Degradative Pathway of L-Leucine in *Proteus vulgaris*

It was reported by Coon *et al.*¹⁻⁴ that in the metabolic pathway of L-leucine, *isovaleryl* coenzyme A was formed from α -ketoisocaproic acid, a deaminated product of leucine, in the presence of coenzyme A (CoA), etc. I postulate that this reaction involves the following two steps:

In this communication it is reported that a cell-free extract which catalyses the reaction (1) was obtained from *Proteus vulgaris*.

Proteus vulgaris was cultivated at 37° C. for 18 hr. on peptone-broth agar slant. The cells were harvested, washed three times with de-ionized water, and suspended in 2×10^{-2} M phosphate buffer, pH 7.2. The washed cell suspension was disrupted by sonic oscillation in a Kubota vibrator, 10 kc./s., for 10 min. After centrifugation the supernatant was made 100 per cent saturated with respect to ammonium sulphate. The precipitate was dialysed against running water. In some cases, this dialysed protein solution was further fractionated with ammonium sulphate or cold acetone.

α -Ketoisocaproic acid, used in the experiments, was prepared by extracting with acidic ether from the reaction mixture in which L-leucine was deaminated oxidatively by *P. vulgaris*. The melting point of the 2,4-dinitrophenylhydrazone of this keto-acid was 140–143° C. In this ether extract no other compound was detected by paper chromatography.

Carbon dioxide output was determined by the conventional Warburg method. The reaction mixture consisted of the following in a final volume of 2.5 ml.: 1.67×10^{-1} M acetate buffer at pH 5.0, 0.5 ml.; 5×10^{-3} M magnesium sulphate, 0.25 ml.; 2×10^{-1} M α -ketoisocaproate, 0.125 ml.; and enzyme, 1 mgm. protein. This reaction was carried out at 30° C. for 10 min.

The reaction of decarboxylation proceeded in nitrogen gas as well as in air ($Q_{\text{CO}_2}^{\text{N}_2}$ and $Q_{\text{CO}_2}^{\text{Air}}$ values were 1,158 and 1,207, respectively). The optimal pH was 5.0, and optimal substrate concentration was found to be 1×10^{-2} M; half maximal activity was obtained with 1.5×10^{-3} M. Though it is not yet clear whether the reaction requires co-factors, coenzyme A, adenosine triphosphate, diphosphothiamine and Mg^{2+} were not required in the case of this crude enzyme.

Moreover, the reaction products were identified with paper chromatography. Three reaction systems were made as follows: (a) complete system (same as above experiments); (b) control system in which the enzyme was omitted; and (c) control system in which the substrate was omitted. The volume of each reaction mixture was 10 ml. Immediately

after incubation for 40 min. at 30° C., 0.5 ml. of mercuric acetate (5×10^{-1} M) was dropped in the reaction mixture. After the precipitate was discarded, 2,4-dinitrophenylhydrazine solution was added and stored at room temperature overnight. 2,4-Dinitrophenylhydrazone formed was washed thoroughly with hydrochloric acid, and dissolved with ether. The ether solution was used for paper chromatography. In experiment c no precipitate was formed. 2,4-

Dinitrophenylhydrazone formed from experiments a and b and *isovaleroaldehyde* solution were developed with phenol (with 20 per cent water), *isoamyl* alcohol, and *n*-propyl alcohol as solvent, respectively. The results were represented in Table 1.

Table 1

Solvent	R_F value		
	Exp. a	Exp. b	Hydrazone of <i>isovaleroaldehyde</i>
Phenol	1.00	0.81	1.00
<i>isoamyl</i> alcohol	0.92	0.83	0.92
<i>n</i> -Propyl alcohol	0.91	0.82 ~ 0.73	0.94

As shown in Table 1, one spot was obtained in experiment a, and the spot had the same R_F value of the hydrazone of *isovaleroaldehyde*. Furthermore, these spots were eluted from the paper with 15 per cent potassium hydroxide solution and were examined spectrophotometrically. It was concluded that *isovaleroaldehyde* was formed from this reaction system. The results reported in this communication and other experiments in which α -ketoisocaproate was oxidized after vigorous decarboxylation, and reduced 2,6-dichlorophenolindophenol or methylene blue by this enzyme system in the presence of coenzyme A (and adenosine triphosphate and diphosphopyridine nucleotide), indicate that α -ketoisocaproate was metabolized via *isovaleroaldehyde* (reaction (1)), and probably via reaction (2). Attempts at solving the latter reaction are in progress. Further details of this work will be published elsewhere.

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