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SUBCELLULAR LOCALIZATION AND LEVELS OF AMINOPEPTIDASES AND DIPEPTIDASE IN *SACCHAROMYCES CEREVISIAE*

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Summary

Three aminopeptidases (L-aminoacyl L-peptide hydrolases, EC 3.4.11) and a single dipeptidase (L-aminoacyl L-amino acid hydrolase, EC 3.4.13) are present in homogenates of *Saccharomyces cerevisiae*. Based on differences in substrate specificity and the sensitivity to Zn^{2+} activation, methods were developed that allow the selective assay of these enzymes in crude cell extracts.

Experiments with isolated vacuoles showed that aminopeptidase I is the only yeast peptidase located in the vacuolar compartment. Aminopeptidase II (the other major aminopeptidase of yeast) seems to be an external enzyme, located mainly outside the plasmalemma.

The synthesis of aminopeptidase I is repressed in media containing more than 1% glucose. In the presence of ammonia as the sole nitrogen source its activity is enhanced 3—10-fold when compared to that in cells grown on peptone. In contrast, the levels of aminopeptidase II and dipeptidase are less markedly dependent on growth medium composition.

It is concluded that aminopeptidase II facilitates amino acid uptake by degrading peptides extracellularly, whereas aminopeptidase I is involved in intracellular protein degradation.

Introduction

In recent years it was shown that, in yeast as well as in other microorganisms, proteolytic activities are not only responsible for general protein turnover, but are also involved in regulatory mechanisms that adapt cell metabolism to changing nutritional conditions [1—3]. Two proteinases (proteinase A and B) and a carboxypeptidase (carboxypeptidase Y) have been purified from yeast [2,4—6]. They are predominantly localized in the cell vacuole whereas specific inhibitors are found in the cytosol [7—10]. In addition, a single dipeptidase and at least three different aminopeptidases from yeast are known [11—15].

Dipeptidase and aminopeptidase I (an enzyme described by Johnson [11] for the first time) were purified and characterized in our laboratory [16—20]. Another aminopeptidase was isolated from yeast autolysates by Hata and coworkers [21], it has some features in common with an enzyme (aminopeptidase II) encountered during the purification of aminopeptidase I [18]. Finally, an aminopeptidase with a molecular weight around 30 000 was repeatedly mentioned [21,22]; little is known, however, on the properties of this enzyme.

To the present date, intracellular levels and distribution of yeast exopeptidases were not systematically investigated. Therefore in the present study attempts were made to ascertain, how many peptidases exist in yeast, and to work out methods for their selective assay in crude cell extracts. Employing these procedures, the dependence of the main peptidase activities on the growth state of cells and medium composition was studied. The results are discussed with regard to their function in vivo.

Materials and Methods

Chemicals. If not otherwise specified, the chemicals used were of p.a.-grade from Merck (Darmstadt, G.F.R.). Most of the peptide substrates were products of Serva (Heidelberg, G.F.R.) or Fluka (Buchs, Switzerland) and used without further purification. Yeast nitrogen base (without amino acids) was supplied by Difco (Detroit, U.S.A.), Ficoll is a product of Pharmacia (Uppsala, Sweden).

Ala-Thr-Gly methyl ester was prepared by Dr. E. Schaich in our laboratory [23]. Ile-Phe was synthetized by coupling Boc-Ile-Onp with phenylalanine and removal of the protecting residue with HCl/dioxan.

Enzymes. Aminopeptidase I [18] and dipeptidase [16] were purified from brewer's yeast as described previously. Aminopeptidase II is a by-product of our dipeptidase purification, from which it is separated on DEAE-cellulose. The resulting preparation was not a homogeneous protein but virtually free of aminopeptidase I and dipeptidase activity.

Yeast strains and cultivation conditions. The experiments were performed with *Saccharomyces cerevisiae* S 288cα, a haploid wild-type strain, kindly provided by Dr. D.H. Wolf, Freiburg. Similar results (not shown) were obtained with *S. cerevisiae* NCYC 366 (The Brewing Research Foundation, Nutfield, Surrey, U.K.). Cells were grown at 30°C with shaking either in a 'minimal medium' composed of 2% glucose and 0.67% yeast nitrogen base (without amino acids) or in a 'complex medium' containing 2% glucose, 2% casein peptone and 1% yeast extract. Cell growth was followed by collecting cells from 2 ml medium on glass fiber filters and measuring dry weights after storage at 110°C for 1 h.

