Characterization of the functional organization of yeast K2 killer preprotoxin gene

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Expression of a cDNA copy of the yeast K2 preprotoxin gene confers the complete K2 killer phenotype on sensitive cells. To determine the functional domains of killer preprotoxin, we analyzed the phenotypes of a set of mutations throughout regions encoding the δ-, α- and β-toxin subunits. Mutations within the β-subunit indicate it to be essential for the killing of sensitive cells and forming of immunity. Mutations within C-terminal region of α causing loss of toxicity also cause loss of immunity, implicating α in expression of both functions. Our results indicate that the leader sequence of preprotoxin is involved in ensuring the immunity.

Key words: *Saccharomyces cerevisiae*, yeast, killer toxin, immunity

INTRODUCTION

Killer strains of *Saccharomyces cerevisiae* secrete a polypeptide toxin to which they are immune themselves. On the basis of killing profiles and missing cross-immunity, toxin-secreting strains have been classified into three major types (K1, K2, K28) [1].

The *S. cerevisiae* K1 killer system is one of the many described among the yeasts. The K1 precursor peptide has a δ-α-γ-β domain organization. Mature K1 toxin is secreted as an α/β heterodimer [2]. Mutational analysis confirmed that the hydrophilic β-subunit of K1 toxin is essential for binding to a cell wall receptor. The α-subunit, in contrast, is multifunctional, having regions necessary for killing, immunity, and cell wall receptor binding that appear to overlap in the polypeptide. The immunity-coding region of K1 extends through the C-terminal half of the α-subunit into the N-terminal part of the γ glycopeptide. β-subunit of K1 is not involved in formation of immunity [3]. The organization of K2 killer system is not so extensively studied. Meškauskas and Čitavičius [4] have synthesized, cloned, sequenced and expressed in yeast the cDNA copy of M2-1 fragment of M2 dsRNA, encoding type K2 killer preprotoxin. Expression of the K2 killer precursor gene by the yeast ADH1 promoter in *S. cerevisiae* conferred both the K2 killer and immunity phenotypes on sensitive host yeast strains. The primary nucleic acids sequence of K2 gene shows no identity with gene coding for the sequence of the K1 toxin, but K2 precursor appears to have a similar overall structure to that of K1 [4, 5].

Here, we report results of a mutational analysis of K2 killer preprotoxin. We show that both the α- and β-subunits appear to be required for toxicity and immunity, and the leader sequence of preprotoxin is necessary for ensuring immunity.

MATERIALS AND METHODS

The *S. cerevisiae* strain α'1 (*MATα leu2 [kil-0]*) [6] was used as a recipient for investigation of phenotypes of K2 gene mutations and as a sensitive tester for killer toxin activity. The *E. coli* strain DH5α [7] was used for the routine growth and maintenance of plasmids. All media for the growth of DH5α and procedures of transformation were standard [8]. Media for the growth of *S. cerevisiae* have been described in [9].

Mutagenesis at restriction sites was accomplished with the K2 killer preprotoxin gene from plasmid pYEX12 [4]. General methods for DNA manipulations involving restriction digestion, Klenow fragment treatment, dephosphorylation, ligation, electrophoresis, extraction of DNA from agarose gel were performed essentially as described in [8] and according to the product manufacturers' recommendations. Transformation into *S. cerevisiae* α'1 was performed by the LiCl procedure [10]. Transformants were selected by complementation of LEU2 auxotrophy and tested for the killer phenotype as described in [11]. Immunity was assessed by spotting 10^7 cells of tester K1, K2 and K28 killer strains onto an agar plate seeded with 10^5 cells of transformant per ml [11]. All plasmid constructions were checked by restriction mapping and retransformation.

