Expression and Secretion of Foreign Proteins in Yeast Using the *ADH1* Promoter and 97 K Killer Toxin Signal Sequence

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Foreign proteins, endo-β-1,4-glucanase of *Bacillus subtilis*, preS1+S2 region of hepatitis B virus large surface antigen, human β$_2$-adrenergic receptor (hβ$_2$AR), and bovine growth hormone (bGH) were expressed in *Saccharomyces cerevisiae* and secreted into the medium. These proteins were expressed using the alcohol dehydrogenase I (*ADH1*) promoter of *Saccharomyces cerevisiae* and secreted by signal sequence of the 97 K killer toxin gene of double-stranded linear DNA plasmid (pGKL1) of *S. cerevisiae*. All these proteins underwent severe modifications; in particular, N-glycosylation in the case of endo-β-1,4-glucanase, hβ$_2$AR, and preS1+S2. Seventy four percent of the expressed endo-β-1,4-glucanase was secreted into the culture medium. Highly modified proteins were detected in the culture medium and in the cell. Expressed hβ$_2$AR, which has seven transmembrane domains, remained in the cell. The degrees of secretion and modification and the states of proteins in the culture medium and in the cell were quite different. These results indicated that the nature of the protein has a critical role in its secretion and modifications.

Keywords: *ADH1* promoter, 97 K killer toxin signal sequence, Modification, Secretion, Yeast.

Introduction

Saccharomyces cerevisiae has been widely used as a host for the production of homologous and heterologous proteins. It is single celled, easily manipulated, and can secrete these proteins into the culture medium through the secretory pathway. Purification of protein from the culture broth is facilitated because *S. cerevisiae* secretes few proteins into the culture medium in relatively low

abundance. Secretion is the preferred route of production of proteins if certain post-translational modifications are required to generate a desired final product. These include disulfide bond formation, glycosylation, and sequence specific endoproteolytic cleavage. Protein secretion in yeast is directed by an amino-terminal leader sequence that induces cotranslational translocation of the protein into the lumen of the endoplasmic reticulum (Novick *et al.*, 1981). The signal sequence is usually composed of about 15–30 amino acids containing a large number of hydrophobic residues (Briggs and Gierasch, 1985). The leader sequence is subsequently removed and modifications occur in the lumen of the endoplasmic reticulum. Homologous and heterologous signal sequences were successful to direct the secretion of foreign proteins from yeast (for review, see Hadfield *et al.*, 1993). *S. cerevisiae* and other yeast cells harboring the double-stranded linear DNA plasmids, pGKL1 and pGKL2, secrete the killer toxin — consisting of 97 K, 31 K, and 28 K subunits — into the culture medium. Signal sequences of the killer toxin subunits have been elucidated by the construction of secretion vectors containing these signal sequences in yeast. The 97 K killer toxin has a 29 amino acid-long secretion signal consisting of a 17 hydrophobic amino acids stretch followed by a relatively hydrophilic 12 amino acids (Hishinuma *et al.*, 1984). This prepro-signal was finally cleaved at the carboxyl side of Lys–Arg residues, most likely by the *KEX2* gene product. Twenty residues from the NH$_2$-terminal Met was used as a secretion signal (Tokunaga *et al.*, 1987). The use of the 28 K killer subunit signal sequence of *S. cerevisiae* (Tokunaga *et al.*, 1988) and killer toxin leader peptide of *Kluyveromyces lactis* has been reported (Baldari *et al.*, 1987).

In this study, we report the expression, secretion, and modification of foreign proteins (endo-β-1,4-glucanase, preS1+S2 region of hepatitis B virus surface antigen, human β$_2$-adrenergic receptor, and bovine growth hormone) in yeast using the alcohol dehydrogenase I (*ADH1*) promoter and *S. cerevisiae* killer signal sequences

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of 97 K toxin of pGKL1. Here, we also showed that the degree of secretion and appropriate conformation of the protein were determined by the polypeptide moiety to be secreted.

Materials and Methods

Strains and media *Escherichia coli* (DH5α) was used for propagation and selection of recombinant plasmids. *E. coli* was grown in Luria-Bertani broth (Sambrook *et al.*, 1989) and the transformants grown in LB supplemented with ampicillin (50 μg/ml).