Preparation of cell extracts. Cells were homogenized by shaking with glass beads in a model MSK cell homogenizer (B. Braun Melsungen, Melsungen, G.F.R.) for 2 min with solid CO_2 cooling. The crude homogenates were centrifuged at 100 000 × *g* for 1—1.5 h, the resulting supernatant solutions ('$10^5 \times g$ supernatants') were used for further experiments. Yeast vacuoles were isolated as described by Kramer et al. [24]: Cells were converted to spheroplasts with snail gut enzymes ('Helicase', l'Industrie Biologique Française,

Gennevilliers, France). After lysis of spheroplasts in hypotonic medium vacuoles were isolated by flotation in discontinuous Ficoll gradients and taken up in 10 mM citrate buffer (pH 6.5). This solutions ('vacuole lysates') and the 10% Ficoll layers of the gradients ('spheroplast lysates') were used further.

Enzyme assays. The hydrolysis of amino acid *p*-nitroanilides was measured spectrophotometrically. 500 µl buffer was preincubated with 10—50 µl cell extract for 5—10 min, then 100 µl substrate stock solution (4 mM in 5 mM H_2SO_4) was added and the mixture incubated at 40°C for an appropriate period of time. The solution was cleared by a short centrifugation at 3000 × *g* and the absorption change was recorded at 405 nm. The hydrolysis of peptides was followed by amino acid analysis as described elsewhere [18]. If the Zn^{2+} concentration of the assay mixtures was to be maintained at 10^{-9} M, buffers were used which (at pH 7.8) contained 10 mM nitrilotriacetic acid and 1.5 mM Zn^{2+} [25].

Results

Peptidases in yeast homogenates

Peptidase activities in yeast cell extracts were separated by sucrose density gradient centrifugation or gel chromatography on Sepharose 6B. Fig. 1a shows the distribution of peptidases in a gradient loaded with a sample of a freshly prepared '10^5 × *g* supernatant' and run overnight at 100 000 × *g*. An analysis of the same sample on Sepharose 6B is shown in Fig. 2. Four major peptidases are discernible according to molecular weights and substrate specificity characteristics. As is shown in detail below, the results strongly suggest that the enzymes giving rise to peaks 1—3 are identical with or closely related to peptidases previously isolated from commercial brewer's yeast in our laboratory.

Peak 1 sediments at the same rate as aminopeptidase I, which has a sedimentation coefficient as high as 22 S and a molecular weight of 640 000 [19]. Peaks 2 and 3 were not clearly separated by both density gradient centrifugation and gel filtration. However, a comparison of substrate specificities confirms that peaks 2 and 3 are due to different enzymes. The features of the peak 2 enzyme correspond to those of dipeptidase which has a molecular weight of 120 000 and a sedimentation coefficient near 6 S [16]. The molecular weight of peak 3 was estimated by gel filtration on a calibrated Sepharose column. A value of 85 000 resulted which is in close agreement with the molecular weight of aminopeptidase II, an enzyme purified about 50-fold from autolysates of brewer's yeast [26].

Another aminopeptidase (peak 4) appeared as a shoulder in peak 3 (Fig. 1a). It was separated from peak 3 more clearly on Sepharose (Fig. 2). The molecular weight of peak 4 is less than 50 000, its sedimentation coefficient amounts to 3—3.5 S.

Substrate specificities

In Table I some representative data on the substrate specificities of purified aminopeptidase I, aminopeptidase II and dipeptidase are summarized. These data are to be compared with the relative turnover rates observed with peaks 1—3 (Figs. 1a and 2).