RESULTS AND DISCUSSION

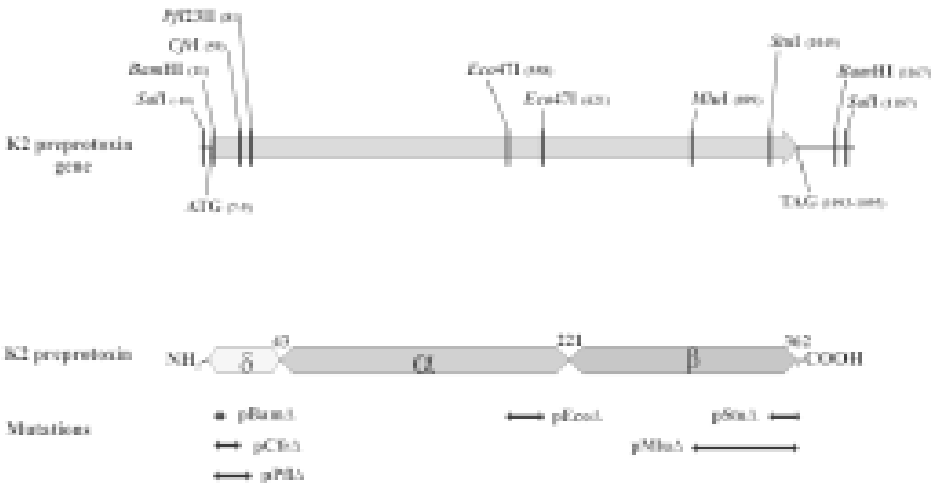

Fig. 1. Localization of deletions introduced into the K2 preprotoxin gene. The restriction map at the top shows the preprotoxin gene as it is cloned into the *Sal*I–*Sal*I sites of pYEX12; only sites used in this work are shown. Below the subunit structure of the K2 killer preprotoxin is shown. The mutations are indicated here by the designations of plasmids containing them. The horizontal arrows indicate the extent of gene deletions

The toxin secretion and immunity exhibited by K2 killer yeast to their toxin are conferred by the precursor gene carried on the cDNA expression plasmid pYEX12. This gene defines 100% killer toxin production and wild-type immunity in this work. Our strategy to determine the functional domains of the K2 preprotoxin was to construct mutations throughtout the δ-, α- and β-encoding regions. By analyzing the ability of mutant toxins to kill sensitive cells and confer immunity, the respective domains could be determined. The actual mutations are summarized in Fig. 1.

C-terminal deletions of preprotoxin were constructed to determine the role of K2 β-subunit in the expression of immunity and killer functions. *Mlu*I restriction endonuclease was used to construct deletion of 56 amino acids in the C-end of β. The obtained plasmid pMluΔ was introduced into the sensitive *S. cerevisiae* strain α'1. All the transformants tested were incapable of killing the sensitive strain α'1 and remained sensitive to killer toxins of K2 type as well as types K1 and K28. Thus, deletion of 56 C-terminal amino acids of β-subunit totally abolished the ability of the toxin to render immunity and kill sensitive cells. Therefore, we decided to construct a shorter deletion in the C-end of β-subunit. We used the restriction endonuclease *Stu*I, removed the 16 C-terminal amino acid region and obtained the plasmid pStuΔ. The α'1-pStuΔ transformants had non-killer phenotypes similar to that of the α'1-pMluΔ, suggesting that the β-subunit of K2 preprotoxin was responsible for the formation of immunity. By contrast, all of the reported mutations in the β-subunit of K1 preprotoxin retained the immunity phenotype – the transformants were fully resistant to toxins type K1 [3]. In addition, deletions in K2 β-subunit were also inactive in killing sensitive cells, while mutations in the same region of K1 allowed secretion of 30–35% of toxin compared with the wild type [3].

The α-subunit of K1 preprotoxin contains two hydrophobic regions separated by a short hydrophilic region [2]. Mutations altering the hydrophobic regions of K1 α-subunit were found to be defective both in killing and immunity [3]. The overall hydrophobic/hydropathic organization is not preserved between K1 and K2 preprotoxins, but the α-subunit of K2 is also relatively hydrophobic [5]. In order to investigate the influence of the hydrophobic C-terminal region of K2 α-subunit on the phenotype, we constructed plasmid pEcoΔ by in-frame deletion of 21 C-terminal amino acids of α. Transformants containing this construct were non-killers and sensitive to K1, K2 and K28 toxins. Based on these findings, we suppose that the C-terminal region (184–205 amino acids) of K2 α-subunit is necessary to ensure killing and immunity like that of K1.