S. cerevisiae strain L2612 (MATα, *leu2, trp1, ura3, can1, cyn2*) was used for expression of the cloned gene. The yeast strain was transformed by lithium acetate treatment of intact cells (Ito *et al.*, 1983). The transformant was selected at 30°C in synthetic medium containing 0.67% yeast nitrogen base without amino acids (Difco Laboratories, Detroit, USA) and supplemented with leucine, tryptophane, and 2% glucose (for SD medium). The rich medium, YP, contained 1% yeast extract and 2% bacto-peptone (Difco) which was supplemented with 2% glucose (for YPD), or 2% glycerol and 2% ethanol (for YPGE).

Construction of YEpACU (Fig. 1A) The 2160 bp *Ava*I fragment of YCp50, containing the *CEN4* region, was eliminated by self-ligation to produce YCpΔCEN. The 2241 bp *Eco*RI fragment of YEp24, containing 2 μm, was treated with Klenow fragment and cloned into *Sal*I, Klenow fragment-treated YCpΔCEN to produce YEpU. The 1.5 kb *Bam*HI–*Eco*RI fragment of pMA56 (Ammerer, 1983), containing the *ADH1* promoter, was cloned into *Bam*HI–*Eco*RI digested YEpU to produce YEpAU. pYeCYC1 (Smith *et al.*, 1979) was digested with *Hin*dIII, treated with Klenow fragment, and digested with *Eco*RI. Then, the 600 bp fragment was eluted and placed in *Ssp*I–*Eco*RI digested YEpAU to produce YEpACU.

Cloning of genes under the control of the *ADH1* promoter (Fig. 1)

1. Endo-β-1,4-glucanase, human β₂-adrenergic receptor (hβ₂AR), and bovine growth hormone (bGH) genes The *Bam*HI fragments of pSECGLU, pSECβ₂AR, and pSECbGH (Hong *et al.*, submitted) were cloned into the *Eco*RI, Klenow fragment-treated YEpACU to produce YEpACUGLU, YEpACUβ₂AR, and YEpACUbGH, respectively.

2. PreS1+S2 region of large hepatitis B virus surface antigen (large HBsAg) PCR primers, M primer: 5'-agGAATTCTTAtgttgtg-3' [containing the *Eco*RI site (GAATTC) and stop codon (TTA)], Killer A primer: 5'-gatccatgaatatattttacatatttttgtt-3', were used to amplify the preS1+S2 region using pSECLSΔ1, a plasmid that contained large HBsAg (Hong *et al.*, submitted), as template. The amplified 570 bp was treated with T4 DNA polymerase and Klenow fragment and cloned into *Sma*I-digested pBluescript to produce pBSPS. The 570 bp *Bam*HI–*Eco*RI digested fragment of pBSPS was treated with Klenow fragment and cloned into *Eco*RI, Klenow fragment-treated YEpACU to produce YEpACUPS.

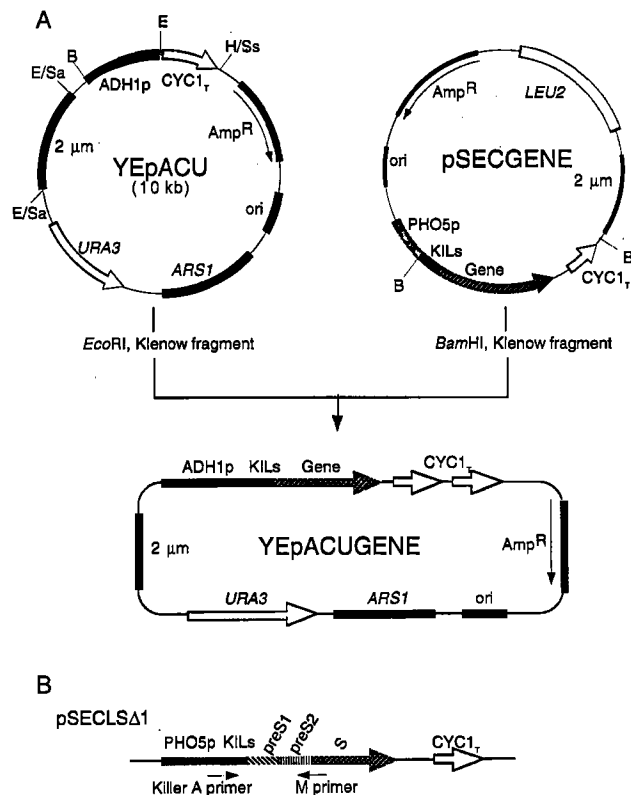**Endo-β-1,4-glucanase assay** For a quantitative assay, yeast