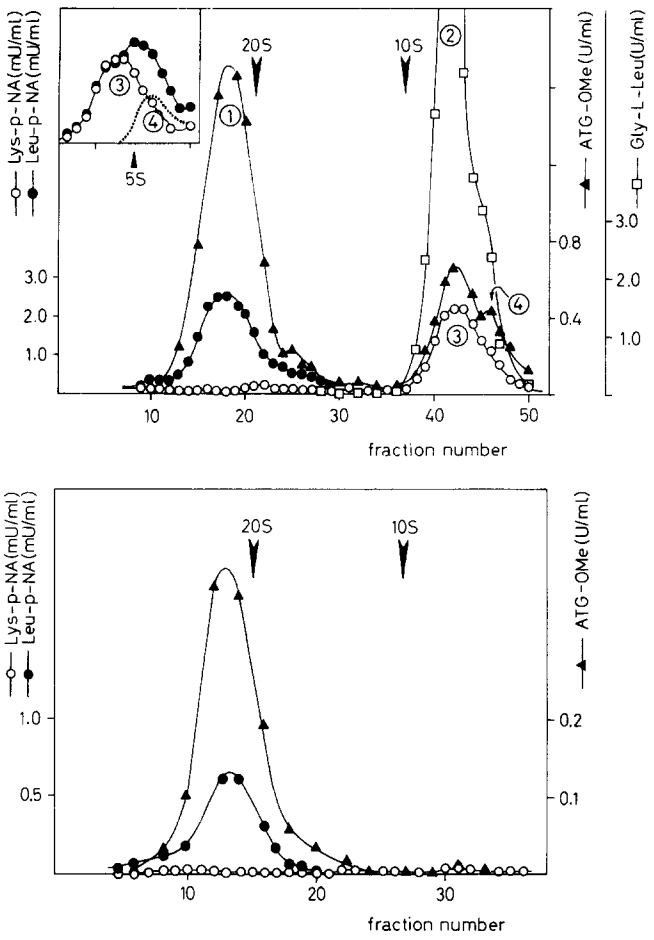

Fig. 1. Separation of yeast peptidases by sucrose density gradient centrifugation. The gradients (5—20% sucrose in 10 mM Tris/succinate buffer (pH 7.3)) were run in an Beckman 41 Ti rotor for 15 h at 30 000 rev./min and fractionated as described previously [19]. The resulting fractions were assayed for activities against: (a) 0.8 mM leucine-*p*-nitroanilide (•) in 0.1 M phosphate/borate buffer (pH 7.8)/ 50 μM Zn^{2+}/0.1 M Cl^-, (b) 0.8 mM lysine-*p*-nitroanilide (○) in 0.1 M phosphate/borate buffer (pH 7.0), (c) 8 mM Gly-L-Leu (□) in 0.1 M Tris-HCl (pH 8.0) and (d) 8 mM L-Ala-L-Thr-Gly methyl ester (▲) in the same buffer as used in a. The bands migrated from the right to the left. The distances travelled are related to sedimentation coefficients in an approximately linear fashion; an abscissa scale in Svedberg units (S) is indicated by arrows. (a) Sample: 200 μl '10^5 × *g* supernatant' from a culture grown for 10 h on 'complex medium', corresponding to 0.5 mg protein. For the sake of clarity, leucine-*p*-nitroanilidase activities of fractions >30 are not shown in the main part, but are depicted in the inset. The dashed line represents the difference of leucine-*p*-nitroanilidase and lysine-*p*-nitroanilidase activities and is to indicate the position of peak 4. Gly-Leu peptidase activities of fractions <30 were negligibly small and have been omitted, too. (b) Sample: 200 μl vacuole lysate from cells grown for 7 h on 'minimal medium', corresponding to about 5 μg protein. Gly-Leu peptidase activities were insignificantly low throughout the gradient and are not shown.

Aminopeptidase I. From Figs. 1 and 2 it is obvious that the relative velocities of peak 1 with the substrates shown (leucine-*p*-nitroanilide, lysine-*p*-nitroanilide, Ala-Thr-Gly-OMe, Gly-Leu) follow the substrate specificity of aminopeptidase I (see also ref. 18). Substrates with charged and/or hydrophilic side chains are hydrolyzed much more slowly than peptides with leucine or other

