Structure-based molecular design for thermostabilization of *N*-acetyltransferase Mpr1 involved in a novel pathway of L-arginine synthesis in yeast

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Previously, *N*-Acetyltransferase Mpr1 was suggested to be involved in a novel pathway of L-arginine biosynthesis in yeast. Our recent crystallographic analysis demonstrated that the overall structure of Mpr1 is a typical folding among proteins in the Gcn5-related *N*-acetyltransferase superfamily, and also provided clues to the design of mutations for improvement of the enzymatic functions. Here, we constructed new stable variants, Asn203Lys- and Asn203Arg-Mpr1, which exhibited 2.4-fold and 2.2-fold longer activity half-lives than wild-type Mpr1, respectively, by structure-based molecular design. The replacement of Asn203 with a basic amino acid was suggested to stabilize α -helix 2, which is important for the Mpr1 structure, probably by neutralizing its dipole. In addition, the combination of two amino acid substitutions at positions 65 and 203 in Mpr1, Phe65Leu, which was previously isolated by the screening from PCR random mutagenesis library of *MPR1*, and Asn203Lys or Asn203Arg, led to further stabilization of Mpr1. Our growth assay suggests that overexpression of the stable Mpr1 variants increase L-arginine synthesis in yeast cells. Our finding is the first report on the rational engineering of Mpr1 for thermostabilization and could be useful in the construction of new yeast strains with higher L-arginine synthetic activity and also improved fermentation ability.

Keywords: L-arginine biosynthesis/*N*-acetyltransferase Mpr1/structure-based molecular design/thermostability/yeast.

Abbreviations: AZC, L-azetidine-2-carboxylic acid; CHOP, *cis*-4-hydroxy-L-proline; GNAT, Gcn5-related *N*-acetyltransferase; GSA, L-glutamate- γ -semialdehyde; IPTG, isopropyl- β -D-thiogalactopyranoside P5C, L- Δ^1 -pyrroline-5-carboxylate; WT, wild-type.

L-azetidine-2-carboxylic acid (AZC) through its *N*-acetylation in the yeast *Saccharomyces cerevisiae* Σ 1278b strain (1, 2) (Fig. 1A). *Saccharomyces cerevisiae* Σ 1278b strain has two genes, *MPR1* and *MPR2* (sigma1278b genes for proline-analog resistance) encoding Mpr1 and Mpr2, respectively. Although there is one base change encoding a single amino acid substitution between two genes (Gly and Glu at position 85 in *MPR1* and *MPR2*, respectively), these genes have similar functions in AZC resistance (1). Genes homologous to *MPR1* have been found in the genomes of various yeasts and fungi, and AZC acetyltransferase activity has been detected in many kinds of yeasts, suggesting that the Mpr1 homologues are widely conserved in yeasts and fungi (3, 4). In addition, Mpr1 was shown to convert *cis*-4-hydroxy-L-proline (CHOP) into *N*-acetyl-CHOP, which has potential to be a new anticancer prodrug (5). However, it is unlikely that AZC and/or CHOP are physiological substrates for Mpr1 because they occur only in certain plants (6–8). Interestingly, our study suggested that Mpr1 is involved in a novel L-arginine biosynthesis pathway (9). It was also suggested that Mpr1 acetylates L- Δ^1 -pyrroline-5-carboxylate (P5C) or its tautomer L-glutamate- γ -semialdehyde (GSA) and then provides *N*-acetyl-GSA, which is an intermediate of L-arginine biosynthesis pathway, promoting it in yeast (Fig. 1B) (9). Previously, the addition of L-arginine was shown to increase the yeast performance such as the fermentation rate (10, 11). These phenomena raise the possibility that the enhancement of L-arginine synthesis mediated by Mpr1 also leads to the construction of new yeast strains with improved fermentation rate.

Previously, we isolated an Mpr1 variant with higher thermostability, Phe65Leu-Mpr1, by the screening from PCR random mutagenesis library of *MPR1* (12). Recently, we determined the three-dimensional structure of Mpr1 by crystallographic analysis (13), and showed that the folding pattern of Mpr1 is similar to those of proteins in the Gcn5-related *N*-acetyltransferase (GNAT) superfamily, to which Mpr1 also belongs. The crystal structure of Mpr1 helped us to understand the mechanism by which Phe65Leu substitution increased its thermostability. It was suggested that the substitution of Phe65, whose side chain is positioned in the hydrophobic region, to Leu improves the packing of the hydrophobic side-chains in this region, leading to the higher thermostability. As described above, the newly clarified crystal structure should assist in the design of new Mpr1 variants with improved enzymatic properties.