Fig. 1. (A) Construction of plasmids containing genes under the control of the *ADH1* promoter. Genes fused to 97 K killer signal sequences in pSEC5 were placed under the control of the *ADH1* promoter. The arrow indicates the direction of transcription from the *ADH1* promoter. (B) Construction of preS1+S2 of large HBsAg. Using PCR primers, Killer A primer and M primer, the preS1+S2 region was amplified. This preS1+S2 was placed under the control of the *ADH1* promoter. Abbreviations: PHO5p, *PHO5* promoter; ADH1p, *ADH1* promoter; KILs, signal sequence of 97 K killer toxin; CYC1T, *CYC1* terminator; AmpR, ampicillin resistant gene; B, *Bam*HI; E, *Eco*RI; E/Sa, *Eco*RI–*Sal*I junction; H/Ss, *Hin*dIII–*Ssp*I junction

cells harboring YEpACUGLU were grown in SD medium and then transferred to YPGE medium to an OD₆₀₀ of 0.1 and incubated at 30°C. Samples were taken in the course of time. After centrifugation, the supernatant was used to determine the secreted glucanase activity and the whole cell extract was used to determine the intracellular glucanase activity. The cell pellet was resuspended in glass bead lysis buffer (10 mM Tris-HCl, pH 7.5, 1 mM EDTA, 1 mM PMSF), disrupted by vortexing with glass beads (0.45 mm), and centrifuged to obtain the cell extract. Samples (0.1 ml) were incubated for 20 min at 45°C with 0.5 ml of 10 mM potassium phosphate, pH 5.8, containing 1% CMC (CarboxyMethylCellulose). Production of reducing sugar was determined by using 3,5-dinitrososalicyclic acid (DNS) as previously described (Skipper *et al.*, 1985). One unit releases 1 μmol of reducing sugar equivalents per min at 45°C.

The molecular weight of secreted glucanase was determined by Congo red-stained agar replica (Beguin, 1983). The yeast cell culture was centrifuged and the supernatant was concentrated

10-fold by ultrafiltration (Centriprep-10 concentrators; Amicon, Inc., USA). The cell pellet was resuspended in glass bead lysis buffer, disrupted with glass beads. The proteins of the concentrated supernatant and the whole cell extract were separated on 12% SDS-polyacrylamide gel. The gel was washed twice for 30 min each with 10 mM potassium phosphate, 25% isopropyl alcohol to remove SDS, and then washed twice for 30 min each with 10 mM potassium phosphate. The SDS-depleted gel was placed on a substrate gel (2% agar, 0.1% CMC, 10 mM potassium phosphate, pH 5.8) for 30 min at 55°C. The agar replica was stained with Congo red to identify the position of CMCase proteins. Tunicamycin was added to yeast culture media to prevent N-glycosylation at the concentration of 2 μg/ml.

Expression of β_2AR, bGH, and preS1 + S2 Yeast cells harboring YEpACUPS or YEpACUβ_2AR or YEpACUbGH were grown as described in the endo-β-1,4-glucanase assay using YPD medium, instead of YPGE.

1. YEpACUβ_2AR and YEpACUbGH Samples were taken in the course of time. After centrifugation, 0.2 ml supernatant was precipitated with 1 ml acetone and resuspended in 20 μl 0.1 N NaOH. 1 ml cell culture was harvested and resuspended in 0.1 ml glass bead lysis buffer, disrupted with glass beads. 5 μl of the concentrated supernatant and the whole cell extract were separated on SDS-polyacrylamide gel.