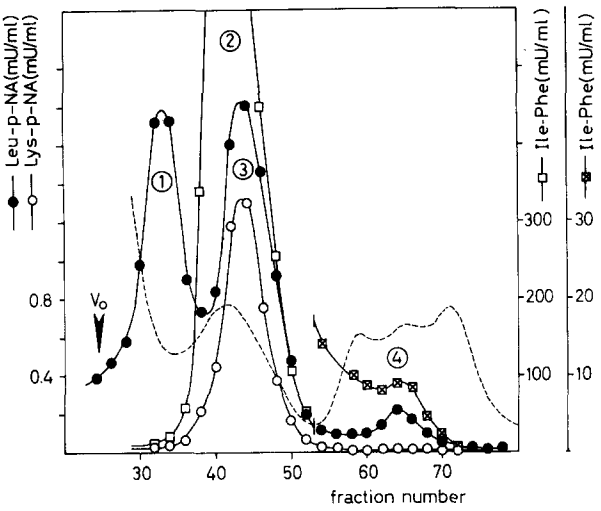

Fig. 2. Gel filtration of a crude yeast homogenate on Sepharose 6B-CL. The column (2 X 50 cm) was equilibrated and eluted with 10 mM Tris/succinate buffer (pH 7.3). Sample: 3 ml '10^5 X *g* supernatant' from a 10 h culture on 'complex medium'. Peptidase activities were measured in 0.1 M phosphate/borate buffer (pH 7.0) with (a) 0.6 mM leucine-*p*-nitroanilide (●), (b) 0.6 mM lysine-*p*-nitroanilide (○) and (c) 7 mM L-Ile-L-Phe (□).

hydrophobic residues in this place. Thus, aminopeptidase I is a typical 'leucine aminopeptidase'.

We have not yet found any selective aminopeptidase I substrates, since all of the peptides that undergo rapid hydrolysis by aminopeptidase I are also

TABLE I

SUBSTRATE SPECIFICITIES OF PURIFIED PEPTIDASES FROM BREWER'S YEAST

Activities are expressed relative to the rate of Leu-Gly hydrolysis = 1. Substrate concentrations were 0.8 mM with amino acid-*p*-nitroanilides and 8 mM with peptides. Enzyme preparations and composition of buffers: Aminopeptidase I, purified according to ref. 18, 0.1 M phosphate/borate buffer (pH 7.8)/0.05 mM Zn^{2+}/0.1 M Cl^-. Aminopeptidase II, partially purified according to ref. 26, 50 mM Tris/HCl (pH 7.6) without Zn^{2+}. Dipeptidase, purified according to ref. 16, 0.1 M Tris-HCl (pH 8.0).

Substrate	Relative activities		
	Aminopeptidase I	Aminopeptidase II	Dipeptidase
L-Leu-Gly	1	1	1
L-Leu-L-Val	0.9	1.8	2.3
L-Leu-L-Leu	0.6	2.3	2.5
Gly-L-Leu	0.02	0.4	4.0
L-Ile-L-Phe	0.03	0.1	1.4
L-Lys-Gly	—	0.65	0.002
L-Lys-*p*-nitroanilide	0.001	0.7	0
L-Leu-*p*-nitroanilide	0.07	1.0	0
L-Ala-*p*-nitroanilide	0.02	0.3	0
Gly-*p*-nitroanilide	0.001	0.06	0
L-Leu amide	0.4	2.1	0
L-Leu-Gly-Gly	4.3	30	0
L-Ala-L-Thr-L-Ala	22	55	0

attacked by aminopeptidase II. However, a rather specific assay of aminopeptidase I in the presence of the other peptidases is feasible if one takes advantage of the fact that aminopeptidase I is strongly activated by Zn^{2+} and Cl^- [18]. Therefore, if the Zn^{2+} concentration is lowered to $1 \cdot 10^{-9}$ M or less, aminopeptidase I is quantitatively inactivated whereas aminopeptidase II is even slightly activated under these conditions. If the activity of cell extracts is determined at high and low levels of Zn^{2+} the difference between the resulting velocities measures aminopeptidase I activity. For the maintenance of Zn^{2+} concentrations around 10^{-9} M the nitrilotriacetic acid/Zn^{2+} metal buffer system described by Cohen and Wilson [25] was used.

Aminopeptidase II. The most conspicuous item in the substrate specificity pattern of both peak 3 and aminopeptidase II is the rapid turnover of lysyl derivatives. Since none of the other yeast peptidases is able to catalyze the splitting of lysine-*p*-nitroanilide at a measurable rate (Fig. 1a) the use of this compound allows a simple and specific assay of aminopeptidase II in crude extracts. Unlike aminopeptidase I aminopeptidase II is not activated by Zn^{2+} or Cl^-.

We have not yet obtained homogeneous aminopeptidase II, the possibility has, therefore, to be excluded that the hydrolysis of lysyl peptides is brought about not by aminopeptidase II itself but by another enzyme in the preparation. For that purpose we have compared the effect of heating on the lysine-*p*-nitroanilidase and leucine-*p*-nitroanilidase activities of aminopeptidase II. The heat stabilities of both activities were the same (Fig. 3). This suggests that they are brought about by a single enzyme.