N-Acetyltransferase Mpr1 was originally discovered as an enzyme which detoxifies the toxic proline analogue

Fig. 1 Reaction mediated by Mpr1. (A) Scheme for the AZC acetyltransferase reaction by Mpr1. (*) L-Arginine biosynthesis mediated by Mpr1. Mpr1 might convert L- Δ^1 -pyrroline-5-carboxylate (P5C)/L-glutamate- γ -semialdehyde (GSA) into N-acetyl-GSA, which is the intermediate of L-arginine synthesis by its reaction. Protein names: Arg2, L-glutamate N-acetyltransferase; Arg5, N-acetyl-L-glutamyl-phosphate reductase; Arg6, N-acetyl-L-glutamate kinase; Arg8, N-acetyl-L-ornithine aminotransferase.

Here, we constructed stable Mpr1 variants by a rational design based on its crystal structure. Substitution of Asn203 to a basic amino acid was suggested to stabilize α -helix 2, which is important for the whole structure of Mpr1, probably by neutralizing its dipole moment, leading to an increase in the thermostability of Mpr1. In addition, we constructed other stable Mpr1 variants with double substitutions, Phe65Leu/Asn203Lys- and Phe65Leu/Asn203Arg-Mpr1. It was also suggested that expression of these Mpr1 variants enhanced the L-arginine biosynthesis in yeast. These findings will lead to the construction of new yeast strains with increased L-arginine synthetic activity and fermentation rate.

Materials and Methods

Strains, plasmids and culture media

The *Escherichia coli* strains DH5 α (F[−], Φ 80d Δ lacZ Δ M15, Δ (lacZYA-argF)U169, *deoR*, *recA1*, *endA1*, *hsdR17*(r_K[−], m_K[−]), *phoA*, *supE44*, λ [−], *thi-1*, *gyrA96*, *relA1*) and BL21(DE3) (F[−] *ompT* *hsdS_B* (r_B[−]m_B[−]) *gal dcm* (DE3)) were used for the construction of plasmids and for the expression and purification of recombinant Mpr1 proteins, respectively. The yeast *S. cerevisiae* strains with a Σ 1278b background used in this study were wild-type (WT) strain L5685 (*MATa ura3-52 trp1 MPR1 MPR2*) and *arg2*-disrupted strain L5685 Δ arg2 (*MATa ura3-52 trp1 MPR1 MPR2 arg2::hph*).

To overexpress the WT and variant Mpr1 in *E. coli*, the pQE2 vector (Qiagen) harbouring *MPR1* in its *SacI*-*HindIII* site under the isopropyl- β -D-thiogalactopyranoside (IPTG) inducible T5 promoter, pQE2-MPR1(*SacI*-*HindIII*), was used. The high-copy-number plasmid pAG426GPD-ccdB (Novagen), which contains the *S. cerevisiae* *URA3* gene, was used to overexpress Mpr1 under control of the *GPD1* promoter. The WT and mutant *MPR1* genes were first cloned into pDONR221 (Invitrogen) by PCR and BP reaction using a Gateway Cloning System (Invitrogen), generating pDONR221-MPR1, and then cloned into pAG426GPD-ccdB by an LR reaction using the Gateway Cloning System (Invitrogen), according to the manufacturer's protocols. The resultant plasmids, pAG426GPD-MPR1 for expression of the WT or variant Mpr1, were introduced into the L5685 Δ arg2 strain for L-arginine synthesis assay *in vivo*. Site-directed mutagenesis into *MPR1* was performed using mutagenic primers (Supplementary Table S1) and pQE2-MPR1(*SacI*-*HindIII*) or pDONR221-MPR1 as a template.

To express Mpr1 in *E. coli*, M9+CA medium containing 0.4% (wt/vol) glucose, 65 mM sodium/potassium phosphate, 8.6 mM NaCl, 18.7 mM ammonium chloride, 1 mM MgSO₄, 100 μ g/ml ampicillin and 2% (wt/vol) casamino acid was used. The media used for *S. cerevisiae* were SC consisting of 2% (wt/vol) glucose as a sole carbon source, 0.67% (wt/vol) yeast nitrogen base without amino acids (Difco), 0.002% (wt/vol) adenine, 0.04% (wt/vol) L-leucine, 0.0008% (wt/vol) *p*-aminobenzoic acid, 0.008% (wt/vol) uracil, L-arginine, L-aspartic acid, L-glutamine, glycine, inositol, L-methionine, L-phenylalanine, L-serine, L-tryptophan, L-alanine, L-asparagine, L-cysteine hydrochloride, L-glutamic acid, L-histidine, L-isoleucine, L-lysine, L-proline, L-threonine, L-tyrosine and L-valine, and drop-out mix lacking uracil and L-tryptophan (SC-Ura-Trp) or lacking uracil, L-tryptophan and L-arginine (SC-Ura-Trp-Arg). To prepare solid medium, 2% (wt/vol) agar was added.