2. YEpACUPS Samples were prepared as described above. Tunicamycin was added to the culture media, if necessary. The yeast cell culture was concentrated 10-fold by ultrafiltration and the whole cell extract was prepared as described above and loaded on 12% SDS-polyacrylamide gel. Protein concentrations were measured by the method of Bradford (1976).

Western blot analysis After electrophoresis, proteins were transferred to nitrocellulose membrane at 300 mA for 1 h (Towbin et al., 1979). This membrane was blocked using phosphate-buffered saline (PBS) containing 5% skim milk and 0.1% Tween 20 for 1 h. Then, 300 ng/ml of the purified mouse anti-preS2 monoclonal antibody (Kim et al., 1996, mAb H8; for yeast harboring YEpACUPS), or 1 : 100 dilution of mouse anti-human β_2-adrenergic receptor monoclonal antibody (mAbβC02; for yeast harboring YEpACUβ_2AR), or a 1 : 2000 dilution of monkey anti-bGH polyclonal antibody (for yeast harboring YEpACUbGH) was incubated with the membrane for 1 h at room temperature. The membrane was washed with the same buffer and incubated with a 1 : 5000 dilution of peroxidase-conjugated goat IgG fraction to mouse immunoglobulins (Cappel, USA; for yeast harboring YEpACUPS or YEpACUβ_2AR), or with 500 ng/ml of affinity-purified goat anti-monkey IgG peroxidase-conjugate (E-Y Laboratories, Inc., San Mateo, USA.; for yeast harboring YEpACUbGH). Signals were detected using ECL (enhance chemiluminescence) Western blotting detection reagents (Amersham, Buckinghamshire, UK).

Results

Cloning of the foreign genes in YEpACUPS YEpACU is a typical yeast–*E. coli* shuttle vector (Fig. 1A)

containing an *E. coli* origin of replication, the ampicillin-resistance gene from pBR322, the yeast 2 μm origin of replication, and the yeast *URA3* structural gene as a selective marker. The features of this vector permit autonomous replication and selection in *E. coli* and yeast. This vector also contains the promoter of the *ADH1* gene and the terminator of the *CYC1* gene.

The genes for endo-β-1,4-glucanase, human β_2-adrenergic receptor (hβ_2AR), and bovine growth hormone (bGH), which were fused to a killer signal sequence (Hong et al., submitted), were placed under the control of the *ADH1* promoter and had two tandem copies of the *CYC1* terminator, as described in Materials and Methods (Fig. 1). The gene for the preS1 + S2 region was placed under the control of the *ADH1* promoter as described in Materials and Methods (Fig. 1). A stop codon (TAA) was introduced into the end of the preS2 region.

Expression of the endo-β-1,4-glucanase gene To determine the degree of secretion of the plasmid-encoded endo-β-1,4-glucanase into the culture medium, cultures of yeast cells harboring YEpACUGLU were fractionated and assayed for CMCase activity (Fig. 2). As the cells grew, CMCase activity increased; i.e., expression and secretion were associated with the cell growth. At 105 h of incubation, the secreted activity was 1.65 units/ml. The activity was found in both secreted (extracellular) and nonsecreted (intracellular) fractions. The proportion of the total recovered activity in the extracellular fraction was about 74% (at 105 h). Instead of YPD, YPGE medium was used in culture because glucose in YPD medium reacts with DNS. Endo-β-1,4-glucanase (395 amino acid) from
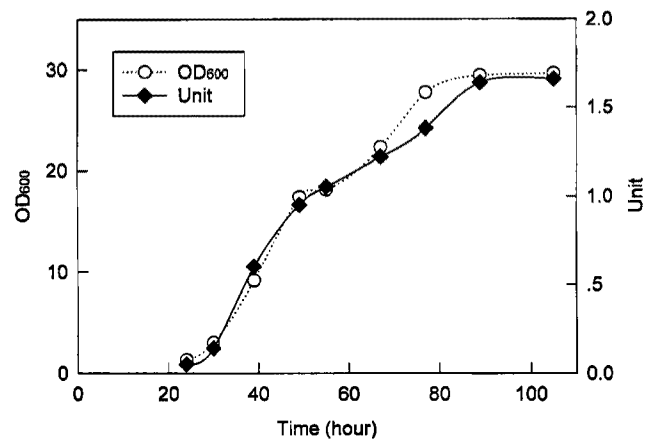