Dipeptidase. The substrate specificity of dipeptidase has been discussed elsewhere [17]. Only dipeptides composed of L-amino acids are hydrolyzed by the enzyme, Gly-Leu is among its best substrates. If Gly-Leu is used for dipeptidase

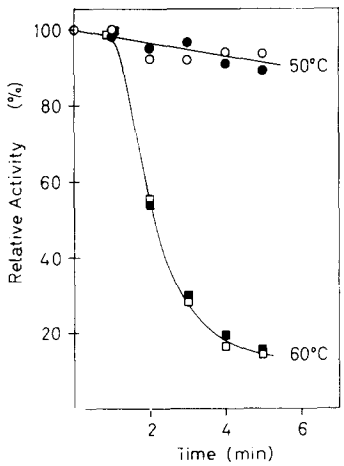

Fig. 3. Effect of heat on aminopeptidase II activities. Ice-cold solutions of aminopeptidase II in phosphate/borate buffer (pH 7.0) were transferred to a water bath held at 50° C or 60° C, respectively. Within the next 5 min samples were removed and rapidly chilled in ice. Then residual activities against leucine-*p*-nitroanilide (■,●) and lysine-*p*-nitroanilide (□,○) were measured as usual and given as percent of the values before heating.

assays with cell extracts the dipeptidase activities of the aminopeptidases may be corrected for. With the yeast strains used, however, the contribution of aminopeptidases to the turnover of Gly-Leu was insignificant when compared to the activity of dipeptidase itself.

Aminopeptidase III. The low molecular weight peak 4 enzyme (aminopeptidase III) is, by an order of magnitude, more active against dipeptides than towards *p*-nitroanilides (Figs. 1a and 2). Ala-Thr-Gly-OMe was also rapidly hydrolyzed. The substrate specificity of aminopeptidase III (the activities of which were low in the yeast strains examined so far) was not yet worked out systematically.

Subcellular localization

Since is known that the proteinases of yeast are enriched in the vacuole [7] we have examined the peptidase contents of vacuole preparations (Fig. 1b). The only activity present was aminopeptidase I, recognizable from its sedimentation behaviour and the ratio of activities towards Ala-Thr-Gly-OMe and amino acid *p*-nitroanilides. Aminopeptidase I is enriched about 20-fold in the vacuole when compared with 'spheroplast lysates' on the basis of specific activity. Significant amounts of aminopeptidase II and dipeptidase were not found in vacuole extracts. It is difficult to ascertain whether the enzyme is exclusively located in the vacuolar compartment, since it is impossible to prevent partial lysis of vacuoles during their separation from spheroplast lysates. Immunological determinations of aminopeptidase I levels in the vacuole indicated that up to 10% of the vacuolar protein may consist of aminopeptidase I (Löffler, H.-G., Frey, J. and Röhm, K.-H., unpublished data).

Aminopeptidase II seems to be (at least partially) localized outside the plasma membrane. With intact yeast cells about 50% of the lysine-*p*-nitroanilidase of homogenates could be measured (Table II). This activity was independent of Zn^{2+} activation and was not secreted by whole cells. Upon digestion of the cell wall it was partially released into the medium. Nevertheless protoplasts showed a higher specific activity than cells. After hypotonic lysis aminopeptidase II activity was not increased when compared to that of intact spheroplasts. Thus, either the interior of the cells is freely accessible to amino acid-*p*-nitroanilides

TABLE II

AMINOPEPTIDASE ACTIVITIES MEASURED WITH WHOLE CELLS AND PROTOPLASTS

Cells (strain NCYC 366) were grown for 3 h on 'complex medium' and, in part, converted to spheroplasts. Untreated cells and spheroplasts were then incubated for 1 h at 30°C with 0.8 mM substrates in 0.1 M sodium phosphate buffer/0.6 M sorbitol (pH 7.0). In addition, spheroplasts were incubated with substrates in phosphate buffer without sorbitol, which induces rapid lysis. Activities are given as munits/10^8 cells (spheroplasts) or as munits in a volume of lysate corresponding to 10^8 spheroplasts.

