Reports

Utilization of Exogenous Proline by the Yeast Candida utilis

Abstract. *Utilization of labeled proline by the yeast* Candida utilis *has been studied. Conversion of the labeled material to biochemically related compounds was observed in the metabolic pools in this organism. The kinetic flow of these molecules into protein was observed, and an explanation is proposed in terms of the absolute cellular concentrations and specific activities of the precursors.*

Carbon metabolism in the yeast *Candida utilis* has been described in a series of papers by Cowie and coworkers. Protein synthesis has been studied from a sugar-supplemented inorganic medium which was the sole carbon source for the cells (*1*) and also where the medium was supplemented with amino acids (*2*) and their analogs (*3*).

Synthetic processes in the cells were described in terms of metabolically active "pools" which could be separately extracted. For example, exogenous threonine was accumulated in yeast in an osmotically sensitive "expandable pool" and transferred to an osmotically insensitive "internal pool" which could be extracted in cold trichloroacetic acid (TCA) (*2*). Intermediary amino acid conversions were found to occur in the latter pool, and the products were utilized directly in protein synthesis. The detailed characteristics of these processes have been published previously (*1–3*).

In the course of studies on the mechanism of action of pyrimidine analogs (*4*, *5*) we were led to consider the effects upon the utilization of exogenous proline in the protein synthetic process. Certain features of the metabolic pool model described above did not accurately describe the flow of C^{14}-proline in uninhibited cells. It is the purpose of this report to describe the characteristics of flow of exogenous proline in *C. utilis* in order to develop a model in which the action of a base analog may be studied.

Cultures of *C. utilis* were obtained from the American Type Culture Collection (No. 9226). Details of growth, media, and methods of extraction have been reported previously (*1–5*). Chromatography and autoradiography techniques were those of Abelson *et al.* (*6*). Uniformly labeled C^{14}-L-proline was obtained from the Nuclear Chicago Corp., Chicago, Ill.

Exponentially growing *C. utilis* cells incubated in the presence of C^{14}-L-proline (uniformly labeled) at concentrations from $4 \times 10^{-6}M$ to $5 \times 10^{-2}M$ were found to incorporate the radioactivity and utilize these molecules for protein synthesis. Transfer of labeled cells to nonradioactive media revealed no loss of C^{14} from the cells or removal of the radioactivity from the protein fraction.

No radioactivity could be found in the distilled water extract of the cells, which indicates that the expandable pool of proline differed from that of threonine described previously (*2*). The cold TCA-soluble fraction of cells grown in various concentrations of labeled L-proline was measured for total pool size by the radioactive content. Results of these experiments are summarized in Fig. 1. The size of the pool as a function of external concentration was similar to that found earlier with phenylalanine, parafluorophenylalanine (*3*), and threonine (*2*) under conditions where both the expandable and internal pools were extracted simultaneously. Both kinds of pools of proline may therefore be found in *C. utilis*, although they cannot be separately extracted by the methods used in the case of threonine.

Both the metabolic pool and the protein were extracted from cells grown in different concentrations of C^{14}-L-proline. After hydrolysis of the protein, both pool and protein fractions were studied by two-dimensional chromatography and autoradiography of the chromatograms. At very low exogenous proline concentrations (carrier-free, $4 \times 10^{-6}M$) both pool and protein contained C^{14} only in the proline residue. At higher external concentrations, however, pool radioactivity was found in spots corresponding to proline, arginine, glutamic acid, ornithine, and citrulline. After very short times of exposure to labeled material, the pool radioactivity was entirely within the proline spot, but within one generation time the pool had reached steady state. This corresponds to (approximately) 15 percent of the radioactivity in proline, 10 percent in arginine, 50 percent in glutamic acid, and the remaining 25 percent principally in ornithine and citrulline.

When the steady state had been reached, analysis of the protein hydrolysates revealed 60 percent of the label in proline, with arginine and glutamic acid also radioactive. These distributions of pool and protein were found to be independent of external proline concentration from $10^{-5}M$ to $10^{-2}M$.

The explanation for the apparent "increase" in radioactivity from precursor pool proline into protein proline lies in the absolute concentrations of amino acids in pool and protein. As an example, yeast cultures incubated with $10^{-3}M$ C^{14}-L-proline were allowed to grow for several generations. The pool and protein were isolated, total radioactivity was determined, and the samples were prepared for chromatography and radioautography. The total radioactivity of the pool indicated 15.7 μM proline per gram of dry cells. However, auto-

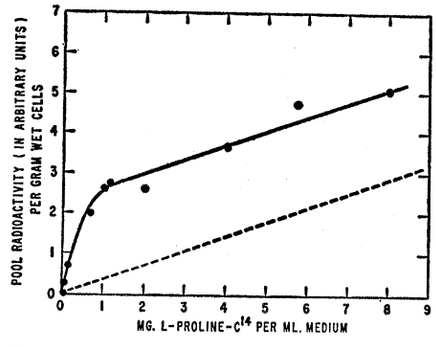


Fig. 1. Accumulation of radioactivity in the pool of *C. utilis* from exogenous supply of C^{14}-L-proline (uniformly labeled). Dashed line shows pool component which is proportional to external concentration.

radiographs showed that only 15 percent of the C^{14} was still chemically in proline, or an absolute pool size of 2.36 μM/g dry cells. The contribution of endogenously produced proline is not greater than 0.17 μM/g dry cells (*5*). The concentrations of arginine and glutamic acid in the pool are 22.7 and 82.5 μM/g dry cells respectively (*5*). If we use the radioactivity distributions mentioned before, then 10,000 count/min of C^{14}/g dry cells in the pool would lead to the specific activities shown in Table 1.

