Efficient Antibody Production upon Suppression of O Mannosylation in the Yeast *Ogataea minuta*^♀^  

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When antibodies were expressed in the methylotrophic yeast *Ogataea minuta*, we found that abnormal O mannosylation occurred in the secreted antibody. Yeast-specific O mannosylation is initiated by the addition of mannos at serine (Ser) or threonine (Thr) residues in the endoplasmic reticulum via protein O mannosyltransferase (Pmt) activity. To suppress the addition of O-linked sugar chains on antibodies, we examined the possibility of inhibiting Pmt activity by the addition of a Pmt inhibitor during cultivation. The Pmt inhibitor was found to partially suppress the O mannosylation on the antibodies. Surprisingly, the suppression of O mannosylation was associated with an increased amount of assembled antibody (H2L2) and enhanced the antigen-binding activity of the secreted antibody. In this study, we demonstrated the expression of human antibody in *O. minuta* and elucidated the relationship between O mannosylation and antibody production in yeast.

Antibodies for pharmaceutical applications have recently received a great deal of attention due to their specific antigen-binding activities, antibody-dependent cellular cytotoxicity, and other useful characteristics. Indeed, sales of antibodies for pharmaceuticals have grown rapidly over the past decade and are expected to exceed 30 billion U.S. dollars by 2010 (1). A large volume of antibodies is required for pharmaceuticals, as antibodies are primarily marketed for chronic conditions. Antibodies possess relatively low potency, which results in the need for accumulating doses over time (10). Although therapeutically antibodies have great potential value, they have also been viewed with some skepticism. Antibodies are expensive to produce, in part because they are commonly manufactured by using batch/fed-batch cultures of mammalian cells. The increasing demand to reduce the cost of antibody production has promoted research into the development of an antibody expression system that is more practical than expression with mammalian cells.  

Development of transgenic plants and animals as alternative hosts is therefore a promising field of study. Monoclonal antibodies have, thus far, been successfully produced from a number of sources, including plants, the milk of transgenic goats, the eggs of transgenic chickens, etc. (4, 12, 32). Moreover, antibodies derived from certain newly developed transgenic systems share physical characteristics that are similar to those of antibodies from mammalian cells such as Chinese hamster ovary (CHO) cells while exhibiting higher antibody-dependent cellular cytotoxicity activity due to the absence of fucose residues in N-linked sugar chains (6, 45). These alternative transgenic expression systems could reduce the cost of large-scale antibody production. However, the prolonged construction of transgenics remains a major disadvantage in terms of market demands.  

The production of antibodies and antibody fragments has been studied by using various microorganism expression systems, including *Escherichia coli*, fungus, and yeast (7, 13, 37, 41). Since microorganisms are known to possess certain advantages as hosts (e.g., rapid growth rate, high heterologous protein productivity, and inexpensive cultivation costs), they have great potential to overcome some of the current hindrances to antibody production. With these advantages in mind, we focused on the construction of an antibody production system using a methylotrophic yeast, *Ogataea minuta*. For the production of pharmaceutical-grade proteins by yeast, it is necessary to convert the yeast-specific, N-linked, high-mannan-type sugar chain, which is modified after protein translation, into a human-compatible type sugar chain, because high-mannan sugar chains can exhibit antigenicity against humans. Therefore, we previously introduced a novel approach to humanizing the yeast-specific N-linked sugar chain (5), and some researchers have shown the usefulness of a yeast-based system for generating a human-compatible N-linked sugar chain, in a report on antibody production in yeast (25).  