Analysis of the amino acid sequence of K2 preprotoxin shows a hydrophobic sequence in δ, between residues 27–43, postulated to act as a secretion leader sequence [5]. This sequence is preceded by a hydrophilic 1–26 amino acid segment whose function remains unknown [4]. We investigated the role of this hydrophilic sequence in the expression of killer phenotype. There are two potential in-frame initiation ATG codons at the start of the K2 preprotoxin gene (codons 7–9 and 76–78) and the most 5' 7-9 ATG is designated as the initiation codon [5]:

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1                 10 BamHI          20                30                40
5'-…GAA AAA ATG GGG ATC CGG GCC ACC AGC CTG GTG CAA GAC GAG

5                 60 CfrI           70                80 Pfl23II
CTG ACA CTA GGT GAG CCG GCC ACC CGA GCA AGG TGC GTA CGT…-3'
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We used the restriction endonuclease *Bam*HI, deleted 11 bp form 5'-end of K2 cDNA and obtained cDNA lacking the first in-phase start codon (7–9 ATG) carried on plasmid pBamΔ-D [12]. Strain α'1 containing this construct was able to kill sensitive cells, but the mutation led to a reduction in the size of the killing zone around the transformants to 66% (Fig. 2, I, line 4) in comparison with the wild-type plasmid pYEX12 (Fig. 2, I, line 3). However, α'1-pBamΔ-D transformants were still sensitive to K2 toxins produced by Rom-K100 (Fig. 2, II, line 1, C) and M437 (Fig. 2, II, line 1, D) killer strains, as well as to K1 (Fig.2, II, line 1, A, B) and K28 (Fig. 2, II, line 2, A, B, C) toxins, and thus exhibited a greatly reduced immunity. The α'1-pBamΔ-D transformants were immune only to their own toxin (Fig. 2, II, 4) and to that of α'1-pYEX12 transformants (Fig. 2, II, line 3).

We deleted 1–59 bp and 1–81 bp from 5'-end of K2 cDNR by using *Cfr*I and *Pfl*23II restriction endonucleases, respectively. Plate tests indicated that the deletions carried on both pCfrΔ and pPflΔ plasmids clearly led to a defective killer phenotype – transformants showed no killing zones on the lawn of the sensitive strain and were sensitive to the action of killer toxins of all types.

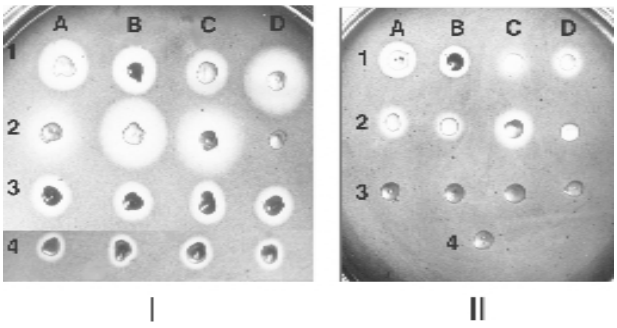

Fig. 2. K2 immunity mutant α'1-pBamΔ-D.
I K2 activity. Test cultures were spotted on a tester α'1 plate. Line 1, A, B – wild type (wt) K1 killer strains, K7, DBY4975; line 1, C, D – wt K2 killer strains, Rom-K100, M437; line 2, A, B, C – wt K28 killer strains, 28, VKM2472, MS300; line 2, D – recipient strain, α'1; line 3 – wt K2 cDNA strain, α'1[pYEX12]; line 4 – pBamΔ-D in a sensitive strain, α'1[pBamΔ-D].
II Immunity. Plasmid pBamΔ-D was transformed into sensitive strain α'1, and the transformed strain was embedded in agar. Then standard tester strains were spotted onto the plate. Line 1, A, B – wt K1 killer strains, K7, DBY4975; line 1, C, D – wt K2 killer strains, Rom-K100, M437; line 2, A, B, C – wt K28 killer strains, 28, VKM2472, MS300; line 2, D – recipient strain, α'1; line 3 – wt K2 cDNA strain, α'1[pYEX12]; 4 – pBamΔ-D in a sensitive strain, α'1[pBamΔ-D]

