Note

Physical and Chemical Characterization of Cell-bound Lipases from *Saccharomycopsis lipolytica*

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Saccharomycopsis lipolytica is known as a lipase-producing and hydrocarbon-assimilating yeast. *S. lipolytica* produces a large amount of lipases bound on the yeast cell when it is cultivated in a medium containing olive oil or a long chain fatty acid (e.g. oleic acid).^{1,2)} Two types of cell-bound lipases were purified and their properties were studied by Ota *et al.*³⁾ One was adsorbed and the other not to CM-Sepharose CL-6B at pH 5.0. Concerning enzymological properties there was little difference between Lipases I and II; however the molecular weight of Lipases I and II was about 39,000 and 44,000 by the methods of gel filtration and SDS polyacrylamide gel electrophoresis respectively. Therefore, we have much interest in comparing the physical and chemical properties of Lipases I and II. In this note, isoelectric focusing, amino acid analysis, carbohydrate analysis, circular dichroism and N-terminal amino acid analysis of Lipases I and II are described.

Isoelectric focusing. Isoelectric focusing was performed in a column (7 × 100 mm) for three hours, according to the standard method,⁴⁾ using a carrier ampholine, in the pH range from 4.0 to 11.0 with the voltage of 200~300 V. Cytochrome *c* and various kinds of acetylated cytochrome *c* purchased from Oriental Yeast Co., Ltd. were used as *pI* markers. In isoelectric focusing the single bands obtained for Lipases I and II calibrated from plots of pH value against mobile distance were pH 5.2 and pH 4.6 respectively.

Amino acid composition. Amino acid analysis of purified Lipases I and II of which specific activity was 10,200 and 8,540 μ /ml/OD₂₈₀ respectively, was performed. Samples were hydrolysed in 6 N HCl solution for 20, 24, and 48 hr at 105°C, and amino acids were determined on a Hitachi

amino acid analyzer. Tryptophan was determined according to the ultraviolet adsorption method.⁵⁾ The results of amino acid analysis of Lipases I and II are presented in Table I. As shown in Table I, both enzymes were rich in hydrophobic amino acids; however, Lipase II had comparatively larger numbers of Glx, Ser, Thr, and Gly.

Carbohydrate analysis. For the carbohydrate analysis, purified lipases were methanolysated in 1 N HCl methanol at 105°C for 24 hr and carbohydrate analysis was performed using a gas chromatography (5% silicone SE-30, 180°C). Only mannose was detected in both enzymes.

The carbohydrate determination was performed according to the phenol-sulfuric acid method⁶⁾ using mannose as the standard carbohydrate. The carbohydrate content of Lipases I and II was 8 and 16% in terms of mannose, respectively. From the amino acid and carbohydrate

TABLE I. AMINO ACID COMPOSITION OF LIPASES I AND II

Amino acid	Lipase I (residues/mol)	Lipase II (residues/mol)
His	15	15
Arg	8	8
Lys	12	12
Asx	40	40
Glx	27	32
Ser	17	22
Thr	16	19
Tyr	17	17
Gly	27	30
Ala	19	20
Val	26	27
Leu	26	26
Ile	23	23
Pro	16	16
Phe	17	16
Try	-4	4
Met	2	2
1/2Cys	7	8
Total	319	337

TABLE II. CARBOHYDRATE CONTENT OF LIPASES I AND II

	Lipase I (daltons)	Lipase II (daltons)
Protein	36,100	37,200
Carbohydrate	3,100	7,100
Molecular weight	39,200	44,300

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TABLE III. COMPARISON OF PHYSICAL AND CHEMICAL PROPERTIES IN CELL-BOUND LIPASES I AND II

	Lipase I	Lipase II
Molecular weight	39,200	44,300
Carbohydrate content	8%	16%
Isoelectric point	pH 5.2	pH 4.6
α -Helix content	40%	45%
β -Sheet content	50%	35%
N-Terminal amino acid	Val-Tyr-	Val-Tyr-

analyses the molecular weight of Lipases I and II was calculated to be 39,200 and 44,300 respectively, as shown in Table II.

Circular dichroism. The circular dichroic spectra in the near-ultraviolet range were analyzed with a Jasco J-20 automatic recording spectropolarimeter in a 50 mM Tris-HCl buffer (pH 7.0). The calculation according to the method of Yang⁷⁾ shows that Lipase I has a secondary protein structure composed of 40% α -helix, 50% β -sheet and 10% random form, while Lipase II contains 45% α -helix, 35% β -sheet and 20% random form. These results suggest that Lipase I has a slightly different secondary structure from Lipase II.

N-Terminal amino acid analysis. N-Terminal amino acid analysis was performed according to the standard method of Edman.⁸⁾ The identification of PTH amino acid was done by gas chromatography (10% silicone SE-30, 220°C) in the first run and amino acid analyzer (Hitachi) in the

second run. The N-terminal dipeptide of both Lipases I and II was identified with Val-tyr-.

Comparison of physical and chemical properties in cell-bound Lipases I and II is presented in Table III. Clearly there were differences between Lipases I and II in physical and chemical properties, though there was no difference in enzymological properties.³⁾

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