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OCCURRENCE OF PROTEINASE A ISOINHIBITORS IN WILD TYPE YEAST STRAINS AND COMMERCIAL BAKER'S YEAST

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Summary: The occurrence of the proteinase A inhibitors 2 and 3 was investigated in wild type strains of Saccharomyces cerevisiae and Saccharomyces carlsbergensis as well as in several strains of commercial baker's yeast. Haploid and diploid strains of Saccharomyces cerevisiae contain only proteinase A inhibitor 3 whereas in Saccharomyces carlsbergensis only proteinase A inhibitor 2 is found. Strains of commercial baker's yeast contain either proteinase A inhibitor 3 or both inhibitors in a constant ratio of 1:3. Single cell cultures isolated from a strain of commercial baker's yeast also contain a mixture of the two inhibitors. Therefore, baker's yeast is not a mixture of two different cell types but the genome for both inhibitors is present in each single cell. In general, the results indicate that the occurrence of the two proteinase A inhibitors is determined genetically and, therefore, they may be called "isoinhibitors".

Introduction: Baker's yeast contains inhibitors against its major endoproteolytic enzymes, proteinases A and B. The proteinases are compartmentalized in the yeast vacuoles whereas the inhibitors are localized in the cytosol which surrounds the vacuoles. Most likely the function of the inhibitors is to protect the cell against harmful proteolysis outside the vacuoles (for a review see ref. 1).

The heat-stable polypeptides which inhibit proteinase A were isolated from a boiled yeast extract and their characteristics were investigated (2-5). Both inhibitors exhibit an identical molecular weight of 7,700 but differ in their isoelectric points being 5.7 for $I^{A}2$ [1] and 6.3 for $I^{A}3$ (2). In the present study the

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[1] Abbreviations: $I^{A}2$, $I^{A}3$, proteinase A inhibitors 2 and 3; $I^{B}1$, $I^{B}2$, proteinase B inhibitors 1 and 2.

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occurrence of the inhibitors in various wild type strains and strains of commercial baker's yeast was investigated. The results demonstrate that the occurrence of the proteinase A inhibitors is determined by the genome of the respective strain.

Experimental Procedures: Proteinase A inhibitor activity was determined according to Saheki et al. (2) or as inhibition of tryptophan synthase-inactivation as described by Ferguson et al. (6). Tryptophan synthase for the assay was purified by the method of Wolf and Hoffmann (7). Protein concentrations were determined by the method of Lowry et al. (8) with crystalline bovine serum albumin as standard.

Saccharomyces cerevisiae X 2180 (aα) was originally isolated by R. Mortimer (Berkeley, CA), Saccharomyces cerevisiae 288c was from G. R. Fink (Ithaca, N.Y.) and Saccharomyces carlsbergensis NCYC 74 from the National Collection of Yeast Cultures (U.K.). Commercial baker's yeast strains (Pleser Hefe, Darmstadt, Sinner Hefe, Karlsruhe and Fala Hefe, Gamshurst) were obtained from Bäko Gesellschaft G.m.b.H. (Freiburg, Germany). The yeast was grown aerobically at 30 °C in a 10 l fermenter (Braun, Melsungen, Germany) on yeast extract-peptone-dextrose medium (YEPD) according to Manney (9) from a 1% (v/v) inoculum. The cells were harvested in the stationary growth phase, washed with water and suspended in water at a concentration of 1 g yeast/ml. The suspension was heated in a boiling water bath for 25 min followed by centrifugation at 4 °C for 30 min at 34,000 x g. The yellow supernatant was filtered through paper on a Buchner funnel, the filtrate was diluted about threefold with water and the pH was adjusted to 4.5 with glacial acetic acid. The extract was applied to a SP-Sephadex C-25 column (2.5 x 8 cm). The column was washed with 50 mM ammonium acetate buffer, pH 4.5, and eluted with a linear gradient from 0-0.6 M sodium chloride in the same buffer (total volume 400 ml). The flow rate was 30 ml/h and fractions of 3.5 ml were collected. The pooled active fractions were lyophilized and subjected to polyacrylamide gel electrofocusing in the pH range 5-8 as described by Wrigley (10). The gels were cut into 2 mm-slices which were homogenized and extracted with 0.3 ml water. In the extracts the pH was measured and proteinase A inhibitor activity was determined with the tryptophan synthase-inactivation assay (6).