On the other hand, yeast-specific O mannosylation also has to be considered in the context of pharmaceutical manufacture. In yeast, protein O mannosylation is initiated in the
endoplasmic reticulum (ER) by the addition of mannose to Ser or Thr residues, and this reaction occurs via protein O mannosyltransferase (Pmt) (38). O glycosylation in mammalian cells is initiated generally by the addition of N-acetylglactosamine via polypeptide N-acetylglactosaminyltransferase activity in the Golgi apparatus or else by the addition of a mannose via members of the Pomt family, which are homologues of yeast Pmt, in the ER (27, 34). The structure of O-linked sugar chains in yeast differs from that of mammalian cells (18, 43). Moreover, since yeasts possess a mechanism with which to increase the solubility of aberrant proteins by O mannosylation in order to suppress aggregation in the ER, heterologous proteins, which are not O glycosylated in mammalian cells, may suffer from O mannosylation in yeast (19, 29).

Previously, we demonstrated the usefulness of protease-deficient strains of O. minuta for the production of antibodies; in this strain, the O. minuta PEP4 (OmPEP4) and the OmPRBI genes, which code for vacuolar protease, and the OmYPS1 gene, which codes for an aspartic protease, were disrupted (23). Additionally, we recently found that abnormal O mannosylation occurred in antibodies secreted from O. minuta. To the best of our knowledge, there have been no reports of O mannosylation occurring in antibodies produced by mammalian cells. For the production of pharmaceuticals, it is generally considered that yeast-specific O-linked sugar chains have to be removed from antibodies, because some mannoses added by Pmt enzymes might possess immunogenicity against humans. To suppress the addition of O-linked sugar chains to antibodies, we examined the suppression of Pmt activity by the addition of a Pmt inhibitor during cultivation.

In the present study, we report the relationship between O mannosylation and antibody production in yeast and discuss the possibility that yeast could be used to produce antibodies identical to those from mammalian cells via the suppression of O mannosylation.

MATERIALS AND METHODS

Yeast strain and media. Cells of O. minuta YK6 (Δomh1 Δomh3 Δomh3 Δomh1), the host strain used in this study, were described as described in our previous study (23). The yeast cells were cultured at 27°C in the following media: YPG medium (containing the following components per liter, 40 g of peptone, 20 g of yeast extract, 13.4 g of glucose, 20 ml of glycerol), and 2× BYPG medium (containing the following components per liter, 40 g of peptone, 20 g of yeast extract, 33.4 g of yeast nitrogen base [YNB] without amino acids, 100 ml of 0.1 M phosphate buffer [pH 6.0], 40 ml of glycerol, 0.2 g of uracil, 0.2 g of adenine, and 0.4 mg of biotin). Transformants were selected on SG agar plates (containing the following components per liter, 6.7 g of YNB without amino acids, 10 ml of glycerol, and 20 g of agar).

DNA methods. E. coli DH5α cells were used for the subcloning of the plasmids. The plasmids were prepared using a QIAprep Spin miniprep kit (Qiagen, Hilden) from E. coli DH5α cells. DNA fragments were recovered from agarose gel using a QIAquick gel extraction kit (Qiagen). The DNA fragments amplified by PCR were subjected to DNA sequence analysis using a DNA sequencer (model 3700; Applied Biosystems, Foster, CA). The plasmid DNA methods.

Construction of the strain expressing antibody genes. The NotI-digested pOMEU1 plasmid was introduced into O. minuta YK6 cells (Δomh1 Δomh3 Δomh3 Δomh1) to complement the OmUR3 marker gene by means of the electric pulse method described previously (24). The strain, which was obtained by selection on SG-plus-Ura plates (SG agar plates with 0.77 g/liter of pyrimidine phosphoribosyltransferase [PPRT] and 0.77 g/liter of uracil) supplemented with 50 μg/ml HYG, and the introduction of the light chain and the heavy chain genes was confirmed by PCR using a genomic DNA template. The obtained strain, in which both the heavy and the light chain genes were integrated into the genome, was designated O. minuta YK6U-HL (Δomh1 Δomh1).

