Note

Protein Sequences of Two Keto Ester Reductases: Possible Identity as Hypothetical Proteins

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We determined the amino acid sequences of two keto ester reductases (YKER-V and -VI) purified from a cell-free extract of Saccharomyces cerevisiae. The N-terminal and internal amino acid sequences of YKER-VI (AcrK) were in agreement with the sequence of hypothetical 36.4-kDa protein (S. cerevisiae chromosome X reading frame ORF YJR105w) in yeast. The N-terminal amino acid sequence of YKER-V was also identical with that of the hypothetical protein coded by yeast chromosome XIV or II. These results suggested that two hypothetical proteins were expressed as keto ester reductases in yeast cells.

Key words: keto ester reductase; bakers' yeast; protein sequence; hypothetical protein

The stereoselective reduction of various carbonyl compounds by oxidoreductases from microorganisms has been widely used for the preparation of optically active alcohols. However, the relationship between the enzymes purified with the reactivity for the synthetic substrates (i.e., keto esters) and these natural substrates in the cells has not been thoroughly analyzed. Recently, we have purified four β-keto ester reductases (1-enzyme-1, 2- and 3-enzyme-1, 2-), seven z-keto ester reductases (YKER-I, VII) and z-acetoxyketone reductase (AcrK) from the cell-free extract of Saccharomyces cerevisiae. Among them, three NADPH-dependent z-keto ester reductases, YKER-I, -VI, and -VII, were identified as 1-enzyme-1, 1-enzyme-2, and 3-enzyme-2, respectively. One of these reductases (named YKER-I) had an excellent stereoselectivity in the reduction of β-keto esters, and also catalyzed asymmetric reduction of z-alkyl-β-keto esters of secondary alcohol accompanied by simultaneous kinetic resolution of dynamic and static optically active centers. The N-terminal amino acid sequence of YKER-I had high sequence similarity (63%) to those of a protein with the molecular mass of 25 kDa coded in a gene of S. cerevisiae. The gene encoded a polypeptide with much sequence similarity to that of aldose reductase, aldehyde reductase, and vertebrate eye lens protein (p-crystalline).

YKER-I and YKER-II, IV, and VI were dimeric, monomeric, and dimeric enzymes, respectively, and their molecular masses were estimated to be 58, 31-39, and 83 kDa, respectively. YKER-V catalyzed exclusively the reduction of many kinds of z-carbonyl compounds such as z-keto esters and methyl and phenyl glyoxal. The N-terminal amino acid sequence of YKER-V was resolved by this study.

Studies on the chromatographic behavior, stereoselectivity, and N-terminal amino acid sequence indicated that AcrK was the same protein as YKER-VI. The enzyme catalyzed the enantiopure reduction of 1-acetoxy-2-alkanones and 1-acetoxy-2-alkanols and β-keto esters to the corresponding β-hydroxy esters. The homology of the amino acid sequences in the N-terminal region of the enzyme and other enzymes was investigated, however no enzyme proteins had significant sequence similarity to YKER-VI. YKER-VI seemed to be identical with the fatty acid synthetase reported by Shieh et al., because the stereospecificities, molecular masses and kinetic constants of the two enzymes were very similar.

Herein, we would like to report the protein sequences of two keto ester reductases, YKER-V and YKER-VI (AcrK), which seemed to be expressed as the hypothetical proteins in yeast cells. We again purified YKER-II, -IV, -V, and -VI from bakers' yeast and analyzed the protein sequence to study the physiological role of these enzyme proteins in yeast from the standpoint of the homology search. The enzymes were purified as described previously, and these isolated enzymes used for sequence analysis were further purified by reverse-phase HPLC on a Sephasil C18 column with a linear gradient from 0 to 80% acetonitrile containing 0.1% trifluoroacetic acid (TFA) for 45 min at a flow rate of 0.1 ml/min in the SMART system.