Fig. 2. Production of reducing sugar by the secreted glucanase in yeast. Cells were grown in SD and transferred to YPGE medium at 30°C. Samples were taken at intervals and the glucanase activity was determined using DNS. Glucanase activities in the supernatant of cells containing YEpACUGLU (♦) and optical density (OD_{600}) of the cell (○) were shown. As the cell grew, secreted glucanase was increased. About 74% of the synthesized glucanase activity was found in the supernatant of cells with YEpACUGLU.

Bacillus subtilis contained the 29 amino-acid long presumptive signal sequence and the first 28 amino acids were deleted (Hong *et al.*, submitted) in YEpACUGLU. Thus secretion was directed only by the killer signal sequence.

To determine the molecular weight (MW) of the secreted glucanase, the method of Congo red-stained agar replica was used. The culture supernatant was concentrated and loaded on an SDS-polyacrylamide gel as described in Materials and Methods. Various sizes were detected and had glucanase activity in the culture medium (Fig. 3A, lane 1). Their approximate MWs were between 37 kDa and 100 kDa. Two major activity bands, at about the 39.2 kDa region, and other minor activity bands, larger than 39.2 kDa, were detected. In yeast, the secreted proteins are modified during their passage through the secretory pathway. *S. cerevisiae* has a tendency to overglycosylate proteins. Endo-β-1,4-glucanase has several potential modification sites including two N-glycosylation sites. Tunicamycin was treated (Fig. 3A, lane 2) to block N-glycosylation and the smallest band (indicated by an arrow) of 34 kDa was enriched. Furthermore, including two bands of about 39.2 kDa, which were also found in the sample without tunicamycin, four discrete activity bands appeared. The secreted glucanase underwent severe glycosylation but the degree of glycosylation was heterogeneous.

Two sizes of glucanase, about 70 kDa and 80 kDa, were detected in the whole cell extract (Fig. 3B, lane 2) and a band of about 70 kDa was detected in the whole cell extract which was treated with tunicamycin (Fig. 3B, lane 3).
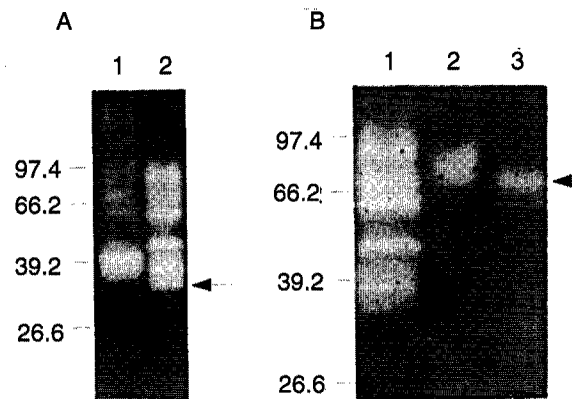

Fig. 3. Activity staining of CMCase from yeast. (A) Glucanase in the culture medium. Cells containing YEpACUGLU were grown in the absence (lane 1) or presence (lane 2, 2 μg/ml) of tunicamycin in YPGE medium. (B) Glucanase in the cell. Cells containing YEpACUGLU were grown in the absence (lane 2) or presence (lane 3) of tunicamycin. Lane 1 is the same sample as in Fig. 3A, lane 2. Protein size markers are shown.

