DEGRADATION OF TOTAL CELL PROTEIN AT DIFFERENT STAGES OF *SACCHAROMYCES CEREVISIAE* YEAST GROWTH

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1. Introduction

The fact that intracellular degradation of proteins is of great physiological importance is beyond doubt. Little is known, however, about the factors controlling protein catabolism. Interesting studies along this line have been carried out by Saheki and Holzer [1,2], Lenney et al. [3] and by Cabib and Ulane [4]. As a result of these investigations, yeast intracellular proteinases and their inhibitors have been isolated and identified and their localization in the cell established. According to the data of Saheki and Holzer [2], the activity of intracellular proteinases increases in the course of aerobic growth of *S. cerevisiae*. It was unknown, however, whether this increase was accompanied by a corresponding change in the rate of degradation of total cell protein of yeast. The present work fills this gap. It is demonstrated that during the exponential phase of growth as the activities of proteinases A and B increase one may observe increase in the rate of degradation of total cell protein of *S. cerevisiae*. It is shown that degradation of proteins in vivo can be suppressed by specific inhibitors of yeast proteinases.

2. Materials and methods

The *S. cerevisiae* strain used in this work as well as the conditions of growth were described previously [5,6]. The yeast was cultivated in all the experiments at 35°C. At certain moments 40 μCi of [^{14}C]leucine (sp. act. 0.5 mCi/mg) were added to 100 ml of the cell suspension. Special experiments have shown that the radioactivity reached a constant level in as soon as 20 min in the early and late exponential phases, the levels, as calculated per unit of the weight of biomass, being rather close.

After a 2 h incubation with labeled leucine, the cells were centrifuged and then washed five times with the non-labeled growth medium for the excess of label to be removed. The washed sediment was suspended in 100 ml of the non-labeled growth medium and then divided into two equal parts. To one of 50 ml aliquots non-labeled leucine (0.5 mg/ml) was added to suppress reincorporation of the label in the proteins to the other – 0.50 mg of non-labeled leucine per ml and inhibitors of proteinases, PMSF* (1 mM) and pepstatin (0.5 μg/ml). The concentration of non-labeled leucine used did not affect the growth of the yeast. At certain time intervals, 1.5 ml aliquots of the suspension were taken, 3- to 4-fold excess of non-labeled yeast and TCA to a final concentration of 10% were added, and the mixture was incubated for 24 h at room temperature to be then centrifuged. The radioactivity of the supernatant (Fraction I) was assumed as being the indicator of the content of free amino acids in the yeast. The sediment was reprecipitated with TCA from 1 N NaOH as described by Hochberg et al [7] and then incubated at 90°C in 7% TCA for 30 min. After being cooled, the suspension was centrifuged and the sediment was successively extracted by the following solvent systems: 7% TCA – 33% ethanol, acetone, a mixture of 96% ethanol–ether (3:1 v/v,

* *Abbreviations:* PMSF, phenylmethyl sulfonyl fluoride; TCA, trichloroacetic acid.

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30 min at 60°C). The total radioactivity of thus extracted TCA-insoluble components is referred to below as that of fraction II and the radioactivity of the final precipitate (Fraction III) is considered to be the radioactivity of total cell protein. The radioactivity was determined in a 'Mark II' liquid scintillation counter (Nuclear Chicago, USA). The activities of yeast proteinases A (pH 3.7) and B (pH 6.2) were measured with haemoglobin denaturated by acid or by urea and alkali, correspondingly [8]. Increase in the content of TCA-soluble products was measured spectrophotometrically with Folin-Ciocalteu's phenol reagent. Tyrosine was used as a standard. The reagents used were purchased: [^{14}C]leucine from Reanal, phenylmethyl sulfonyl fluoride from Sigma. Pepstatin (microbial peptide inhibitor of acid proteinases [9]) was a generous gift from Professor H. Umezawa.

3. Results and discussion

The rate of degradation of cell proteins is usually determined by following the decrease in the radioactivity level in the protein fraction or the accumulation of the label in the free amino acid fraction by means of pulse-chase technique [10,11]. In the present work the accumulation of label in the TCA-soluble fraction (Fraction I) was followed. The rate of the increment of the label divided by the total radioactivity of the cell protein gave the specific rate of protein degradation.