Cultivation conditions. A rhodamine-3-acetic acid derivative, the Pmt inhibitor used in this study, was designated R3AD and was prepared according to the method described in a report by Orchard et al. (31). The R3AD compound was dissolved in a 10 mM concentration of dimethyl sulfoxide (DMSO). The 2× BYPG3RD medium was prepared by the addition of 10 μl of R3AD to 50 ml of 2× BYPG medium. O. minuta YK6U-HL cells were precultured in 100 ml of YPG medium for 24 h at 27°C. Cells were harvested by centrifugation and grown at an initial optical density at 600 nm of 10 in 50 ml 2× BYPG3RD medium or in 50 ml DMSO, which was the containing 10 μl of R3AD in the BYPG medium as that used in the case of 2× BYPG3RD. The cell density at OD600 was measured at 24 h intervals, and 1 μl of R3AD in DMSO was added to the 2× BYPG3RD medium for every 1-unit increase of cell density at OD600 (e.g., if the detected absorbance at OD600 was 30 in 50 ml of medium, then 20 μl of R3AD was added). As a control, 1 μl of DMSO without R3AD was also added to 2× BYPG medium consecutively for the 1-unit increase of cell density at OD600. These cultures were maintained for 72 h at 27°C. The cells were then harvested by centrifugation, and the supernatants were analyzed as described below.

Western blotting analysis. Twenty microliters of the supernatant or 3 μl of the cell suspension in phosphate-buffered saline (PBS) buffer (Gibco, Grand Island, NY) at a concentration of OD600 50 was subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis, SDS-PAGE) using a Mini-Protean II (Bio-Rad Laboratories, Hercules, CA) in the presence of dithiothreitol (DTT). In order to detect deglycosylated antibody, N-glycanase treatment was carried out according to the manufacturer’s protocol. Samples, including antibodies, were boiled at 100°C for 5 min in the presence of 0.5% SDS and 50 mM 2-mercaptoethanol and were then incubated with 1.25% Nonidet P-40 and N-glycosidase F (Roche, Indianapolis, IN) at 37°C overnight. Samples equivalent to 4.5 μl of supernatant were also subjected to SDS-PAGE under the reducing condition. One microliter of the supernatant or 3 μl of the cell suspension described above was subjected to SDS-PAGE under the nonreducing conditions in the absence of DTT. The polyclinivinylidene difluoride membranes on which the proteins were electroblotted were blocked by using Block Ace (Dainippon Sumitomo Pharma, Osaka, Japan), and the samples were incubated with horseradish peroxidase (HRP)-conjugated goat polyclonal anti-human Fc antibody (1:5,000 dilution; Sigma-Aldrich, St. Louis, MO) and alkaline phosphatase (AP)-conjugated goat anti-human kappa antibody (1:5,000 dilution; Sigma-Aldrich). The antibodies were detected using a Supersignal West Dura kit (Pierce, Rockford, IL) for HRP and an alkaline phosphatase conjugate substrate kit (Bio-Rad Laboratories, Hercules, CA) for AP. The control, human anti-TRAIL-R antibody (hMAb) was produced at Kirin (Takasaki, Japan) by using CHO DG44 cells.

Quantification of the secreted antibody by EnVision. The antibody productivity in supernatants cultivated with or without R3AD was measured using EnVision, based on fluorescent resonance energy transfer (FRET) technology. Supernatants were diluted 20 times in 20 mM Tris-HCl buffer (pH 7.2) including 10% Block Ace. Two microliters of diluted samples were incubated with 12 μl of 20 mM Tris-HCl buffer (pH 7.2) including 0.97 μg/ml LANCE europium (Eu)-
Detection of the heavy and light chains of secreted antibody. Supernatants prepared with and without R3AD from *O. minuta* YK6U-HL (*Δoch1 Δypsl*) cell cultures and the purified antibody from CHO cell cultures were treated with N-glycanase, and the samples were subjected to WB analysis under the reducing conditions in the presence of DTT. HRP-conjugated anti-human Fc antibody (A) and AP-conjugated anti-human kappa antibody (B) were used for antibody detection. Lanes 1, control antibody from CHO cells; 2, control antibody from CHO cells with N-glycanase treatment; 3, supernatant from *O. minuta* YK6U-HL cells; 4, supernatant from *O. minuta* YK6U-HL cells with N-glycanase treatment; 5, supernatant from *O. minuta* YK6U-HL cells with the addition of R3AD; 6, supernatant from *O. minuta* YK6U-HL cells with the addition of R3AD and N-glycanase treatment.

