presence of lysine. Dinitrophenol prevented this induction of the oxidative activity.

Preliminary evidence for the presence of a racemase in lysine-grown cells of the pseudomonad was obtained in several ways: (a) the addition of freeze-dried pseudomonads (which did not oxidize lysine) to suspensions of fresh corynebacteria allowed the complete oxidation of DL-lysine, i.e., with the same total oxygen uptake as on the equivalent amount of L-lysine; (b) freeze-dried pseudomonads converted L-lysine to a form only one-half oxidized by the corynebacteria; and (c) most definitively, cell-free extracts of lysine-grown cells of the pseudomonad allowed the complete oxidative deamination of all of the DL-amino acid by the L-amino acid oxidase of Neurospora crassa (Thayer and Horowitz, J. Biol. Chem., 192, 755, 1951). Data on the last type of experiment are presented in table 1. The doubling of the total oxygen uptake on DL-lysine, as illustrated there, could also be effected as a two-step process, with the racemase preparation added after cessation of oxygen uptake.

TABLE 1
Oxidation of L- and DL-lysine by L-amino acid oxidase: effect of addition of pseudomonad racemase

Racemase preparation. Cells grown on 0.3 per cent L-lysine medium (plus 0.05 per cent MgSO4 and 0.2 m phosphate, pH 6.8); 10.8 g wet cells disintegrated in Raytheon 9 ke magnetostriction oscillator, fraction precipitated between 0.25 and 0.6 saturated ammonium sulfate dissolved in 25 ml 0.01 m phosphate, pH 6, and stored frozen until used. Three-tenths ml per vessel; preincubated 2 hr with lysine solution before addition to L-amino acid oxidase in main compartment.

L-Amino acid oxidase. Isolated from Neurospora culture medium by ammonium sulfate precipitation. One and five-tenths ml per vessel in 0.1 m phosphate, pH 6.

Gas phase. O2; temperature, 28 C; KOH in center well of Warburg vessel.

<table>
<thead>
<tr>
<th>Substrate (8 μmoles)</th>
<th>Racemase</th>
<th>Net O2 Uptake (μL)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-Lysine</td>
<td>Absent</td>
<td>0</td>
</tr>
<tr>
<td>DL-Lysine</td>
<td>Absent</td>
<td>44</td>
</tr>
<tr>
<td>L-Lysine</td>
<td>Absent</td>
<td>88</td>
</tr>
<tr>
<td>D-Lysine</td>
<td>Present</td>
<td>87</td>
</tr>
<tr>
<td>DL-Lysine</td>
<td>Present</td>
<td>85</td>
</tr>
<tr>
<td>L-Lysine</td>
<td>Present</td>
<td>84</td>
</tr>
</tbody>
</table>

* Theoretical (in presence of catalase) = 89 μL/8 μmoles L-lysine.
uptake had indicated complete oxidation of the L-isomer that was originally present.

Similar L-amino acid oxidase experiments showed no racemase activity for the racemic forms of methionine, alanine, leucine, and histidine in the cell-free extract of lysine-grown pseudomonads. A suggestion of ability to racemize ornithine was observed. In preliminary studies of the induction of the ability of this pseudomonad to metabolize lysine, and to form the induced racemase, it was found that either ornithine or lysine as a growth substrate would induce, at least partially, the ability to metabolize the other. Freeze-dried cells from pseudomonad cultures grown on ornithine did have the ability to convert n-lysine to a form completely oxidized by the corynebacteria, and L-lysine to a partially inert form. It is thus possible that the same enzyme could be responsible for the racemization of lysine and ornithine. Cells grown on other amino acids showed this ability to a much lesser degree, and those grown on succinate plus ammonium nitrogen were devoid of it. It is therefore not clear whether a specific lysine racemase is involved, or whether coupled transamination reactions as suggested by Thorne et al. (J. Bacteriol., 69, 357 and 70, 420, 1951) are involved. However, the fact that the racemization occurs in ammonium sulfate precipitated cell-free preparations, without the addition of a co-substrate, strongly indicates that a single racemizing enzyme is involved.

DETECTION OF DIPHOSPHOPYRIDINE NUCLEOTIDASE-FORMING STREPTOCOCCI

ALAN W. BERNHEIMER

Department of Microbiology, New York University College of Medicine, New York, New York

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Among streptococci belonging to groups A, C, and G, some, but not all, strains produce an extracellular enzyme that catalyzes the cleavage of diphosphopyridine nucleotide (DPN) at the nicotinamide-ribose linkage (Carlson, Kellner, Bernheimer, and Freeman, J. Exptl. Med., 106, 15, 1957; Bernheimer, Lazarides, and Wilson, J. Exptl. Med., 106, 27, 1957). Among group A streptococci the capacity to form this enzyme is correlated with the serological type of streptococcus as determined by the antigenically specific M protein formed (Lazarides and Bernheimer, J. Bacteriol., 74, 412, 1957). In the course of our studies it became desirable to develop a method that would expedite the differentiation of colonies that produce diphosphopyridine nucleotidase from colonies failing to do so.

Agar plates containing DPN are prepared as follows: 1.8 ml of a 1 per cent solution of DPN, previously sterilized by passing through an ultrafine fritted glass filter, and 1.7 ml sterile horse serum are aseptically mixed with 15 ml melted sterile meat-infusion agar containing 1 per cent neoepetone and previously brought to 45 C. The mixture is poured into a flat-bottom petri dish, streaked, and incubated for 16 hr at 37 C. In most of our experiments, colonies on plates containing no DPN have been replicated to DPN-containing plates by printing from velvet (Lederberg and Lederberg, J. Bacteriol. 63, 399, 1952) and under these conditions 8 hr incubation is sufficient for elaboration of enzyme.

The plates are sprayed with m NaCN, and after 5 to 10 min are removed to the darkroom where ultraviolet contact-prints are made using the plate as the negative. Since cyanide reacts with the quaternary nitrogen form of DPN to yield an addition product having an absorption maximum at 325 mμ (Colowick, Kaplan, and Cioti, J. Biol. Chem., 191, 473, 1951) the background agar containing unhydrolyzed DPN becomes relatively opaque to ultraviolet light of this wave length while areas of agar in which the DPN has been split remain ultraviolet-transmissible. An ultraviolet source having a suitable emission spectrum (Thiers and Vallee, Ann. N. Y.