Results: The occurrence of the proteinase A inhibitors was investigated in a variety of yeast strains and the properties of the inhibitors were compared to those isolated from baker's yeast (2). Wild type strains of Saccharomyces cerevisiae and Saccharomyces carlsbergensis were grown aerobically on YEPD medium (9). The cells were harvested in the stationary growth phase where the highest proteinase A inhibitor levels are found (11,12). The heat-stable inhibitors were isolated from boiled yeast extracts

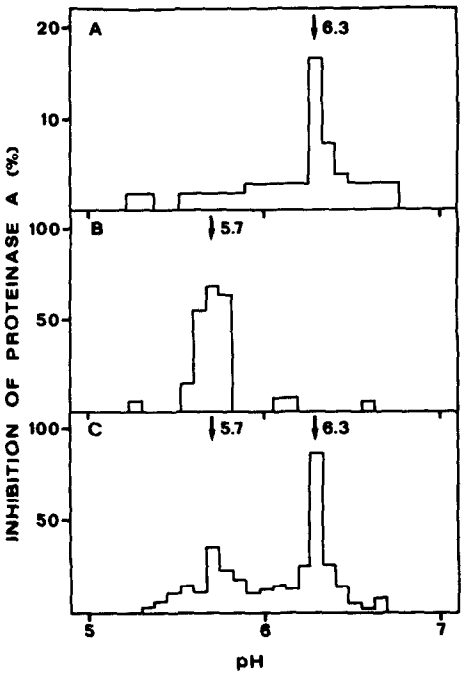

Figure 1: Analytical gel electrofocusing of proteinase A inhibitors from (A) Saccharomyces cerevisiae 288c, (B) Saccharomyces carlsbergensis NCYC 74, and (C) baker's yeast (Pleser Hefe). The inhibitors were isolated from stationary yeast cells and subjected to gel electrofocusing as described in the experimental section. Arrows indicate where maximal inhibitor activities were detected.

by SP-Sephadex C-25 chromatography which results in about 15-fold purification. Under the chromatography conditions employed the proteinase A inhibiting material is completely recovered. Since the inhibitors differ in electric charge only, they were separated and identified by analytical gel electrofocusing. In the haploid strain Saccharomyces cerevisiae 288c only one polypeptide with proteinase A inhibitor activity is found which exhibits an isoelectric point of 6.3 (Figure 1A). The diploid strain Saccharomyces cerevisiae X 2180 (aα) also contains only one inhibitor with an isoelectric point of 6.3 (not shown). In contrast, the inhibitor isolated from Saccharomyces carlsbergensis NCYC 74 shows an isoelectric point of 5.7 (Figure 1B). For comparison the

proteinase A inhibitors were also isolated from baker's yeast (Pleser Hefe). This yeast strain contains the two inhibitors, I^A2 and I^A3, described by Saheki et al. (2) which have isoelectric points of 5.7 and 6.3, respectively (Figure 1C). Thus, it appears that the inhibitor isolated from Saccharomyces cerevisiae corresponds to I^A3 and that from Saccharomyces carlsbergensis to I^A2.

We also isolated proteinase A inhibitors from strains of commercial baker's yeast. The specific proteinase A inhibitor activities in the boiled yeast extracts are very similar for the strains under investigation (Table I). After SP-Sephadex C-25 chromatography of the boiled extracts in Sinner Hefe two peaks of proteinase A inhibitor activity with isoelectric points of 5.7 and 6.4 were identified by electrofocusing whereas Fala Hefe contains only one inhibitor with an isoelectric point of 6.2 (Table I). Although about the same specific inhibitor activities are found in boiled extracts of all three strains the distribution of the inhibitor forms differs.