As seen in fig.1, the content of the label in the TCA-soluble fraction in the early exponential growth phase increased linearly up to 4×10^3 dpm within an hour, and at the end of the phase - up to 8×10^3 dpm within an hour. Under the same conditions the levels of radioactivity of the protein fraction (Fraction III) were constant and equal to 12×10^4 dpm and 8.5×10^4 dpm*, respectively.

These results indicate that the rate of degradation is 3.3% of total cell protein per hour at the early exponential phase of growth and 9.4%** per h at the late exponential phase. This means that as the rate of growth decreases, the rate of degradation

* A certain decrease in the radioactivity level of the total cell protein in the late exponential phase of growth may be explained by the fact that the labeled amino acid in the precursor pool is strongly diluted. It follows from the amino acid analysis data that in the late exponential phase, the content of most individual amino acids, including leucine, increases 2- to 4-fold.

** These values are in accord with the data of other authors for bacterial cells [10,12–14].

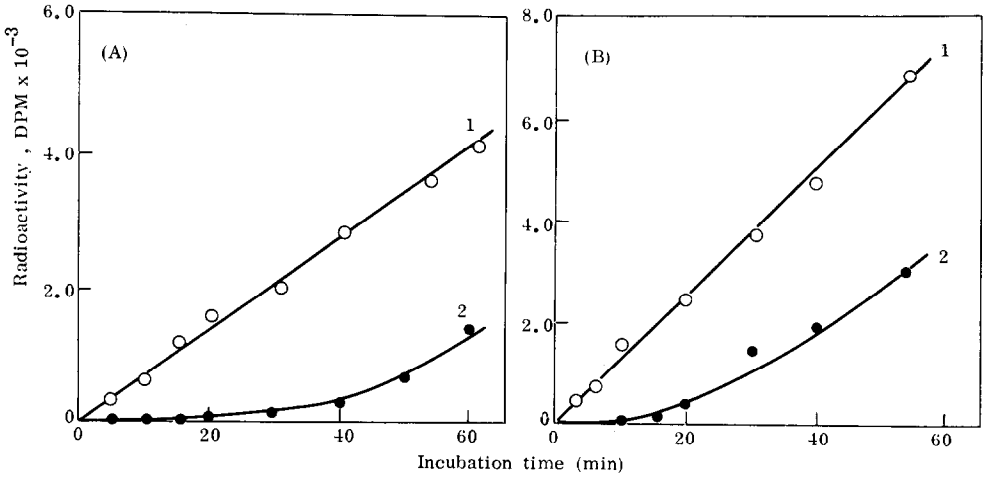

Fig.1. Accumulation of [^{14}C]leucine in the TCA-soluble fraction in the course of incubation of an 8-hour (A) and 15-hour (B) culture of labeled yeast in a nonradioactive medium. *Saccharomyces cerevisiae* yeast labeled in the early and late exponential phases (see Materials and methods) were transferred in a nonradioactive medium containing a 20 000-fold (with respect to the initial concentration of [^{14}C]leucine) excess of nonlabeled leucine and incubated in the absence (1) and in the presence (2) of proteinases inhibitors, PMSF (1 mM) and pepstatin (0.5 μg/ml of the suspension).

Table 1
Activities of proteinases A and B in cell homogenates of yeast harvested at the early and the late exponential phases

	Early exponential phase (8 h of growth)	Late exponential phase (15 h of growth)
	μg Tyrosine per mg of yeast homogenate per 60 min[a]	
Proteinase A	14	28
Proteinase B	37	110

[a]The measurements were made at 35°C.

increases approx. 3-fold. This brings to mind the work of Pine [10] and Goldberg et al. [11] who stated that there was an inverse correlation between the rate of degradation of the total cell protein and the rate of growth of *E. coli*.