The purified enzymes were reduced and S-
pyridylethylated. The S-alkylated proteins were obtained as the
holopirrases after desalting with 0.1% TFA. The polypeptides
were digested at 37 C as shown below [buffer, incubation time,
and enzyme-to-substrate ratio (mol:mol)]; Lys-C (10 mm Tris-HCl
buffer at pH 8.5, 18 h, and 1:20), Asp-N (50 mm potassium
phosphate buffer at pH 8.0, 18 h, and 1:60). The peptides obtained
by proteolytic digestion were separated by reverse-phase HPLC
on a Sephasil C18 column by monitoring at 214 nm (data not
shown).

The amino acids of peptides sequenced were performed by
automated Edman degradation with a protein sequencer (Applied
 Biosystems, 476A). The sequence of the N-terminal amino acid
region of YKER-VI (aka R) was 1 Thr-Ala-Pro-Leu-Val-Val-Leu-
Glu-Asn, 1 Pro-Leu-Leu-Asp-Phe-Glu-Ala. Similarity of the se-
qued of YKER-VI with those of other proteins in yeast was
looked for by a computer search of the EMBL GenBank DDBJ
databases. The N-terminal amino acid sequence of YKER-VI
was the same sequence as that of the hypothetical EMBL with
molecular mass of 36.4 kDa coded by a bacterial gene of S. cerevisiae
(ORF YJR105w in chromosome X, SOD-CAP2 intergenic region).
Furthermore, the internal sequences of YKER-VI were also identical with
those of the hypothetical 36.4 kDa protein (Fig. 1).

The amino acid residues of Thr2-Ala17, Asp43-Phe49, Asp91-144, His150-Lys158, Pro187-Lys202, Asp227-Leu231, and
Asp237-Glu262 in the hypothetical protein were the same as
sequences of YKER-VI. The identified sequences were 120 amino
acid residues (37% of the full sequence). The molecular mass
(36.4 kDa), and the N-terminal and internal sequences of
YKER-V were identical to those of the hypothetical protein.
These results indicated that YKER-V was the same protein as
the hypothetical 36.4 kDa protein. No enzymes with sequence similarity
to the hypothetical 36.4 kDa protein were found by a computer search of the National Biochemical Research Foundation
(NBRF) data banks.

On the other hand, the N-terminal amino acid sequences of
YKER-II, -IV, and -V were also analyzed, however the N-terminal
amino acid residues of YKER-II and -IV were blocked.
The sequence of the N-terminal amino acid region of YKER-V
was 1 Asn-Ile-Val-Glu-Asn-Met-Leu-His-Pro.1 Lys-Thr-Thr-Glu-Ile-
Tyr-Phe-Ser-Leu-Asn.20 Asn. Similarity of the sequence of the
enzyme to those of other proteins was looked for by a computer search of the NBRF data banks. As the result, completely
identical sequence was found in a hypothetical protein encoded
upstream of the DNA sequence of a repetitive CAT region in
the yeast chromosome XIV (yeast ORF 1 gene). In addition, a
polypeptide chain encoded in yeast chromosome II (yeast ORF
YBR149w) was also identical to the sequence of the N-terminal
amino acid region of YKER-V. The nine amino acids from
N-terminal region in these two open reading frame (Met-Ser-Ser-
Val-Ser-Thr-Glu) were lacking in YKER-V (Fig. 2).

The polypeptide might have been deleted during the maturing
of the enzyme protein. The molecular masses of these hypothetical
proteins encoded in chromosome XIV and II from the amino acid
sequence data were calculated to be 38022 and 38027 Da,
respectively. The molecular mass of YKER-V was the same as those of
the hypothetical proteins. Therefore, it is necessary that the
complete amino acid sequence of YKER-V is resolved to find
whether this enzyme is expressed from chromosome XIV or II.

From these results, these hypothetical proteins seemed to be
expressed as the two keto ester reductases (YKER-V and -VI)
from the S. cerevisiae genome. Further detailed studies including
the gene expression of these enzyme proteins are now under
investigation and will be reported in our following paper.

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