GENETIC AND BIOCHEMICAL STUDIES ON THE UREASE ENZYME SYSTEM OF *SCHIZOSACCHAROMYCES POMBE*

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12 residues of the N-terminal sequence, extending the N-terminal sequence to 18 residues. The 18 N-terminal amino acids had 55.6% identity and 83.3% similarity (exact plus conservative replacements) with the jack bean urease N-terminal sequence. The seven amino acids of T21 had 42.9% identity and 100% similarity with the urease from *Klebsiella aerogenes*. Peptide T40 (25 amino acids) had only very poor identity with other sequenced ureases.

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ABSTRACT

Two indicator media were developed to detect urease activity in *Schizosaccharomyces pombe* colonies. These media were more sensitive than previously published media, permitted the rapid identification of urease mutants, were suitable for identifying urease positive transformants and were not affected by amino acid and nucleotide supplements.

Four genes, designated *ure1*, *ure2*, *ure3*, and *ure4*, are required for urease activity in *S. pombe*. Each of the genes was mapped to an approximate genetic location by induced haploidization and meiotic recombination: *ure1* on the left arm of chromosome III, 32 cM from *fur1* and 50 cM from *ade6*; *ure2* on the right arm of chromosome I, 69 cM from *ura2* and 100 cM from *ade4*; *ure3* on the right arm of chromosome I, 31 cM from *ade4* and 91 cM from *ura2*; *ure4* on the left arm of chromosome I, 100 cM from *lys1*.

The lithium chloride method for *S. pombe* transformation was modified to improve the transformation frequency up to 100-fold by using carrier DNA and resuspending the cells in 0.9% NaCl after transformation. Urease mutants for each of the four *ure* genes were transformed with a *S. pombe* gene bank. Three different plasmid clones, each of which specifically complemented one of the *ure1*, *ure3*, or *ure4* mutants, were isolated by complementation of the *ure*$^-$ phenotype. A gene bank clone complementing the *ure2* mutant was not found.

S. pombe urease was purified and characterized. The enzyme was intracellular and only one urease enzyme was detected by non-denaturing PAGE. The urease was purified 3,939-fold, with a 34% yield, by acetone precipitation, ammonium sulfate precipitation and DEAE-Sepharose ion exchange column chromatography. The native enzyme had M_r = 212,000 (Sepharose CL6B-200 gel filtration). One subunit was detected, with M_r =102,000 (SDS-PAGE), indicating the undissociated enzyme contains two identical subunits. The specific activity was 709 μmol urea per min/mg protein. The enzyme was stable between pH 5.0 and pH 9.0. The optimum pH range for enzyme activity was pH 7.5 - pH 8.5. The K_m for urea was 1.03 mM. The sequences of the amino-terminus and three tryptic peptides of the enzyme were determined: N-terminus - Met Gln Pro Arg Glu Leu His Lys Leu Thr Leu His Gln Leu Gly Ser, peptide T21 - Phe Ile Glu Thr Asn Glu Lys, peptide T40 - Leu Tyr Ala Pro Glu Asn Ser Pro Gly Phe Val Glu Val Leu Glu Gly Glu Ile Glu Leu Leu Pro Asn Leu Pro, peptide T43 - Glu Leu His Lys Leu Thr Leu His Gln Leu Gly Ser Leu Ala. The sequence of T43 overlaps the last

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