STUDIES ON THE FORMATION OF BIOTIN FROM DESTHIOBIOTIN AND SULFATE IN *SACCHAROMYCES CEREVISIAE* I. ON BIOTIN FORMATION FROM DESTHIOBIOTIN BY WASHED CELL SUSPENSION OF YEAST

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It has been known that pimelic acid (1), pelargonic acid derivatives (2) and desthiobiotin are intermediates in the biosynthesis of biotin. The previous investigations of this biosynthesis have dealt almost exclusively with the carbon source. The present investigation was undertaken to elucidate the sulfur source. Dittmer *et al.* (3) reported that resting yeast cells did not convert any measurable amount of desthiobiotin to biotin. Therefore the optimal conditions for maximal biotin formation by washed cells were studied by the authors, and the results are reported in this paper.

EXPERIMENTAL

1. Yeast

Saccharomyces cerevisiae G.M. ATCC 7752 was used. It was maintained on the slope of the stock medium.

2. Stock Medium

Atkin's medium (4), modified by adding, in 100 ml, 100 μ g of DL-desthiobiotin, 250 μ g of nicotinic acid, 0.1 g of ammonium sulfate and 0.5 g of vitamin-free casein hydrolysate, but no biotin, was used.

3. Growth Medium

The composition of this liquid medium was identical with the stock medium except that it contained a suboptimal amount (20 m μ g per 100 ml) of DL-desthiobiotin and no agar. Fifty ml portions of the medium were placed in 300 ml Erlenmeyer flasks, and sterilized for 5 minutes at 121° and 15 pounds.

4. Washed Cell Suspension

Five loops of *Sacch. cerevisiae* was inoculated from the stock culture in the growth medium which was incubated for 20 to 24 hours at 30° without shaking.

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The cells were harvested by centrifugation, washed three times with saline, and resuspended and diluted with saline to desired turbidity. The suspension contained 10 mg of dry cells per ml.

5. Tests for Biosynthesis of Biotin by Washed Cell Suspension

The reaction system for the biosynthesis of biotin consisted of 2 ml of 0.1 *N* $\text{Na}_2\text{HPO}_4\text{-KH}_2\text{PO}_4$ buffer solution at pH 6.9, 1 ml of 2×10^{-6} *M* DL-desthiobiotin solution, 25 mg of glucose, 2 ml of cell suspension and sufficient water to make 10 ml. The reaction mixture was incubated for 1 hour at 37° without shaking in 125 ml Erlenmeyer flask, and heated in a boiling water bath for 15 minutes to stop the reaction.

6. Determination of Biotin

Total biotin (free and conjugated) was determined after the cells and reaction mixtures were hydrolyzed in 4 *N* H_2SO_4 for 2 hours at 121° and 15 pounds, followed by neutralization with NaOH. Only the free biotin was extracted in 15 minutes at 121° and 15 pounds from the washed cells without acid hydrolysis. The assay was carried out according to the method of Niimura (5) using *Lactobacillus arabinosus*.

RESULTS

1. Effect of the Amount of Biotin Originally Present in Washed Cells

The influence of the amount of desthiobiotin in the growth medium on the amount of biotin in the cells grown in the medium was studied (Table I).

TABLE I.
Effect of the Amount of Biotin Originally Contained in Washed Cells.

DL-Desthiobiotin in growth medium	Biotin in cells before reaction	Biotin produced	Blank ^a
<i>mμg/100 ml</i>	<i>mμg/mg</i>	<i>mμg</i>	<i>mμg</i>
100,000	1.1	5.2	9.6
200	0.2	28.9	0.4
20	0.1	39.6	0.3
5	0.1	32.2	0.1
0	0.1	32.2	0.1

^a Amount of biotin determined after incubation without addition of desthiobiotin

Increasing the amount of desthiobiotin in the growth medium up to 100 μg per 100 ml caused an increase in the intracellular level of biotin, but in the case of less than 100 $\text{m}\mu\text{g}$ of desthiobiotin per 100 ml, the biotin level was kept constant (0.1 $\text{m}\mu\text{g}$ per mg of dry cells), and the cells might be called deficient in biotin.

The biotin-deficient cells formed more biotin in the reaction mixture than the sufficient cells (Table I). Therefore, in the experiments of the biosynthesis of biotin, the cells grown in the medium containing a suboptimal amount of DL-desthiobiotin should be used.

Recently, a controlling system was found to act in this biotin-deficient condition, by which the biosynthesis of biotin was accelerated. This observation was similar

to that by Miyagawa (6) that the yeast cells grown in a thiamine-deficient medium produced the largest amount of thiamine.

2. *Effect of the Age of Cells*

The effect of the age of the cells was studied, because the age of cells is one of the most important factors for the activity. As summarized in Table II,

TABLE II.
Effect of the Age of Cells on Biotin Formation.

Age of cells	Biotin produced
<i>hr</i>	<i>mμg</i>
22	36
46	36
70	39
118	27

the biotin-producing ability of the cells was almost constant from 22 to 70 hours, and it decreased after 70 hours.