**RESULTS**

Effect of R3AD on O mannosylation in antibody production. *O. minuta* YK6U-HL cells (*Δoch1 Δypsl*), in which light and heavy antibody chains were expressed, were cultivated in 2× BYPG medium at 24°C for 72 h, and the supernatant was subjected to Western blotting (WB) analysis under the reducing condition. The WB analysis revealed that although heavy and light chains of the antibody were secreted from *O. minuta* YK6U-HL cells, the smear signals were detected at a higher molecular weight than that of the corresponding intact heavy and light chains (Fig. 1A and B, lanes 3 and 4). Incidentally, the anti-TRAIL-R antibody used in this study has only one N-glycosylation site in the CH2 region. The smear signals in the heavy chain, which had not completely disappeared after N-glycanase treatment, were thought to indicate that O mannosylation had occurred in the antibodies. Alternatively, since the molecular weight shift of the antibody heavy chain to the lower molecular weight was detected after the N-glycanase treatment in WB analysis (Fig. 1A, lanes 4 and 6), it was confirmed that the secreted antibody had an N-linked sugar chain. Next, the Pmt inhibitor R3AD was added to the culture medium in order to verify its efficacy for inhibiting cell growth and O mannosylation. The inhibitor used in this study was a rhodamine-3-acetic acid derivative designated 1c by Orchard et al. (31). The inhibitor 1c was found to inhibit *Candida albicans* Pmt1p with a 50% inhibitory concentration at a dose of 2.3 μM, and this inhibitor was shown to be effective at inducing morphological changes in *C. albicans* associated with a loss of Pmt activity in vitro. (31). The addition of R3AD had no effect on cell growth (data not shown), and a decrease in smear signals at the higher molecular weight was observed (Fig. 1A and B, lanes 5 and 6). Although the remaining smear signals for both antibody chains indicated that O mannosylation had not been completely suppressed, O mannosylation in the antibody was inhibited by the addition of R3AD. On the other
hand, the signal of the heavy chain detected in the antibody obtained from *O. minuta* YK6U-HL cell cultures with R3AD appeared to be increased. This may not indicate the enhanced secretability of the single heavy chain but the increment of the assembled antibody, because the heavy chain is thought to be secreted after it has assembled with the light chain in the ER (2).

**Suppression of O mannosylation results in an increase in secreted antibody.** Supernatants prepared with and without R3AD were subjected to WB analysis under nonreducing conditions. The amount of secreted assembled antibody (H2L2) was clearly increased in the antibody obtained from *O. minuta* YK6U-HL cell cultures with R3AD (Fig. 2A and B, lanes 2 and 3). This result indicates that the suppression of O mannosylation enhances the secretability of the assembled antibody. Moreover, the increase of the secreted assembled antibody was quantified by EnVision analysis, which detects assembled antibodies in supernatants by measuring the interaction between the Eu-labeled anti-human Fc and biotin-labeled anti-human kappa antibody, using FRET technology (Fig. 3A). The titer of

![FIG. 2. Detection of the secreted assembled antibodies. The supernatants prepared with and without R3AD from *O. minuta* YK6U-HL (*Δoch1 Δyps1*) cell cultures and the purified antibody from CHO cells were subjected to WB analysis under nonreducing conditions in the absence of DTT. HRP-conjugated anti-human Fc antibody (A) and AP-conjugated anti-human kappa antibody (B) were used for antibody detection. Lanes 1, control antibody from CHO cells; 2, supernatant from *O. minuta* YK6U-HL cells; 3, supernatant from *O. minuta* YK6U-HL cells with R3AD added.](http://aem.asm.org/)

