and analysed on a Dowex-1-borate column as shown in Fig. 1. Radioactivity was concentrated in the first orcinol-positive peak. Experiments with non-labeled gluconate (100 μmoles in an otherwise identical experiment) gave rise to an orcinol-positive peak in the same position. These peaks were concentrated after being passed through a Dowex-50-hydrogen column, and boric acid was removed as the methanol complex. The resulting syrup showed a violet color by spot test with anthrone, and its orcinol reaction showed a ratio of absorbancy at 670 mμ to that at 540 mμ[5], which suggested the presence of keto-pentose. The autoradiograph of the syrup thus obtained showed the same R_F value as that of ribulose. The product was also subjected to reaction with *o*-nitrophenylhydrazine according to GLATTHAAR's procedure[4]. The resulting hydrazone was fractionated on an alumina column and its radioactivity was counted. The first yellow band eluted with ethyl acetate was *o*-nitrophenylhydrazine. Following that, elution by acetone, *n*-propanol, ethanol, and methanol produced peaks but no radioactivity was detected in them. Radioactivity was eluted with methanol–water (1:1), and this orange peak appeared in the same position as authentic ribulose *o*-nitrophenylhydrazone (m.p. 168°). 10 mg of ribulose *o*-nitrophenylhydrazone was added to the eluate and the specific activity of the crystal was determined during 5 recrystallizations. The weight of the hydrazone was estimated from the molar extinction coefficient ($6.0 \cdot 10^3$) at 432 mμ, and the samples were counted in the state of infinite thinness. The specific radioactivity was found to be almost constant during the recrystallization as follows. 1, 170.1 $\pm$ 17.4; 2, 138.5 $\pm$ 9.1; 3, 166.0 $\pm$ 11.9; 4, 158.3 $\pm$ 10.2; 5, 180.9 $\pm$ 13.4 counts/min/mg. This enzyme also dehydrogenated 6-phosphgluconate[5] slowly, but slight alkaline phosphatase activity was also detected in the preparations used. Further purification of this enzyme is now going on.

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[1] Y. MACHIYAMA, Y. MANO AND N. SHIMAZONO, *Biochim. Biophys. Acta*, 42 (1960) 564.
[2] J. D. SMILEY AND G. ASHWELL, *Federation Proc.*, 19 (1960) 85.
[3] S. ISHIKAWA, *J. Biochem. (Tokyo)*, 46 (1959) 347.
[4] C. GLATTHAAR AND T. REICHSTEIN, *Helv. Chim. Acta*, 18 (1935) 80.
[5] B. L. HORECKER AND P. Z. SMYRNIOTIS, *J. Biol. Chem.*, 193 (1951) 371.

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Selective formation of α-amylase by non-growing cells of *Pseudomonas saccharophila*

Several years ago, RICKENBERG AND LESTER[1] reported that a mutant of *Escherichia coli* produced 5 % as much β-galactosidase when induced under certain non-growing conditions as was produced during logarithmic growth. They suggested that the

enzyme formed in non-growing cells indicated preferential synthesis of β-galactosidase in relation to other cellular proteins. Subsequent investigations by MANDELSTAM[2] and others[3] have demonstrated that a significant fraction (5–6 %/h) of the proteins of non-growing cells of *E. coli* undergoes turnover, and as a result of these studies, it was presumed that the observations of RICKENBERG AND LESTER could still be explained on the basis of a constant "differential rate of synthesis" of β-galactosidase[4]. Similar assumptions could be made on analogous inducible systems in resting yeast cells[5,6].

Recently we reported that *Pseudomonas saccharophila* forms an extracellular α-amylase in phosphate buffer when incubated with starch under conditions where there is no detectable increase in protein. Furthermore, in periods of up to 4 h, such cells produce as much enzyme as do comparable quantities of growing cells[7]. It was also found that this enzyme is formed at a differential rate of 1:1200 in cells growing with starch as the carbon source[8], *i.e.*, α-amylase represents 0.08 % of the total proteins synthesized.

It seemed obvious that α-amylase, if it is formed *de novo*, is not being made at the same differential rate with respect to other proteins in resting cell suspensions as in growing cultures. Evidence has already been presented[8] in this connection, supporting the view that this enzyme is not formed from high-molecular-weight precursors, but rather *de novo*. On the basis of these observations, it seemed certain that the formation of α-amylase in resting cells represents a selective (preferential) synthesis of this protein.

It is the purpose of this communication to give details regarding this contention and to measure the extent of this selectivity. The objective was to introduce a radioactive amino acid into the free amino acid pool[9] of *P. saccharophila* simultaneous with induction and, after a suitable period of time, to compare the radioactivity of the cellular proteins with that of the isolated, purified enzyme.