Substrate	Activity (munits) corresponding to		
	10^8 cells	10^8 spheroplasts	lysate from 10^8 spheroplasts
Lysine-*p*-nitroanilide	1.1	1.9	1.9
Leucine-*p*-nitroanilide	1.0	2.3	2.5

or, most probably, the enzyme is localized in the cell wall. Evidence in favour of the second interpretation was furnished by preliminary results of experiments on the cytochemical demonstration of aminopeptidase II activity (Frey, J. and Röhm, K.-H., unpublished data).

Influence of carbon and nitrogen source on peptidase levels

We measured specific activities of individual peptidases in '$10^5 \times g$ supernatants' as a function of medium composition and growth state of the culture. In Fig. 4 the levels of dipeptidase and aminopeptidases I and II are plotted against the time of growth. The media contained glucose as a carbon source and either NH_4^+ ('minimal medium', Fig. 4a) or casein peptone ('complex medium', Fig. 4b) as sources of nitrogen. Each of the main peptidases behaved differently in response to the nutritional state of the cells.

The intracellular levels of dipeptidase were substantially higher in cultures growing exponentially on a poor nitrogen source than those in cells kept on peptone. With increasing age of the cells dipeptidase activities steadily decreased and became rather low in resting cells.

In contrast, the levels of aminopeptidase II changed little with the growth phases. Maximal activities were attained in the early stationary state, somewhat lower activities were found during exponential growth. In the experiment shown in Fig. 4, specific aminopeptidase II activities were essentially the same in both media. In other cases, they were by 50—100% higher in cells growing on the rich nitrogen source.

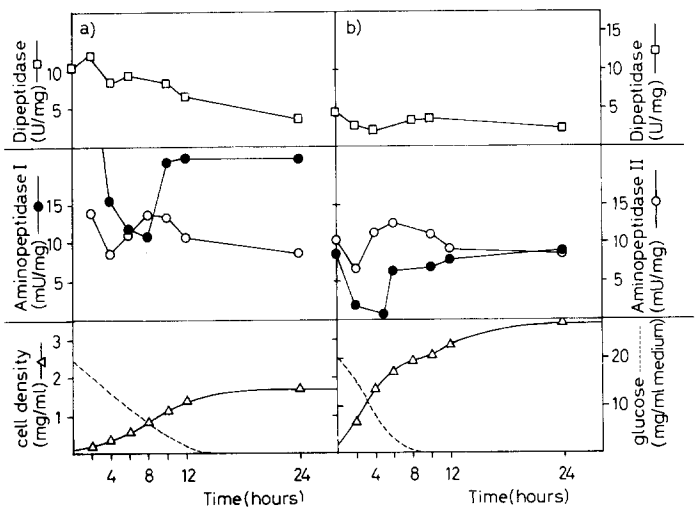

Fig. 4. Variation of peptidase levels with cell growth. Cells were cultivated and homogenized as described in Materials and Methods. a, contains data on cells grown on 'minimal medium' with NH_4^+ as the sole nitrogen source; b, refers to cells grown in the presence of casein peptone. Cell densities (△, mg dry weight/ml), the concentration of medium glucose (- - - - -, mg/ml) and specific activities of dipeptidase and aminopeptidases I and II (munits or units per mg supernatant protein) are plotted against the time of growth (h). Specific aminopeptidase activities were determined as follows: Dipeptidase (□) with 8 mM Gly-Leu in Tris-HCl (pH 8.0); aminopeptidase II (○) with 0.8 mM lysine-*p*-nitroanilide in 0.1 M phosphate/borate (pH 7.0); aminopeptidase I activities (●) were calculated from the total leucine-*p*-nitroanilidase activity (measured at pH 7.8 in the presence of 50 μM Zn^{2+}) minus the leucine-*p*-nitroanilidase activity of aminopeptidase II.

The response of aminopeptidase I activity to growth medium composition was more conspicuous. At zero time, when cell proliferation was induced by addition of fresh medium to an almost stationary culture, aminopeptidase I activity was high, but dropped to one third or less within the following 2 h. The same effect took place when only glucose was added. As soon as the concentration of medium glucose had fallen again below 1%, aminopeptidase I activity increased again. Its original level was reattained within 2 h and maintained throughout the state of glucose starvation. This 'glucose effect' took place in both 'minimal' and 'complex' medium.

The repressive influence of glucose on aminopeptidase I formation was superimposed by a similar effect of the nitrogen source. When cells were grown on glucose and ammonia (i.e. in 'minimal medium') aminopeptidase I levels were enhanced 3—10-fold throughout the growth cycle. This effect is most probably due to the release of aminopeptidase I synthesis from repression by amino acids. The chemical nature of the repressing metabolite(s) was not yet established, however.