![FIG. 3. Quantification of secreted assembled antibodies by EnVision. Schematic diagram of EnVision (A) shows the interaction of Eu-labeled anti-human Fc antibody with the sample antibody under excitation from 360 nm to 615 nm. Fluorescence at 665 nm, which is the emission wavelength of allophycocyanin (APC), was generated by the energy transfer of the wavelength at 615 nm to APC conjugated with streptavidin, which bound to the biotin-labeled anti-human kappa antibody associated with the target antibody. The wavelength generated at 665 nm was measured to quantify the antibody concentration in the supernatant. Supernatants from *O. minuta* YK6U-HL (*Δoch1 Δyps1*) cells were incubated with anti-Fc and anti-kappa antibodies, and the samples were analyzed by EnVision (B). Data are averages from quadruplicate experiments, and error bars are mean maximum and minimum values.](http://aem.asm.org/)
the secreted antibody reached 60 μg/ml, i.e., threefold higher than that of the secreted antibody produced without the addition of R3AD (Fig. 3B).

Increase in the productivity of intracellular antibodies by the suppression of O mannosylation. The intracellular antibodies in O. minuta YK6U-HL cells were detected by WB analysis. Production of both the light and heavy chains of the antibody was enhanced by the suppression of O mannosylation (Fig. 4A and B, lanes 2 and 3). Moreover, the addition of R3AD facilitated the efficient formation of the assembled antibody (H2L2), while only a small amount of assembled antibody (H2L2) was detected in samples prepared without O mannosylation inhibition (Fig. 4C, lanes 2 and 3). The O-mannosylated light and heavy chains of the antibody could be sorted into the protein degradation pathway in the cell, as a result of their inefficient assembly. Alternatively, the secretory capacity of the yeast is not likely to be sufficient to secrete whole assembled antibody formed in the cell with the suppression of O mannosylation.

Effect of O mannosylation on the affinity of the secreted antibody. Antibodies from O. minuta YK6U-HL cells prepared with and without R3AD were purified by means of protein A column chromatography, and the purified products were subjected to FACS analysis to verify their abilities to bind to the antigen, which was displayed on the surface of the L929/DR5ADD cells. Antibodies produced by yeast or CHO cells, which interact with antigen on the cell surface, were detected using R-phycocerythrin-conjugated rabbit polyclonal anti-human kappa antibody. As a result, the suppression of O mannosylation by R3AD enhanced the antibody binding activity (Fig. 5). However, inhibited binding activity was observed for the antibodies from O. minuta YK6U-HL cell cultures with R3AD, compared to that of antibodies from CHO DG44 cells. This is possibly caused by the incomplete suppression of O mannosylation by R3AD or by the O mannosylation due to the remaining activity of some Pmt proteins, which are not suppressed by R3AD.

DISCUSSION

The O mannosylation that occurs in the ER increases the solubility of aberrant proteins to prevent aggregation in yeast cells (29). In this study, we showed that the yeast secreted O-mannosylated antibodies. The antibody is likely to be secreted after the assembly of the light and heavy antibody chains in the ER because the interface between the chains is highly hydrophobic (2, 9). Since the hydrophobic interface causes aggregation in the ER, the antibody may suffer from O mannosylation, which is initially helpful to suppress the protein aggregation in yeast cells. In addition, inefficient secretion of antibody has been observed with yeast cells. It is conceivable that the inhibition of antibody assembly in the ER occurred by the addition of mannoses to the interface of the antibody chains via O mannosylation. Moreover, an increase in intracellular antibody was associated with the suppression of O mannosylation. This result indicated that antibodies with O-linked mannoses cannot assemble efficiently, resulting in their aggregation in the ER, and were then degraded by delivery to vacuoles (23, 44). Secreted antibodies with O mannosylation