Since these molecules are the precursors of proteins (*1, 2*), the specific activity of the protein residues will approach the pool levels after several generations. At that time, the radioactivity distribution of the protein will be as shown in Table 2.

The distribution of radioactivity in the proteins alone would indicate that most of the added proline remained as that residue. As is seen from this example, very little of the C^{14}-proline taken up by the cells remained in that form. Because of the large pool sizes of the products, the specific activities of arginine and glutamic acid were low and do not contribute as much C^{14} to the protein as the high specific-activity proline.

Accumulation of exogenous proline by cells of *Candida utilis* has been followed. Incorporation of material in the TCA-soluble fraction of the cells has been found to be similar to that already found for other amino acids. These results indicate the existence of two pools within the cell (*2, 3*) termed the "expandable" and "internal" pools. In contrast with the studies on threonine (*2*), the expandable pool of proline was found to be osmotically stable, and therefore could not be removed by extraction in distilled water.

Table 1. Pool levels.

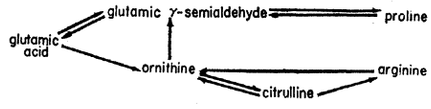
Pool content (μM/g dry cells)	Radioactivity (count/min per gram dry cells)	Specific activity (count/min per μM)
	Proline	
2.36	1500 (15%)	636
	Arginine	
22.7	1000 (10%)	44
	Glutamic acid	
82.5	5000 (50%)	61
	Other compounds	
	2500 (25%)	

Table 2. Protein levels.

Protein content (μM/g dry cells)*	Specific activity (count/min μM)	Total radioactivity		
		Count/min	Calc. (%)	Found (%)
		Proline		
235.5	636	150,000	75	59
		Arginine		
247.5	44	11,000	5	6
		Glutamic acid		
648.8	61	40,000	20	21

* See *5*.

Pathways of proline synthesis in *Candida utilis* have been described by Abelson *et al.* (*6, 7*) in the following schema:



The results of the experiments reported here are in agreement with this plan. Although proline is an "end product" in amino acid biosynthesis (*7*), most of the exogenous proline was converted in the pool to other compounds, principally arginine and glutamic acid. Because of the small pool size of proline, the unconverted radioactivity led to a high specific activity of proline in the pool. However, the very large concentrations of C^{12}-arginine and C^{12}-glutamic acid greatly diluted the radioactive molecules derived from C^{14}-proline, resulting in low specific activities of these compounds.

The amino acid composition of the proteins differs from that of the pool (*1, 2, 5*), requiring different rates of turnover of the individual amino acid pools in protein synthesis. Under conditions of the experiments reported here, a doubling of protein would require the proline pool to turn over 100 times, arginine 11 times, and glutamic acid 8 times. The effects of different specific activities and different rates of turnover are multiplicative, resulting in a protein radioactivity distribution concentrated in proline. An analysis of the radioactivity in protein alone would therefore indicate little conversion of the exogenous labeled material to other products (*6, 7*).

The radioactivity distribution of proteins calculated from known pool sizes and specific activities is in reasonable agreement with the observed values. One source of error lies in the labeled ornithine and citrulline in the internal pool which was ignored in these calculations. If these compounds contribute most of their radioactivity to protein proline, the calculated figures would more closely approach the observed protein values. The principal conversion of ornithine and citrulline to proline has already been shown by Abelson *et al.* (*7*).

It is therefore concluded that two pools of proline may be found in *Candida utilis*, supplying the demands of cellular protein synthesis. Most of the exogenous proline is converted to other amino acids in the glutamic acid family when the external concentration is $10^{-5}M$ or greater. Because the pool concentrations of these compounds vary widely, the specific activity of these precursors also differs greatly. This leads to a distribution of radioactivity in protein which is concentrated in proline, in spite of the fact that most of the exogenous material was converted to other compounds.

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12 December 1962

Xenon Tetrafluoride: Crystal Structure

Abstract. *On the basis of a three-dimensional x-ray analysis, the xenon tetrafluoride molecule in the solid is planar; the approximate symmetry is* D_{4h}. *The average distance between the xenon and the fluorine is 1.92 ± 0.03 Å.*

The structure of xenon tetrafluoride is of great interest as a basis for the theoretical explanation of its existence. The present report describes the crystal structure of this substance.

Xenon tetrafluoride was prepared in these laboratories in the manner described by Claassen *et al.* (*1*). The ma-