3. *Effect of the Addition of Several Sulfur Compounds*

The addition of several sulfur compounds did not increase the production of biotin as shown in Table III. Therefore, the sulfur compounds originally con-

TABLE III.
Additive Effects of Sulfur Compounds on Biotin Formation.

Sulfur compounds (10^{-4} M)	Biotin produced
	<i>mμg</i>
None	36
Na ₂ SO ₄	38
Na ₂ SO ₃	39
Na ₂ S	38
DL-Methionine ^a	33
L-Cysteine	38
DL-Homocysteine ^a	36
L-Glutathione	36
Methylmercaptan	37

^a 2×10^{-4} M

tained in the cells seemed to be enough for the biosynthesis of biotin from desthiobiotin.

4. *Effect of Temperature and pH*

The temperature was shown to have a great effect on the yield of biotin as shown in Table IV. The amounts produced at 30° and 20° were 67 and 22 per cent respectively of that at 37°. The production was kept almost constant within a broad pH range.

5. *Effect of the Concentration of DL-Desthiobiotin*

The effect of the concentration of DL-desthiobiotin in the range from 2×10^{-8} to 5×10^{-6} M was studied, and the optimum concentration was found to be 2×10^{-7}

TABLE IV.
Effect of Temperature

Temperature	Biotin produced
$^{\circ}\text{C}$	$\text{m}\mu\text{g}$
20	8.5
30	25.4
37	38.0

TABLE V.
Effect of the Concentration of DL-Desthiobiotin.

Concentration of DL-desthiobiotin	Biotin produced
M	$\text{m}\mu\text{g}$
10^{-8}	0.2
5×10^{-8}	21.5
10^{-7}	35.9
2×10^{-7}	43.1
10^{-6}	32.9
5×10^{-6}	23.3

TABLE VI.
Stimulatory Effect of Glucose.

Glucose added	Biotin produced
mg	$\text{m}\mu\text{g}$
0	3.9
5	9.5
10	20.3
25	43.1
50	46.1

M as shown in Table VI.

6. *Effect of the Amount of Cells*

The yield of biotin was plotted against the amount of cells in Fig. 1. As the amount of the cells increased, the yield of biotin at first increased proportionally, then gradually approaching to a constant value.

7. *Stimulatory Effect of D-Glucose*

The formation of biotin by washed cells increased markedly, approximately 4 to 10-fold, by the addition of glucose as given in Table VI. Glucose is possibly assumed to be participated as a energy source in the biosynthesis of biotin from the following reasons. Other compounds such as sodium acetate and ethanol could be substituted for glucose under aerobic condition. Further, sufficient supply of air by shaking resulted in the reduction of the amount of glucose necessary for stimulation of biotin formation.

Lichstein (7) suggested that an active transport mechanism was involved in the permeability of *L. arabinosus* to biotin, but it has not yet been known concerning the permeability of *Sacch. cerevisiae* to desthiobiotin. It remains to be settled on this point.

FIG. 1 *Effect of the Amount of Cells*

8. *Effect of the Incubation Time on Biotin Formation*

The amounts of biotin produced by incubation with and without shaking were plotted against the incubation time in Fig. 2. It was found that more biotin was

FIG. 2 *Effect of Incubation Time*

○, addition of 25 mg glucose, without shaking; ×, addition of 25 mg glucose, with shaking; ●, without glucose, without shaking.

produced by incubation without shaking than by shaking for 30 to 60 minutes. The reason remains still obscure.

9. *Distribution of the Synthesized Biotin*

The synthesized biotin was practically restricted in the cells and 87 per cent of the vitamin was present in a bound form.

SUMMARY

1. The best condition for the biosynthesis of biotin from desthiobiotin by the washed cells of *Saccharomyces cerevisiae* was found as follows: The cells cultured in a liquid medium containing a suboptimal amount (20 μg per 100 ml) of DL-desthiobiotin for 1 to 3 days were incubated in the reaction mixture containing DL-desthiobiotin (2×10^{-7} M) and glucose at 37° without shaking.

2. Sulfur necessary for the biosynthesis of biotin seems to be sufficiently present in the cells, because the addition of several sulfur compounds did not increase the formation of biotin. The synthesized vitamin was practically restricted in the cells and 87 per cent of it was present in a bound form.

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REFERENCES

1. du Vigneaud, V., Dittmer, K., Hague, E., and Long, B.; *Science* **96**, 186 (1942).
2. Okumura, S., Tsugawa, R., Tsunoda, T., and Motozaki, S.; *J. Agri. Chem. Soc. (Japan)* **36**, 599 (1962) **36**, 605 (1962).
3. Dittmer, K., Melville, D. B. and du Vigneaud, V.; *Science* **99**, 203 (1944).
4. Atkin, L., Schultz, A. S., Williams, W. L. and Frey, C. N.; *Ind. Eng. Chem. Anal. Ed.* **15**, 141 (1943).
5. Niinura, T.; *Vitamins* **12**, 106 (1957).
6. Miyagawa, K.; *ibid.*, **16** 680 (1959).
7. Lichstein, H. C. and Ferguson, R. B.; *J. Biol. Chem.* **233**, 243 (1958).