62. An Enzymic Transcarboligation

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In their recent communication on the biochemical rôle of acetoin-diacetyl system, Akabori and Shimazu¹⁾ supposed that the enzymic formation of acetoin and acetic acid from diacetyl and acetaldehyde, which is apparently an oxido-reduction, is actually a transcarboligation, i. e. an exchange reaction between an acetyl group of diacetyl and acetaldehyde molecule as follows :

The purpose of the present work is to confirm the supposed mechanism using C¹⁴ labelled acetaldehyde and unlabelled diacetyl as substrates.

Enzyme preparation : Fresh pig heart mince was homogenized in a β -glycerophosphate buffer by a glass homogenizer and centrifuged. The cloudy supernatant was brought to pH 4.6 by adding 10% acetic acid. The precipitate thereby formed was centrifuged down and suspended in phosphate buffer (pH 7.4). This enzyme preparation has the diacetyl-mutase²⁾ activity dismutating diacetyl to acetoin and acetic acid in the anaerobic condition in the presence of cocarboxylase and Mn⁺⁺.

Diacetyl : Commercial diacetyl was purified by distillation and the fraction boiling at 87.5–88° was used.

Acetaldehyde-1,2-C¹⁴ : Acetylene-C¹⁴ was evolved from barium carbide-C¹⁴, converted to acetaldehyde using mercuric sulfate as catalyser and purified by distillation. 10,266 c.p.m./mM.

Experiments

5 mM of unlabelled diacetyl and 5.11 mM of acetaldehyde-C¹⁴ were added to 100 ml of the enzyme preparation containing Mn⁺⁺ and cocarboxylase and incubated at 37.5°, air in the gas phase being replaced by nitrogen. After 4.5 hours incubation the reaction mixture was acidified by adding 3N H₂SO₄, heated to 60°, unchanged diacetyl and acetaldehyde were removed by aeration and finally distilled with FeCl₃. Diacetyl in the distillate was precipitated as Ni-dimethylglyoxime by usual method. The precipitate was collected

and its radioactivity was measured by a Geiger-Müller end window counter. In the control experiment labelled acetaldehyde only was incubated with the same enzyme preparation.

In the both experiments reaction vessels contained 100 ml of enzyme preparations, 2.5 mg of cocarboxylase, 10 mg of MnCl_2 and the substrates as shown in the following table.

Substrate	Acetoin formed	Total activity	Specific activity
I. Acetaldehyde C^{14} 5.11 mM	1.57 mg	11,773 c.p.m.	c.p.m./mM 7,500
Diacetyl 5.00			
II. Acetaldehyde C^{14} 5.11	0.29	5,635	19,431

It is obvious from the above figures that the total yield and the total activity of acetoin in exp. I is much higher as compared to the control experiment. From these results it could be concluded that the larger part of acetoin in the exp. I has been produced not by the oxido-reduction but by the transcarbolication.

References

- 1) S. Akabori and Y. Shimazu: Proc. Japan Acad., **28**, 36 (1952).
- 2) D. E. Green, P. K. Stumpf, and K. Zarudnaya: J. Biol. Chem., **167**, 811 (1947).