Thus, elimination of the first initiation codon (plasmid pBamΔ-D) had a dramatic effect on the formation of immunity, but the gene was still expressed. Deletion of 1–59 bp region preceding the second potential initiation codon (plasmid pCfrΔ) resulted in a sensitive phenotype as did the deletion of the second initiation codon (plasmid pPflΔ). These findings enabled us to suppose that the second potential in-frame initiation codon 76–78 ATG could be used as the initiation codon for protein synthesis, and the 11–59 bp region was important as well. This resulted in an assembly of the mutant N-terminal region of the leader sequence, implicating the hydrophilic region (1–26 amino acids) of the leader sequence in the expression of immunity.

Our results indicate some differences in the functional organization of K1 and K2 killer toxins. We have demonstrated that the β-subunit of K2 killer toxin is involved in forming the K2 immunity – mutations in the β-subunit C-terminal-coding region resulted in a sensitive phenotype. The leader sequence of K2 preprotoxin was important in the expression of immunity as well – mutations of the leader peptide-coding region partially or totally inactivated the immunity. We also demonstrated that the hydrophobic C-terminal region of α-subunit was necessary for immunity and toxic functions.

References

1. Schmitt MJ, Eisfeld K. Recent Res Devel Virol 1999; 1: 525–45.
2. Bostian KA, Elliot Q, Bussey H et al. Cell 1984; 36: 741–51.
3. Zhu H, Bussey H. Mol Cell Biol 1991; 11 (1): 175–81.
4. Meškauskas A, Čitavičius D. Gene 1992; 111: 135–9.
5. Dignard D, Whiteway M, Germain D et al. Mol Gen Genet 1991; 227: 127–36.
6. Читавичюс Д, Инге-Вечтомов СГ. Генетика 1972; 1 (1): 95–102.
7. Woodcock DM, Crowther PJ, Doherty J et al. Nucleic Acids Res 1989; 17 (9): 3469–78.
8. Sambrook J, Fritsch EF, Maniatis T. Molecular Cloning: a Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1989.
9. Sherman JF, Fink GR, Hiks JB. Methods in Yeast Genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1986.
10. Ito H, Fukuda Y, Murata K et al. J Bacteriol 1983; 153: 163–8.
11. Наумова ТИ, Наумов ГИ. Генетика 1973; 9 (4): 85–90.
12. Gulbinienė G, Jokantaitė T, Melvydas V. Biologija 2001; 4: 7–9.

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MIELIŲ K2 KILERINIO PREPROTOKSINO GENO FUNKCINĖS ORGANIZACIJOS CHARAKTERISTIKA

S a n t r a u k a

Siekdami nustatyti K2 kilerinio preprotoksino funkciškai svarbias sritis, tyrėme lyderinę seką δ, taip pat α ir β toksino subvienetus koduojančių geno regionų mutacijų fenotipus. Nustatyta, kad *Saccharomyces cerevisiae* K2 tipo kilerinio preprotoksino funkcinė organizacija skiriasi nuo K1 tipo preprotoksino. K2 preprotoksino β subvienetas yra svarbus užtikrinant toksiškumą ir imunitetą. Tuo tarpu K1 preprotoksino β subvieneto mutacijos tik sumažina kilerinio toksino produkciją ir neturi įtakos imunitetui. K2 preprotoksino hidrofilinė 1–26 aminorūgščių seka, esanti prieš signalinį peptidą, dalyvauja imuniteto sudaryme: šios sekos mutacijos lemia tik dalinį atsparumą, arba visišką K2 imuniteto praradimą. K2 preprotoksino α subvieneto C-galinė hidrofobinė sritis (184–205 aminorūgštys) dalyvauja užtikrinant ir imunitetą, ir kilerinį aktyvumą.