Protein expression and purification

The Mpr1 protein was overexpressed as previously reported (13). Briefly, the *E. coli* strain BL21(DE3) harboring pQE2-MPR1(*SacI*-*HindIII*) was cultured at 37°C in M9+CA medium. When the absorbance of the culture at 600 nm reached 0.8, Mpr1 was overexpressed by induction with 0.1 mM IPTG at 18°C for an additional 18 h. The bacterial cell pellet was washed by and suspended in 20 mM sodium phosphate buffer (pH 7.4) containing 500 mM NaCl (buffer A) and then disrupted by sonication using Sonifier 450 (BRANSON). The cell lysate was subjected to centrifugation at 6,000 $\times$ g for 20 min and filtration to remove cell debris. The resulting cell-free extract was applied to a Ni-Sepharose 6 Fast Flow (GE Healthcare Bio-Sciences) equilibrated with buffer A, contaminant proteins were washed away using buffer A containing 80 mM imidazole, and then the bound proteins were eluted with buffer A containing 500 mM imidazole. Protein concentrations were determined by Bradford assay using bovine serum albumin as a standard protein.

Acetyltransferase assay

The acetyltransferase activity of Mpr1 was assayed as described previously (13) using a DU-800 spectrophotometer (Beckman Coulter) with slight modifications. The reaction mixture contained 50 mM Tris-HCl (pH 7.5), 5 mM AZC, 100 μ M acetyl-CoA (AcCoA) and an appropriate amount of enzyme. One unit of enzymatic activity was defined as the amount of enzyme catalysing the degradation of 1 μ mol of AcCoA per minute.

Evaluation of thermostability

Thermostability of the WT and variant Mpr1 were evaluated by measuring the remaining activity after incubation at 50°C. The concentrations of purified enzymes were adjusted to 0.25 mg/ml by buffer A containing 500 mM imidazole. Each enzyme solution was incubated at 50°C and the specific activity was measured as described above at the indicated time point. The remaining activity was calculated by the activity at 0 min as 100%. The half-life time of the remaining activity for WT, Asn203Asp, Asn203Glu, Asn203Lys and Asn203Arg was calculated by the curve-fitting method following Eq.1, assuming that their time courses follow this curve.

$$A = A_0 \times e^{-kt} \quad (1)$$

Here, A is the remaining activity, A_0 is the initial activity (100%), k is the kinetic constant for denaturation and t is the incubation time.

Mpr1-dependent L-arginine synthesis *in vivo*

The L5685 Δ arg2 strain harboring pRS414 and pAG426GPD-ccdB or expressing the WT or variant Mpr1 was grown in SC-Ura-Trp medium until the stationary phase, then washed twice with distilled water. The serial dilutions were spotted onto an SC-Ura-Trp or SC-Ura-Trp-Arg plate and cultured at 30°C.

Results

Enhancement of the thermostability of Mpr1 by structure-based molecular design

Our previous studies demonstrated that α -helix 2 (α 2) containing Phe65 is important for the stability of Mpr1 (12, 13). Therefore, we designed amino acid

substitutions to stabilize $\alpha 2$. From the crystal structure, the side chain of Asn203 interacts with the carbonyl oxygen of the Glu70 and Ser71 backbones in $\alpha 2$, suggesting this side chain stabilizes $\alpha 2$ by neutralizing its dipole (Fig. 2). An Asn residue at position 203 was replaced with a basic amino acid, lysine or arginine, to enhance the ability to neutralize the negative charge of the dipole.