Expression of preS1 + S2 of the large HBsAg gene Cells harboring YEpACUPS grown in YPD broth and samples were taken as described in Materials and Methods. At an OD$_{600}$ of 1.5, preS1+S2 was detected in the culture medium and in the cells (Fig. 4A). As the cells grew, the amount of expressed preS1+S2 increased both in the culture medium and in the cells. The secreted protein had a MW larger than 100 kDa (labeled as b) while a 27 kDa protein was detected (labeled as a) in the cells. As the cells grew, proteins smaller than 27 kDa were detected (lane 7, 8). PreS1+S2 has two potential N-glycosylation
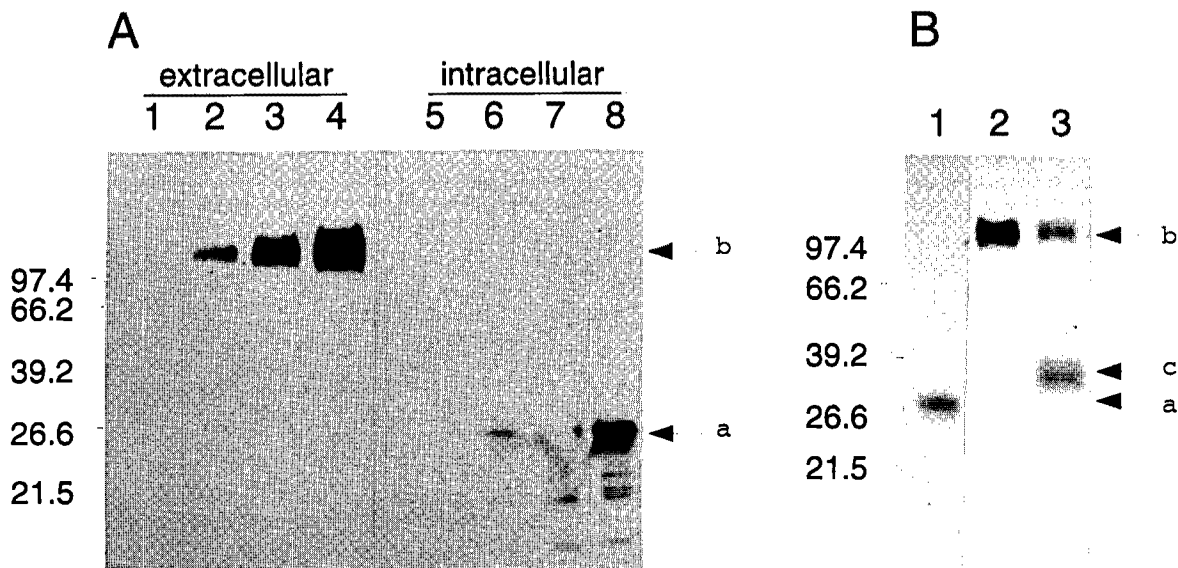

Fig. 4. Expression and secretion of preS1+S2. (A) Cells containing YEpACUPS were grown in YPD medium and samples taken at intervals. 12% SDS-polyacrylamide gel was used and Western blot analysis was carried out as described in Materials and Methods. Lanes 1 through 4 were samples in the culture medium at OD$_{600}$ = 0.6, 1.5, 2.9, 5.1, respectively. Lanes 5 through 8 were samples in the cell at the same OD$_{600}$ of lanes 1–4. (B) Cells containing YEpACUPS were grown in the absence (lane 2) or presence (lane 3) of tunicamycin. 10 μg of the whole cell extract was loaded in lane 1. Protein size markers are shown.

sites. When tunicamycin-treated, a 35 kDa band was detected in the culture medium (Fig. 4B, lane 3, labeled as c), while only a protein larger than 100 kDa was detected in the sample that was not treated with tunicamycin (Fig. 4B, lane 2, labeled as b). However, there was no differences in the intracellular forms of preS1+S2 (27 kDa, labeled as a) whether treated with tunicamycin or not. The first 19 amino acids of large HBsAg work as an endoplasmic reticulum retention signal and the first 14 amino acids of it was deleted (Hong et al., submitted) in YEpACUPS.

Expression of the bovine growth hormone (bGH) gene

Cells harboring YEpACUbGH were grown in YPD medium and samples taken as described in Materials and Methods. At 15 h (OD$_{600}$ = 1.8) after transfer to YPD broth, secreted bGH was detected with a MW larger than 200 kDa (Fig. 5, labeled as a). In addition to that size (labeled as a), a 45 kDa form was detected (labeled as b) in the cell. bGH (190 amino acid) contained a 27 amino-acid long signal peptide and the first 27 amino acids of it was deleted (Hong et al., submitted) in YEpACUbGH.