Two explanations for the occurrence of two proteinase A inhibitors in strains of commercial baker's yeast are plausible. These

Table I: OCCURRENCE OF PROTEINASE A ISOINHIBITORS IN STRAINS OF COMMERCIAL BAKER'S YEAST[a]

Yeast Strain	Specific Proteinase A Inhibitor Activity (U/mg)	Isoelectric Points of Proteinase A Inhibitors
Pleser	10.5	5.7, 6.3
Sinner	12.4	5.7, 6.4
Fala	11.6	6.2

[a]Specific proteinase A inhibitor activities were measured in boiled yeast extracts by the method of Saheki et al. (2). Isoelectric points of the inhibitors were determined by analytical gel electrofocusing as described in the experimental section.

strains either consist of a mixture of two genetically different cell types or each baker's yeast cell contains the genome for both inhibitors. To test these possibilities single cells were isolated and grown under the conditions described above. Cultures from the different single cell isolates contained the inhibitors in a constant ratio of 1:3 (Table II). This result agrees with the explanation that genes for both inhibitors are present in each single baker's yeast cell and that they are expressed in a constant ratio.

Discussion: Both haploid and diploid strains of Saccharomyces cerevisiae contain only $I^{A}3$ whereas in Saccharomyces carlsbergensis $I^{A}2$ is exclusively found (Figure 1). Since the yeast cultures were grown under identical conditions and the inhibitors isolated by the same procedure it is unlikely that the restriction of $I^{A}3$ to Saccharomyces cerevisiae and of $I^{A}2$ to Saccharomyces carlsbergensis, respectively, may be caused by a transcriptional or translational control or by an artificial modification during the isolation. The results rather suggest that the genetic information for the proteinase A inhibitors differs in these strains.

Table II: PROTEINASE A ISOINHIBITORS IN CULTURES FROM SINGLE CELLS ISOLATED FROM A STRAIN OF COMMERCIAL BAKER'S YEAST[a]

Isolation No.	Proteinase A Inhibitor Activity ($A_{530} \times min^{-1} \times ml^{-1}$)		$I^{A}2 : I^{A}3$
	$I^{A}2$	$I^{A}3$	
1	0.26	0.85	1 : 3.3
2	0.33	1.10	1 : 3.3
3	0.18	0.54	1 : 3.1

[a]Proteinase A inhibitors were isolated from single cell cultures of baker's yeast (Pleser Hefe) as described in the experimental section. After separation by gel electrofocusing activities of the inhibitors were measured by the method of Ferguson et al. (6).

The distribution of the proteinase A inhibitors in the various yeast strains is a counterpart to the occurrence of the proteinase B inhibitors. Baker's yeast contains both I^B1^1 and I^B2 in a constant ratio of 1:3 whereas in Saccharomyces carlsbergensis only I^B1 and in Saccharomyces cerevisiae exclusively I^B2 are found (13,14). Studies on the amino acid composition and the amino acid sequence have revealed that Val-32 and Lys-34 in I^B1 are replaced by Leu-32 and Lys-34 in I^B2 (15-17). These results have confirmed that the occurrence of the two proteinase B inhibitors is determined genetically and, therefore, they are classified as "isoinhibitors". The coincidence in the occurrence of the proteinase B and A inhibitors in the various yeast strains is obvious. Most likely the two proteinase A inhibitors are also coded by different genes and thus they may also be called "isoinhibitors".

In the original purification procedure of the proteinase A inhibitors from baker's yeast, Saheki et al. (2) separated four peaks of inhibitor activity by ion exchange chromatography. Peaks 2 and 3 were further purified to homogenity and were designated I^A2 and I^A3. In the present study only two proteins with inhibitor activity were detected in the same yeast strain. Their isoelectric points are identical to those found for I^A2 and I^A3. The appearance of additional peaks with inhibitor activity in the original purification may be due to the chromatography conditions or to artificial modification of the proteins by trichloroacetic acid precipitation. Peak 1 in the purification by Saheki et al. (2) may reflect a chromatography breakthrough fraction because it is no longer observed under the modified chromatography conditions employed in the present study. Precipitation with trichloroacetic acid may result in artificial modification and appearance of additional forms of proteinase A inhibitors. This effect of acid

treatment has been observed with the proteinase B inhibitors (14). Therefore, trichloroacetic acid precipitation was omitted in the present isolation procedure. From the present study it is evident that only two natural proteinase A inhibitors, $I^{A}2$ and $I^{A}3$, exist in baker's yeast.

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