First, we measured the specific activity of the WT and variant Mpr1 purified from *E. coli* cells as a recombinant enzyme using AZC as a model substrate of Mpr1 *in vitro* (Fig. 3A). All variants showed a similar degree of activity, although Phe65Leu-Mpr1 and Asn203Lys-Mpr1 exhibited slightly lower and higher activity than the WT-Mpr1, respectively, indicating that the mutations examined in this study were not critical for the specific activity itself. Next, we evaluated the thermostability of the WT and variant Mpr1 by measuring the remaining activity after incubation at 50°C (Fig. 3B). The results showed that the remaining activity of each variant decreased with the incubation time. The half-life of the activity of Phe65Leu-Mpr1 (32.2 ± 9.0 min) was longer than that of the WT-Mpr1 (11.4 ± 4.8 min), as previously reported (12). Interestingly, Asn203Lys-Mpr1 and Asn203Arg-Mpr1 similarly exhibited 2.4-fold (27.0 ± 1.0 min) and 2.2-fold (23.8 ± 4.8 min) longer activity half-lives than WT-Mpr1. On the other hand, the Asn203Asp substitution reduced the activity half-life (4.2 ± 1.1 min) by 2.6-fold compared to that of the WT-Mpr1. These results indicate the introduction of positively charged residues at this position increases the thermostability of Mpr1, whereas the introduction of a negative charge at this position decreases it, probably because neutralization of the negative charge of the dipole moment stabilizes $\alpha 2$, leading to stabilization of the whole protein structure as expected (Fig. 4A and B). Unexpectedly, the Asn-to-Glu substitution at position 203 increased the activity half-life (16.3 ± 2.1 min), even though the negative charge of Glu induces an electrostatic repulsion like Asp, indicating that this substitution stabilizes the Mpr1 structure by an unknown mechanism.

Our previous study revealed that Mpr1 forms a dimer in solution (13). In the crystal structure, Asn203 is located at the interface of two molecules of Mpr1, which seem to be a pair of dimers. Asn203 might interact with Arg129 on the other protomer to contribute to formation of a dimer, even though the distances between them are varied from 3.1 Å to 6.2 Å on each dimer in the asymmetry unit of the crystal structure of Mpr1 with PDB ID code of 3W6X (Supplementary Fig. S1A). Some proteins in the GNAT superfamily exert their functions as oligomers (14). To exclude the possibility that a Glu residue introduced at position 203 enhances the thermostability by promoting dimer formation through an electrostatic interaction with Arg129 on the other protomer, we analysed the oligomeric state of Mpr1 by native-PAGE (Supplementary Fig. S1B). Two bands were observed for each variant, one corresponding to a monomer and the other to a dimer. Both bands for WT-Mpr1 appeared to weaken with increasing

Fig. 2 Stabilization of α -helix 2 containing Phe65 by Asn203. In the crystal structure of Mpr1, the side chain of Asn203 interacts with the carbonyl oxygen of the Glu70 and Ser71 backbones in α -helix 2 ($\alpha 2$) to stabilize it by neutralizing the negative charge of the dipole moment. Mpr1 structure is shown in a cartoon model. Some residues or the substrate CHOP are shown in a stick model or sphere model, respectively. Electrostatic charge of the dipole moment of $\alpha 2$ is shown by $\delta +$ and $\delta -$. The numbers show the distances of hydrogen bonds (Å).

incubation time, probably because the proteins denatured at high temperature were no longer soluble and electrophoresed. Bands for two highly stable variants, Asn203Lys- and Asn203Arg-Mpr1, were still observed even after the incubation for 30 min. In contrast, an unstable variant, Asn203Asp-Mpr1, rapidly disappeared after the incubation for 15 min. Asn203Glu-Mpr1 exhibited an electrophoresis pattern similar to that of WT-Mpr1. Although the results of the native-PAGE seemed to reflect the thermostability of each variant, the ratios of monomer and dimer did not change with the incubation time for each variant, indicating that the effects of these amino acid substitutions on the thermostability did not arise through an intermolecular interaction. Therefore, it was suggested that the stability of Asn203Glu-Mpr1 is enhanced by an unidentified intramolecular interaction.

Construction and characterization of the Mpr1 variants with additional stabilization enhancements

Based on our previous report that Phe65Leu-Mpr1 is more stable than WT-Mpr1 (12, 13), we constructed and evaluated new variants with double substitutions, Phe65Leu/Asn203Lys- or Phe65Leu/Asn203Arg-Mpr1. These variants showed a slightly lower activity than WT-Mpr1; Phe65Leu/Asn203Arg-Mpr1 in particular exhibited a 36% reduction in activity from

Fig. 3 Specific activities and thermostabilities of the Mpr1 variants at position 203. (A) The specific activity of each Mpr1 variant was determined. (B) The remaining activity after incubation at 50°C was plotted with the incubation time. The values are the means and standard deviations of at least two independent experiments.