Expression of the human β$_2$-adrenergic receptor (hβ$_2$AR) gene

Cells harboring YEpACUβ$_2$AR were grown in YPD medium and samples were taken as described in Materials and Methods. Any detectable signals were not detected in the culture medium. Most of the expressed hβ$_2$AR remained in the cell. At 15 h (OD$_{600}$ = 1.9) after transfer to YPD broth, the expressed hβ$_2$AR was detected with a MW of about 66 kDa (Fig. 6, indicated by arrow).
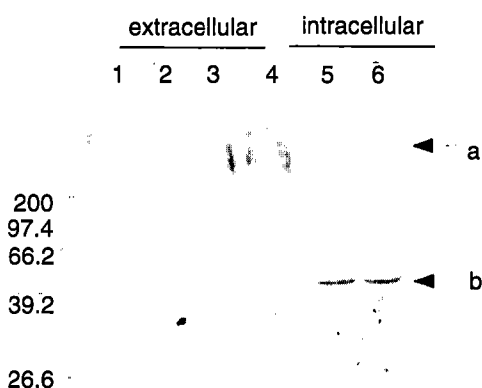

Fig. 5. Expression and secretion of bGH. Cells containing YEpACUbGH were grown in YPD medium and samples taken at intervals. 8% SDS-polyacrylamide gel was used and Western blot analysis was carried out as described in Materials and Methods. Lanes 1 through 4 were samples in the culture medium at 7 h, 15 h, 21 h, 26 h, respectively. Lanes 5, 6 were samples in the cell at 15 h and 21 h. Protein size markers are shown.
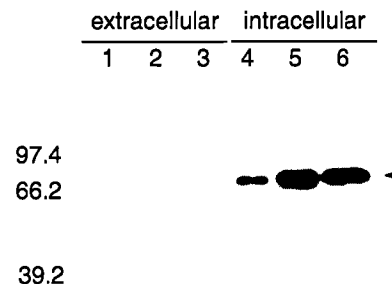

Fig. 6. Expression and secretion of hβ$_2$AR. Cells containing YEpACUβ$_2$AR were grown in YPD medium and samples taken at intervals. 10% SDS-polyacrylamide gel was used and Western blot analysis was carried out as described in Materials and Methods. Lanes 1 through 3 were samples in the culture medium at 15 h, 21 h, 26 h, respectively. Lanes 4 through 6 were samples in the cell of lanes 1–3. Protein size markers are shown.

Discussion

In this paper, we reported the expression, secretion, and modification of foreign proteins in yeast. For constitutive expression through the entire growth phase, the ADH1 promoter was used. The ADH1 gene encodes a glycolytic enzyme (ADHI) which converts acetaldehyde to ethanol, regenerating NAD+ (from NADH), and is expressed in high levels— more than 1% of the total RNA — during growth on glucose. The ADH1 was considered to be a constitutive gene, but expression is repressed about 2–10 fold when the cells are growing on a nonfermentable carbon source (Denis et al., 1983). However, expression is regained during the stationary phase or in ethanol-grown cells after a lag phase, yielding much higher final product levels than on glucose. The efficient expression of ADH1 is dependent upon a cis-acting regulatory element — UAS$_{RPG}$ (Tornow and Santangelo, 1990). UAS$_{RPG}$ is found in the promoter of many genes, including glycolytic genes (e.g., ADH1, PYK1, TPI1, ENO1, TDH3; Tornow and Santangelo, 1990), most of the ribosomal protein genes, and the genes encoding elongation factor 1α [TEF1 and TEF2 (Huet et al., 1985; Huet and Sentenac, 1987)]. Expression of glycolysis genes, including ADH1, is dependent on several trans-acting factors, namely, GCR1 (Baker, 1986; Holland et al., 1987), GCR2 (Uemura anf Fraenkel, 1990), and RAP1/GRF1/TUF (Huet et al., 1985; Shore and Nasmyth, 1987; Buchman et al., 1988).