Growth conditions, requirements for induction, and methods for isolation and purification of the enzyme have been reported elsewhere[10,11]. Labeled aspartate or glutamine were introduced into cells by first subjecting them to a period of starvation (aeration for 4 h at 30° in complete medium, containing 0.2 % sucrose but without a nitrogen source), and then suspending the cells with 0.01 % (final concn.) of the labeled compound in phosphate buffer (0.033 *M*, pH 6.8) plus starch (0.2 % final concn.). Under these conditions, these particular compounds were taken up into the pool more effectively than without prior starvation. When labeled methionine was used, it was simply added (0.03 % final concn.) during induction in buffer. In all cases, cells were harvested and their proteins extracted and counted for radioactivity. The supernatants were treated to obtain purified α-amylase, which was similarly counted.

The results (Table I) reveal that the ratio of labeled amino acid incorporated into enzyme to that into cellular protein is approximately 1:40. An experiment in which cells were grown on starch in the presence of radioactive aspartate is also included and it is evident that the incorporation ratio thus obtained is compatible with the finding that α-amylase represents 0.08 % of the total protein in such cultures.

The results substantiate the contention that α-amylase is selectively formed in non-growing cells of *P. saccharophila*. In contrast to other cases in which preferential synthesis of specific enzymes has been shown[12,13], this case is not accountable for on

TABLE I

INCORPORATION OF LABELED AMINO ACIDS INTO CELLULAR AND α-AMYLASE PROTEINS

Amino acid used	*Medium*	*Incubation period* (min)*	*Total radioactivity*		*Incorporation ratio***
			Amylase counts/min × 10⁻²	*Cell protein counts/min × 10⁻²*	
[^{14}C]-L-aspartic	Buffer	90	300	10400	35
[^{14}C]-L-glutamine	Buffer	90	35.7	1710	48
[^{35}S]-methionine	Buffer	180	13.0	569	44
[^{14}C]-L-aspartic	Complete	18 h	30.3	31400	1040

* Cells induced in buffer showed no increase in total proteins during the periods of incubation. Cells grown in the presence of labeled aspartic acid were derived from a small inoculum and allowed to reach a turbidity which measured 400 in the Klett colorimeter at 540 mμ. At this time, the cells were in the late logarithmic phase.

** Incorporation ratio = counts/min in cellular proteins:counts/min in α-amylase.

the basis of a relief from repression[14]. In this system, the absolute amounts of amylase protein formed by growing and non-growing cells in short-term experiments are comparable. Thus, the selectivity here concerns not the increased rate of formation of one enzyme against the background of a constant synthesis of other cellular proteins, but rather the decreased rate of formation of all cellular proteins against the constant synthesis of α-amylase. These bacteria therefore appear to possess a control mechanism of a more generalized nature than others that have been described[15].

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1 H. V. RICKENBERG AND G. LESTER, *J. Gen. Microbiol.*, 13 (1955) 279.
2 J. MANDELSTAM, *Biochem. J.*, 69 (1958) 110.
3 E. BOREK, L. PONTICORVO AND D. RITTENBERG, *Proc. Natl. Acad. Sci.*, 44 (1958) 369.
4 J. MONOD, G. COHEN-BAZIRE AND M. COHN, *Biochim. Biophys. Acta*, 1 (1951) 585.
5 M. STEPHENSON AND J. YUDKIN, *Biochem. J.*, 30 (1936) 506.
6 S. SPIEGELMAN AND R. DUNN, *J. Gen. Physiol.*, 31 (1948) 153.
7 J. A. SCHIFF, J. M. EISENSTADT AND H. P. KLEIN, *J. Bacteriol.*, 78 (1959) 124.
8 J. M. EISENSTADT AND H. P. KLEIN, *J. Bacteriol.*, 77 (1959) 661.
9 A. MARKOVITZ AND H. P. KLEIN, *J. Bacteriol.*, 70 (1955) 649.
10 A. MARKOVITZ AND H. P. KLEIN, *J. Bacteriol.*, 70 (1955) 641.
11 A. MARKOVITZ, H. P. KLEIN AND E. H. FISCHER, *Biochim. Biophys. Acta*, 19 (1956) 267.
12 L. GORINI AND W. MAAS, in W. D. MCELROY AND B. GLASS, *Chemical Basis of Development*, Johns Hopkins Press, Baltimore, 1958.
13 A. TORRIANI, *Biochim. Biophys. Acta*, 38 (1960) 460.
14 H. J. VOGEL, *Handbuch der Pflanzenphysiologie*, 1959, p. 117.
15 B. MAGASANIK, A. K. MAGASANIK AND F. C. NEIDHARDT, (Ciba Symposium) *Relation of Cell Metabolism*, Little, Brown & Co., Boston, 1959, p. 334.

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