Fig. 4 Mechanism for the stabilization of destabilization by the introduction of amino acid residues at position 203. The crystal structures of (A) Asn203Lys-, (B) Asn203Arg-, (C) Asn203Glu- and (D) Asn203Asp-Mpr1. The Mpr1 structure is shown in a cartoon model. Some residues or the substrate CHOP are shown in a stick model or sphere model, respectively. The numbers show the distances of hydrogen bonds (Å).

WT-Mpr1 (Fig. 5A). On the other hand, these two substitutions at positions 65 and 203 drastically increased the thermostability of Mpr1 so that the curves of inactivation versus incubation time for these variants did not follow Eq. 1 (Fig. 5B). Therefore, we succeeded in constructing highly stable variants of Mpr1 with double substitutions.

Effects of the stable Mpr1 variants on L-arginine synthesis in yeast

To evaluate the effects of stable Mpr1 variants isolated here on L-arginine synthesis *in vivo*, we performed spot

assays using *S. cerevisiae* L5685Δarg2 harboring an empty vector or overexpressing the WT- or variant Mpr1 on SC-Ura, Trp medium in the presence or absence of L-arginine. The *ARG2* gene encodes N-acetylglutamate synthase, which acetylates L-glutamate as the first step of L-arginine synthesis from L-glutamate. The disruption of *ARG2* does not cause L-arginine auxotrophy because the genomic *MPR1/2* genes can function alternatively (9); however, the growth of the Δarg2 strain on L-arginine-depleted medium was much slower than that of the WT strain (data not shown). Overexpression of WT-Mpr1 in the

Fig. 5 Specific activities and thermostabilities of the Mpr1 variants with double amino acid substitutions at positions 65 and 203. (A) The specific activity of each Mpr1 variant was determined. (B) The remaining activity after incubation at 50°C was plotted with the incubation time. The values are the means and standard deviations of at least three independent experiments.

L5685 Δ arg2 strain increased its growth rate on L-arginine-depleted medium, although the growth of this mutant on L-arginine-containing medium was almost the same, suggesting that the overexpression of Mpr1 enhances L-arginine synthesis in yeast (Supplementary Fig. S2). The subsequent spot assay showed that overexpression of Phe65Leu-, Asn203Lys-, Phe65Leu/Asn203Lys-, or Phe65Leu/Asn203Arg-Mpr1 accelerated the growth of L5685 Δ arg2 on L-arginine-depleted medium compared with WT-Mpr1 without any difference in the growth on L-arginine-containing medium (Fig. 6), indicating that stabilization of the Mpr1 structure greatly contributes to L-arginine synthesis *in vivo*, even though the mechanism is still unclear.

Discussion

We previously isolated a stable variant, Phe65Leu-Mpr1, by the screening from PCR random mutagenesis library of *MPR1* (12). A recent structural analysis clarified the mechanism by which the substitution of Leu for Phe65 increases the thermostability of Mpr1, and also provided clues to the design of novel mutations for improvement of the enzymatic functions (13). Here, we successfully constructed new stable Mpr1 variants by structure-based molecular design, and indicated that some of them had increased abilities to contribute to L-arginine biosynthesis. This is the first report on the rational engineering of Mpr1 and to show that the stability of Mpr1 is important for L-arginine synthesis *in vivo*. Our finding in this study could lead to the construction of new industrial yeast strains with higher L-arginine synthetic activity.

The replacement of Asn203 with lysine or arginine stabilized the Mpr1 structure (Fig. 3B), probably because the introduction of positively charged amino acids at that position neutralized the dipole moment in α 2 more effectively than the original residue, asparagine, in WT-Mpr1 (Supplementary Fig. S2A and B).

Fig. 6 Effects of the Mpr1 variants on L-arginine synthesis in yeast. *Saccharomyces cerevisiae* L5685 Δ arg2 strains harboring an empty vector or overexpressing the WT or variant Mpr1 were cultured, and the serial dilutions were spotted onto SC-Ura-Trp or SC-Ura-Trp-Arg solid medium, and then incubated at 30°C. The pictures for SC-Ura-Trp or SC-Ura-Trp-Arg were taken after incubation for 3 or 8 days, respectively.

