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Use of novel selenomethionine-resistant yeast to produce selenomethionyl protein suitable for structural analysis

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Introduction

Proteins whose methionine residues are replaced with selenomethionine (SeMet) are useful for solving phase problems for X-ray crystallography by SAD or MAD phasing methods. Expression of SeMet derivatives has been achieved routinely in *Escherichia coli*, however, eukaryotic proteins that receive post-translational modifications, such as glycosylation, often failed to be expressed as functional proteins. Studies have reported the production of SeMet derivatives in eukaryotic cells, such as CHO cells, insect cells and yeasts. Most of the reports presented methodology for SeMet substitution in each host; however, SeMet toxicity on recombinant protein expression was rarely studied. Unlike higher eukaryotic cells, yeasts have the potential to minimize or eliminate SeMet toxicity through SeMet-resistant mutation.

In budding yeast *Saccharomyces cerevisiae,* SeMet-resistant mutants have been isolated. However, such mutants showed enhanced sulfate assimilation [1]. This phenomenon is not suitable for SeMet incorporation because of inhibition by newly synthesized Met. Therefore, it is important to isolate SeMet-resistant mutants without enhanced sulfate assimilation. Here, we report the isolation of SeMet-resistant yeast *Pichia pastoris* that is suitable for SeMet incorporation into the recombinant protein.

Results

P. pastoris wild-type cells did not readily proliferate in the presence of 3.13 μg/ml SeMet on synthetic medium lacking Met (SC-Met), and did not grow at all in the presence of 12.5 μg/ml SeMet. By subculturing wild-type cells on medium containing SeMet, mutants that grew on SC-Met medium containing 12.5 μg/ml SeMet were isolated. Because SeMet-resistant mutants with an enhanced ability to assimilate sulfate were isolated by a spontaneous mutation in *S. cerevisiae* [1], it is likely that the mutants we obtained also increase the Met pools. Such mutants, unlike wild-type cells, possess a sensitivity toward selenate, which is used as a toxic sulfate analogue [2]. A selenate resistance assay revealed that most of the SeMet-resistant mutants were sensitive to selenate. A strain, SMR-94, possessed resistance to selenate, and the sulfate assimilation ability of the strain was probably negligible.

To assess the mutant availability, human lysozyme (HLY) was expressed by the mutant under the control of constitutive promoter. The productivity of SeMet derivatives of HLY was equal or slightly less than the SeMet-unlabeled HLY produced by parental wild-type cells. An incorporation rate of SeMet into HLY was estimated to be 65% based on the amino-acid analysis.

Next, we attempted to determine the crystal structure of SeMet-labeled HLY by SAD phasing method. The X-ray absorption spectrum of the crystal showed the selenium *K* edge at 0.97929 Å; X-ray diffraction data then were collected at that wavelength. The two selenium sites were clearly identified by the SHELXD program and used for phase calculation. The electron density map obtained after phase refinement was clear enough for model building (Fig. 1A). Indeed, the ARP/wARP program was able to trace 95% of the HLY polypeptide chain. To confirm the sites of selenium atoms in HLY molecules expressed by SMR-94 cells, an anomalous difference Fourier map was created (Fig. 1B). The electron densities contoured at 10σ were observed at the sulfur atoms of both Met residues, indicating that replacement of Met with SeMet using SMR-94 cells was sufficient for SAD phase determination.

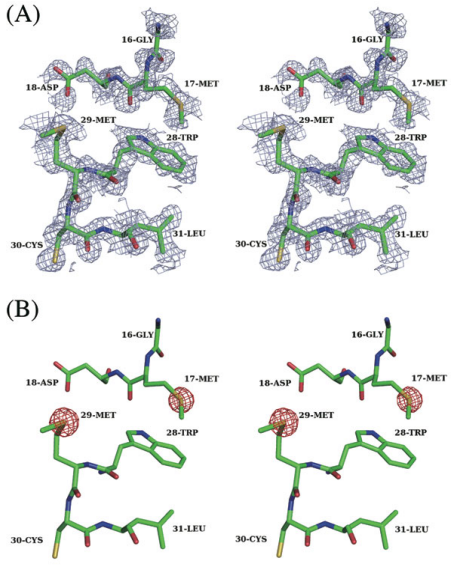

Fig. 1. Stereoview of electron density maps with the refined HLY model (stick representation). (A) A SAD experimental electron density map contoured at 1.0σ. (B) An anomalous difference Fourier map contoured at 10σ.

References

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