According to the experimental data represented in table 1, at the late exponential growth phase, the activities of proteinases A and B† increases 2- to 3-fold, a fact that agrees with the above data on the rates of degradation of total cell protein. Addition of pepstatin and PMSF resulted in inhibition of the proteinases and in decrease in the rate of protein degradation (fig.1). These results should be interpreted as indicating that proteinases A and B are involved in digestion of cell proteins, and the increase in the rate of degradation is associated with the increase in the activities of these proteolytic enzymes. It should be noticed that inhibition of protein degradation took place only during 20–40 min of incubation. Decrease in inhibitory action of PMSF and pepstatin could be explained by some modifications of the inhibitors in the yeast cells.

Estimation of the true rate of degradation was not an easy matter. In fact, TCA-soluble precipitate contains significant amounts of labeled products that can be extracted with different solvents (table 2). The label could have been incorporated in the complex of leucine with tRNA and lipids (see for example [16]), as well as in hydrophobic polypeptides (pooled Fraction II). The latter were present in TCA–ethanol and ethanol–ether extracts. These extracts, when treated with pronase, yielded free leucine and other products of proteolysis. Some of the polypeptides could be considered as being intermediate products of protein degradation. This conclusion may be inferred from the data of table 2: the levels of radioactivity in the TCA–ethanol and

† Ferguson et al. [15] as well as Saheki and Holzer [2] had demonstrated that the activity of a proteolytic enzyme which can inactivate tryptophan-synthase increases in the course of the exponential phase of *S. cerevisiae* growth approx. 4-fold.

Table 2
The radioactivity of the fractions prepared by successive treatment of yeast by TCA and organic solvents

Fractions	Conditions of extraction	Early exponential phase of growth		Late exponential phase of growth	
		Without proteinase inhibitors, DPM	With proteinase inhibitors, DPM	Without proteinase inhibitors, DPM	With proteinase inhibitors, DPM
I	TCA	2000	330	4000	800
II	NaOH; TCA (30 min, 90°C)	11 500	10 500	18 000[a]	16 500
	TCA–ethanol	10 500	12 500	49 000	55 000
	ethanol–ether (30 min, 60°C)	2400	3800	2000	4200
III	Protein residue	120 000[a]	120 000[a]	85 000[a]	85 000[a]

[a]These values did not change in time. The rest of the values are average ones for a 60 min interval.

ethanol–ether fractions are dependent on the presence of porteinase inhibitors (pepstatin and PMSF) in the incubation medium. However, it is quite possible that Fraction II contains also polypeptides which are intermediate products of protein synthesis. Thereby there arises a question of how one is to estimate the specific rate of degradation of cell protein. If the rate of accumulation of the label in Fraction I is referred to the radioactivity of Fraction III, one obtains the values of 3.3% and 9.4% of total cell protein per hour at the early and late exponential phases of growth, respectively (see above). However, the rate of accumulation of the label in the TCA-soluble fraction may be referred to the total radio-activity of Fractions II + III, as Fraction II contains a certain amount of TCA-insoluble polypeptides. In this case the rates of degradation are 2.8% and 5.2% per h, respectively.

One of the possible reasons for the rate of degradation becoming higher is the increase in the activities of proteolytic enzymes due to the lower content of their natural inhibitors in the cell. However, according to the data of Lenney et al. [3] and of Saheki and Holzer [2], the concentration of natural inhibitors increases as does the activity of yeast proteinases. It is not impossible that the increase in the rate of protein degradation in the late exponential phase was the result of starvation of the yeast [11]. In any case, it should be emphasized that there was no deficiency in free amino acids at the late exponential phase. For example, at this phase the content of free leucine in the cells increases 4-fold.

As revealed by Goldberg [17] addition to the growth medium of *E. coli* of amino acid analogs induces formation of abnormal proteins and, as an aftermath, increase in the rate of degradation of the total cell protein. One could therefore suggest that in the late exponential phase of growth the concentration of defective proteins, possessing higher sensitivity to proteolysis, increases; but there exists no evidence for such proteins accumulating in the normal conditions of yeast growth. The point of view that increase in the rate of degradation of total cell protein is the result of intensified synthesis of the proteolytic enzymes of the cell and/or altered membrane permeability of vacuoles where yeast proteinases are concentrated, seems to be the most plausible. It is not yet known how these processes are regulated.

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