However, the Glu substitution at position 203 also stabilized Mpr1, even though the thermostability of Asn203Asp-Mpr1 was lower than that of WT-Mpr1, probably due to the electrostatic repulsion of aspartate with the dipole of α 2. Our oligomeric state analysis suggests that the Asn203Glu substitution stabilizes Mpr1 through an unidentified intramolecular interaction. It is possible that a glutamate residue introduced at position 203 interacts with Lys73 positioned near α 2 (3.1 Å) (Fig. 4C), whereas Asp203 is unlikely to interact with this residue because the distance is too far (4.2 Å) (Fig. 4D). The interaction between Glu203 and Lys73 is suggested to increase the stability of Mpr1 enough to compensate for the destabilization induced by its electrostatic repulsion with the dipole in α 2 and/or the omission of the stabilization by the original residue asparagine.

We also constructed more stable variants by the combination of Asn203Lys or Asn203Arg with Phe65Leu, which has been reported previously (12). All of the single Mpr1 variants including Phe65Leu-

Mpr1 showed a similar pattern in denaturation with the incubation time at 50°C, following Eq. 1. However, two variants with double mutations, Phe65Leu/Asn203Lys- and Phe65Leu/Asn203Arg-Mpr1, did not show the inactivation curve based on Eq. 1. These variants kept almost 80% of their activity after incubation for 50 min, and then were denatured quickly. These double mutations might affect the maintenance of the structure and/or stability of Mpr1 through unknown mechanisms.

Overexpression of the Mpr1 variants increased the growth of the *S. cerevisiae* $\Delta arg2$ strain on L-arginine-depleted medium; expression of the stable variant Mpr1 enhanced the growth more than expression of WT-Mpr1 (Fig. 6). The expression of the stable Mpr1 variants could increase the ability to synthesize L-arginine in yeast, although there was no significant difference in intracellular L-arginine contents among the strains overexpressing WT-Mpr1 or stable Mpr1 variants (data not shown). The L-arginine biosynthesis might be the rate-limiting step for growth in these strains because of the disruption of the *ARG2* gene, as suggested by the result of our growth assay on SC medium with or without L-arginine (Fig. 6). It is possible that L-arginine synthesized via the Mpr1-dependent pathway is used for cell growth, suggesting that L-arginine is not accumulated in yeast cells overexpressing stable Mpr1 variants even if they had the higher ability to produce L-arginine. However, the mechanism by which the stabilization of Mpr1 enhances L-arginine biosynthesis is still unclear. When using an artificial expression system, the level of transcription and translation should be the same among variants. Therefore, the stabilization of Mpr1 might confer resistance against its protease-dependent degradation *in vivo*, because the thermostability of the protein is correlated with protease resistance (15). Further analyses are required to clarify the mechanism by which the amount of Mpr1 is controlled through protease-dependent degradation under L-arginine-depleted conditions. An improved understanding of this mechanism could lead to further enhancements of Mpr1-dependent L-arginine biosynthesis. Expression of Asn203Arg-Mpr1 did not increase the growth on L-arginine-depleted medium, even though Asn203Arg-Mpr1 was much more thermostable than WT-Mpr1 and had the same enzymatic activity as WT-Mpr1. There might be a threshold level of thermostability *in vitro*, which will enhance L-arginine synthesis *in vivo*, because Asn203Arg-Mpr1 showed the shortest half-life time among the five stabilized mutants, whose effects on L-arginine biosynthesis were examined (Fig. 6). In contrast, the Mpr1 activity *in vitro* was measured using AZC as a model substrate, raising the possibility that the Asn203Arg substitution affects *N*-acetyltransferase activity of Mpr1 *in vivo* for the intracellular substrate.

Another substrate for the reaction by Mpr1, CHOP, which is a collagen metabolism modulator (16, 17), has potential to be a new anti-cancer drug (18). However, the lower solubility of CHOP to water limits its therapeutic application because it is rapidly excreted, and thus chemical modifications to enhance its water

solubility are required. Conjugation of CHOP with polyethylene glycol has been examined as a means of increasing its water solubility (19). Recently, production systems for *N*-acetyl-CHOP, which is another chemically modified form of CHOP, were developed *in vitro* and *in vivo* (5, 20). The construction of a highly stable variant Mpr1 might lead to more efficient production and further analyses of *N*-acetyl-CHOP as a prodrug.

It was reported that L-arginine used as the sole nitrogen source increased the fermentation rate more effectively than ammonium sulphate or L-glutamine (11). Furthermore, the addition of L-arginine increased the fermentation rate of yeast (10). These phenomena suggest that the synthesis of L-arginine promote the fermentation performance. Our finding can be applied to construct new yeast strains with enhanced L-arginine synthetic activity, therefore, might lead to the improvement of the fermentation rate.

Supplementary Data

Supplementary Data are available at *JB* Online.

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Conflict of Interest

None declared.

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