Discussion

Our results show that *S. cerevisiae* contains three major soluble aminopeptidases (active against a variety of peptides and amino acid derivatives) and a single dipeptidase. All of these enzymes have been described previously, but, to the present date, a generally accepted denomination of yeast exopeptidases does not exist. To avoid further confusion, we propose the nomenclature adopted in the present paper (Table III) denominating the aminopeptidases by the roman numerals I, II and III according to molecular weights.

Aminopeptidase I is the largest peptidase in yeast and, at the same time, the

TABLE III

EXOPEPTIDASES OF YEAST

The peptidases characterized in the present paper (left column) and enzymes described previously are compared. As discussed in the text, some of the presumed identities still have to be conclusively demonstrated. Carboxypeptidase, although not within the scope of this study, was also included.

Proposed denomination	Molecular weight	References	Other names
Aminopeptidase I	640 000	18—20	Polypeptidase [11] Lysosomal aminopeptidase ('AP III') [27]
Aminopeptidase II	85 000	18	Aminopeptidase I [21] Aminopeptidase [31] (External)Leucylaminopeptidase [32]
Aminopeptidase III	~30 000	this paper	Aminopeptidase II [21] Amino acid naphthylamidase [22]
Carboxypeptidase	61 000	6	Acid Carboxypeptidase [30] Peptidase α [33]

first one obtained in a pure state [11]. The presence of an aminopeptidase with remarkably high molecular weight in the yeast vacuole was discovered by Matile and coworkers [27]. The levels of this enzyme (which is, undoubtedly, identical with aminopeptidase I) were highest in stationary cultures, but decreased upon addition of glucose. An enhancement of this 'lysosomal aminopeptidase activity' was observed when protoplasts were incubated under conditions of nitrogen starvation. This is in agreement with our observation that aminopeptidase I synthesis is repressed by both glucose and amino acids. Glucose repression also controls the activity of yeast proteinases A and B which share the vacuolar localization of aminopeptidase I [28]. The influence of the nitrogen source on proteinase and peptidase levels in yeast was studied by Hansen et al. [29]. These workers did not find substantial differences between proteolytic activities in cells grown on $(NH_4)_2SO_4$, valine and peptone, respectively. As far as peptidase activities are concerned, the seeming contradiction of this findings to our results may be explained by the assay conditions chosen by Hansen et al. [29]. In this study activities were measured against leucine-*p*-nitroanilide and Ile-Phe in the absence of aminopeptidase I activators. Thus, mainly aminopeptidase II and dipeptidase activities were detected the dependence of which on medium composition is not very conspicuous.

The repression of enzymes by glucose is widespread among microorganisms. However, its regulatory significance is not obvious in the case of proteolytic enzymes. The derepression of proteolytic activities during amino acids starvation, on the other hand, stimulates intracellular protein breakdown and thus serves to maintain protein synthesis under conditions of insufficient amino acid supply. In yeast, aminopeptidase I seems to be involved in this process.

The molecular and catalytic properties of aminopeptidase II are still incompletely characterized. It is, therefore, difficult to ascertain whether aminopeptidase II is identical with 'aminopeptidase I' isolated by Masuda et al. [21]. Although the substrate specificities of both enzymes are similar (unfortunately lysine-*p*-nitroanilide was not tested as a substrate of 'aminopeptidase I') the molecular weights are different. An interesting feature of aminopeptidase II, offering a clue to its function, is its obviously external localization. In yeast, several hydrolases (e.g. invertase and acid phosphatase) occur in closely related external and internal forms. When it became evident that the aminopeptidase activity of yeast is in part exocellular, a similar relationship between vacuolar and external aminopeptidase was discussed [27,32]. In the present report it is demonstrated that the respective activities are brought about by different enzymes and are independently regulated.

The particular localization of aminopeptidase II indicates that the enzyme participates in peptide utilization. Although certain peptides may enter the yeast cell without previous degradation [34,35] an external aminopeptidase strongly promotes amino acid uptake by furnishing additional amino acids to the more efficient amino acid transport systems. Moreover, the size of peptides which can be taken up by yeast is limited, whereas such a restriction is not imposed on the uptake via aminopeptidase and amino acid transport.