FIG. 4. Detection of intracellular antibodies. O. minuta YK6U-HL (Δochl Δyps1) cells resuspended in PBS buffer and purified antibody from CHO cells were subjected to WB analysis under the reducing (A and B) or nonreducing (C) conditions. Antibodies were detected by HRP-conjugated anti-human Fc antibody (A and C) and AP-conjugated anti-human kappa antibody (B). Lanes 1, control antibody from CHO cells; 2, resuspension of O. minuta YK6U-HL cells; 3, resuspension of O. minuta YK6U-HL cells prepared with R3AD.

FIG. 5. FACS binding analysis of the secreted antibodies. The binding activities of the antibodies were evaluated by flow cytometry. L929/DR5ADD cells were incubated with antibodies produced by CHO cells (closed triangles), and O. minuta YK6U-HL cells were treated without R3AD (closed circles) or with R3AD (closed squares). The geometric means of the fluorescence intensities were calculated by CellQuest.

Properties of antibodies produced with and without R3AD. The antibodies purified by protein A column chromatography were subjected to gel permeation column chromatography. The major peak was detected in the high-molecular-weight fraction of the antibody prepared from cultures without R3AD (Fig. 6A). In the case of the antibody prepared from cultures with R3AD, the main peak was detected in the same fraction as that of the antibody derived from CHO cells, although in the former cultures, the polymer fraction was present (Fig. 6B). These results suggest that O mannosylation causes antibody aggregation; however, it is not clear that the aggregation of the antibody detected in the gel permeation column chromatography occurred in the cell. The suppression of O mannosylation enables the production of antibodies at a level similar to that of antibodies from mammalian cells. Although the polymer fraction detected in the antibody prepared from cultures with R3AD could have been due to the residual O-linked mannoses, the usefulness of the R3AD was clearly demonstrated by these results.
exhibited low antigen-binding activity and were of higher molecular weight than expected, whereas antibodies in which \( \text{O} \) mannosylation had been suppressed by R3AD showed increased antigen-binding activity and formation of the oligomeric species (H2L2). In yeast, \( \text{O} \) mannosylation generally stabilizes the endogenous secretory protein (26, 42). However, in antibody secretion, \( \text{O} \) mannosylation could work not as a stabilizer but as a destabilizer by inhibiting the assembly of both chains. These results indicate that with the suppression of \( \text{O} \) mannosylation, yeast can be used to produce antibodies similar to those produced by mammalian cells.

In mass spectrometric analysis of the secreted antibody, \( \text{O} \)-linked sugar chains on light chain in the regions of amino acid residues 1 to 18 and 25 to 45 and on heavy chain in the region of amino acid residues 149 to 211 were detected for the antibody produced in \( O. \ minuta \) YK6U-HL cells without R3AD. On the other hand, the \( \text{O} \)-linked sugar chains on the light chain in the region of amino acid residues 1 to 18 disappeared in the antibody produced with R3AD; however, other \( \text{O} \)-linked sugar chains still existed. Additionally, any \( \text{O} \) mannosylation could not be detected in the Fc region. Since mass peaks corresponding to the fragments of VH1 to CH1 region, as opposed to those of the Fc region, were not clearly detected, the VH-to-CH1 region could be highly \( \text{O} \) mannosylated, forming high-molecular-weight species. The detailed analyses of the \( O. \ minuta \) \( \text{O} \)-linked sugar chains and the \( \text{O} \)-mannosylated Ser and/or Thr residues in the antibody are in progress.

It is possible that other Pmt proteins, not suppressed by R3AD, could be responsible for the remaining \( \text{O} \) mannosylation. Generally, in yeast, Pmt proteins have been categorized as belonging to the Pmt1p, Pmt2p, and Pmt4p subfamilies (17, 33, 39). The Pmt1p subfamily (i.e., Pmt1p and 5p) interacts with the Pmt2p subfamily (i.e., Pmt2p and 3p) to form active heteromeric complexes, while the Pmt4p subfamily forms a homomeric complex (17). Pmt1-2p subfamily complexes have different substrate specificities from that of the Pmt4p subfamily complex (21). Although R3AD was developed as a \( C. \ albicans \) Pmt1p inhibitor and the rhodanine inhibitors were shown to be relatively specific for \( C. \ albicans \) Pmt1p (3), it is not understood whether the inhibitors have an effect on the other Pmt proteins. Some compounds have been developed as \( C. \ albicans \) Pmt1p inhibitors (31). These compounds, designated as 1d and 4a, strongly inhibit the proliferation of \( C. \ albicans \) in the presence of G418, while 1d, 4a, and R3AD are almost equally effective at the inhibition of \( C. \ albicans \) Pmt1p. It is likely that 1d and 4a are more effective than R3AD at suppressing cell wall biosynthesis. Since some Pmt proteins are responsible for cell wall integrity (16), it is possible that these inhibitors inactivate not only \( C. \ albicans \) Pmt1p but also other Pmt proteins that are not inhibited by R3AD. Since triple- or double-knockout \( PMT \) gene strains lead to severe growth defects (16), it is difficult to disrupt the entire \( PMT \) genes, a factor which might be related to \( \text{O} \) mannosylation of the antibody. Therefore, the use of these Pmt inhibitors, which can control the inactivation of the other Pmt proteins (with the exception of Pmt1p) by the additive volume, is thought to be useful in antibody production by yeast.

Some reports have addressed the production of antibody by yeast, although in those studies, productivity was either low or not mentioned (15, 20, 25, 30, 44). For the production of antibodies in yeast, it will be necessary to increase productivity. To attain this goal, the introduction of chaperones may improve the assembly of the antibody in the ER, resulting in efficient secretion. However, introduction of the chaperones was not sufficient to yield high amounts of full-length antibody in yeast, while the overexpression of chaperones such as disulfide isomerase have been reported to contribute partially to the enhancement of the secretability of the antibody fragment (8, 14, 36). In contrast, an increase in secretion was confirmed with several kinds of different antibodies by the addition of R3AD to \( O. \ minuta \) cultures (data not shown). Therefore, the suppression of \( \text{O} \) mannosylation, as shown in this study, is expected to be useful for increasing antibody productivity in yeast; indeed, this is the first report to demonstrate a successful enhancement of secreted full-length antibody in yeast.

The overexpression of the antibody fragment referred to as 4-4-20 scFv induced an unfolded protein response (UPR) (22). Additionally the Pmt inhibitor or the \textit{pmt1} mutant stimulates...
the UPR pathway in *C. albicans* (3). Since the UPR system allows for an increase in the capacity for protein folding, secretion, and degradation by the up-regulation of various genes, to prevent the accumulation of unfolded proteins (40), up-regulation of the UPR pathway could be beneficial for the production of some secretory proteins. However, an increase in the secretion of human insulin-like growth factor was not detected in the pmt-deficient strain in which the UPR may be up-regulated, while improvement in the quality was confirmed (11). Therefore the UPR may not always contribute to the enhancement of the productivity of secretory proteins. On the other hand, the overexpression of whole-antibody genes may also induce the UPR and result in an enhancement of O mannosylation in the antibody because up-regulation of *PMT* genes occurs in the UPR (40). Therefore, the inactivation of Pmt proteins could improve the quality and secretability of the overexpressed and overproduced antibody.

Although the improved secretion of the protein by suppression of O mannosylation might not be a general phenomenon, the suppression of O mannosylation could be beneficial for the production of proteins forming either homomeric or heteromeric complexes through their hydrophobic interaction in yeast. Further study is needed to characterize the Pmt proteins in *O. minuta* and to inactivate the Pmt proteins associated with O mannosylation in the antibody by the construction of the *PMT* knockout strains and the use of various inhibitors.

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