The specific activities of dipeptidase in yeast are usually much higher than those of the aminopeptidases, but conclusive evidence as to its function is not available. The influence of amino acid starvation on dipeptidase levels may be

taken to indicate an involvement in intracellular proteolysis. However, the enzyme is not encountered in the vacuole, its subcellular localization still remains to be established.

Acknowledgement

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References

1 Holzer, H. (1976) Trends Biochem. Sci. 1, 178—181
2 Holzer, H., Betz, H. and Ebner, E. (1975) Curr. Top. Cell. Regul. 9, 103—156
3 Switzer, R.L. (1977) Annu. Rev. Microbiol. 31, 135—157
4 Lenney, J.F. (1956) J. Biol. Chem. 221, 919—930
5 Hata, T., Hayashi, R. and Doi, E. (1967) Agric. Biol. Chem. 31, 150—159
6 Hayashi, R., Moore, S. and Stein, W.H. (1973) J. Biol. Chem. 248, 2296—2300
7 Lenney, J.F., Matile, P., Wiemken, A., Schellenberg, M. and Meyer, J. (1974) Biochem. Biophys. Res. Commun. 60, 1378—1383
8 Saheki, T., Matsuda, Y. and Holzer, H. (1974) Eur. J. Biochem. 47, 325—332
9 Betz, H., Hinze, H. and Holzer, H. (1974) J. Biol. Chem. 249, 4515—4521
10 Matern, H., Hoffmann, M. and Holzer, H. (1974) Proc. Natl. Acad. Sci. U.S. 71, 4874—4878
11 Johnson, M.J. (1941) J. Biol. Chem. 137, 575—586
12 Grassmann, W. and Dyckerhoff, H. (1928) Hoppe-Seyler's Z. Physiol. Chem. 179, 41—78
13 Grassmann, W., Emden, L. and Schneller, K. (1934) Biochem. Z. 271, 216—228
14 Nishi, A. (1958) J. Biochem. Tokyo 45, 991—1004
15 Cordonnier, R. (1966) Thesis, University of Aix-Marseille, France
16 Röhm, K.-H. (1974) Hoppe-Seyler's Z. Physiol. Chem. 355, 675—686
17 Herrmann, V., Knack, I. and Röhm, K.-H. (1978) Hoppe-Seyler's Z. Physiol. Chem. 359, 47—61
18 Metz, G. and Röhm, K.-H. (1976) Biochim. Biophys. Acta 429, 933—949
19 Metz, G., Marx, R. and Röhm, K.-H. (1977) Z. Naturforsch. 32c, 929—937
20 Marx, R., Metz, G. and Röhm, K.-H. (1977) Z. Naturforsch. 32c, 938—943
21 Masuda, T., Hayashi, R. and Hata, T. (1975) Agric. Biol. Chem. 39, 499—505
22 Tjeder, A. (1966) Acta Chem. Scand. 20, 1442—1444
23 Schaich, E. (1973) Thesis, University of Marburg, G.F.R.
24 Kramer, R., Kopp, F., Niedermeyer, W. and Fuhrmann, G.F. (1978) Biochim. Biophys. Acta 507, 369—380
25 Cohen, S.R. and Wilson, I.B. (1966) Biochemistry 5, 904—909
26 Metz, G. (1976) Thesis, University of Marburg, G.F.R.
27 Matile, P., Wiemken, A. and Guyer, W. (1971) Planta 96, 43—53
28 Saheki, T. and Holzer, H. (1975) Biochim. Biophys. Acta 384, 203—214
29 Hansen, R.J., Switzer, R.L., Hinze, H. and Holzer, H. (1977) Biochim. Biophys. Acta 496, 103—114
30 Kuhn, R.W., Walsh, K.A. and Neurath, H. (1974) Biochemistry 13, 3871—3878
31 Cordonnier, R. (1967) Ann. Nutr. Aliment. 21, B247—273
32 Matile, P. (1969) Proceedings of the 2nd Symposium on Yeasts, Bratislava 1966, pp. 503—508
33 Felix, F. and Brouillet, N. (1966) Biochim. Biophys. Acta 122, 127—144
34 Becker, J.M. and Naider, F. (1977) Arch. Biochem. Biophys. 178, 245—255
35 Marder, R., Becker, J.M. and Naider, F. (1977) J. Bacteriol. 131, 906—916