During synthesis of a eukaryotic secretory protein, the signal sequence allows binding of the translation complex to a signal-recognition particle which mediates the binding of the polysomes to the endoplasmic reticulum via the docking protein. The nascent protein is then cotranslationally translocated across the membrane of the endoplasmic reticulum and the signal peptide is cleaved by a signal peptidase. In the endoplasmic reticulum proteins are modified, processed, and then transported to the Golgi

apparatus where further modification of the glycosylated structure occurs. From here, the proteins are packaged into vesicles, delivered to the cell surface, and finally released into the culture medium. We used the 17 amino acid-long signal sequence (MNIFYIFLFLLSFVQGL) of the 97 K killer toxin as a signal sequence for secretion.

Endo-β-1,4-glucanase (395 amino acid) has a 29 amino-acid long presumptive signal sequence and bGH (190 amino acid) contains a 27 amino-acid long signal peptide. When these genes were fused to the killer signal sequence, their signal sequences were deleted. Therefore, secretion in yeast was driven only by the killer signal sequence.

S. cerevisiae has a tendency to overglycosylate proteins, although glycosylation is not necessary for secretion. The difference in outer oligosaccharide chain composition of foreign proteins secreted from yeast could affect their folding, stability, or activity. Some naturally glycosylated proteins may not fold properly, or be secreted, or be active, if glycosylation is eliminated. The expressed glucanase, hβ_2AR, and preS1+S2 protein, which have two, seven, and two potential N-glycosylation sites (Asn-X-Ser/Thr), respectively, underwent glycosylation (Figs. 3, 4, 6). Tunicamycin was used to study the glycoylation of these proteins. This is the well-known antibiotic which inhibits N-glycosylation of secretory proteins due to its inhibition of the formation of dolichol-pyrophosphoryl-N-acetylglucosamine (Lehle and Tanner, 1976).

In the case of glucanase, a 34 kDa form appeared and other discrete bands were shown when tunicamycin was added to the culture medium (Fig. 3A). On the other hand, glucanase activities were detected broadly upto 100 kDa, except for two distinct bands, in the sample that was not treated with tunicamycin. The degree of glycosylation in yeast was heterogeneous and various sized glucanases appeared. Including N-glycosylation, other modifications, e.g., O-glycosylation, occurred in the expressed glucanase because other discrete forms, which were larger than 40 kDa, were made in the culture medium when it was tunicamycin-treated. Also, a 70 kDa protein, not 34 kDa, appeared in the cell when tunicamycin was added. The predicted size of glucanse is 40 kDa (Seo et al., 1985) but 34 kDa was the smallest, unglycosylated form in the culture medium. This may be due to considerable processing or degradation of the primary translation product beyond signal peptide cleavage, which occurs in many bacterial cellulolytic enzymes (Mackay et al., 1986; Robson and Chambliss, 1897; Park et al., 1991).

Large HBsAg (preS1+S2+S) has four transmembrane domains. When this large HBsAg expressed in yeast, most of the expressed proteins remained in the cell (Hong et al., submitted). We expressed only a preS1+S2 region (preS2 region is involved in antigenicity), in which the transmembane domains were removed. preS1+S2 has two potential N-glycosylation sites and the secreted form was highly N-glycosylated (Fig. 4B). The expected MW of preS1+S2 is 15 kDa; however, a 27 kDa protein was detected in the cell (Fig. 4B). This was not due to N-glycosylation because there were no differences in size whether tunicamycin was added or not. Perhaps other modifications were responsible for that. Smaller proteins (<27 kDa) were also detected in the cell although in small amounts (Fig. 4A). A 35 kDa form was detected at the samples taken from the culture medium when treated with tunicamycin. This protein was different from the intracellular form (27 kDa) in size. Perhaps O-glycosylation or phosphorylation or myristylation other than N-glycosylation had occurred during secretion. bGH has no N-glycosylation site and the expected MW is 19 kDa. A high MW of bGH (larger than 200 kDa) was detected in the culture medium. Including this form, a 45 kDa protein was also detected in the cell. Perhaps severe modifications had occurred during secretion.

There are reports that proteins with a transmembrane domain are difficult to secrete to the medium due to the hydrophobic domain (Jabbar, 1985). This is the case in hβ_2AR which has seven transmembrane domains and so most of the expressed hβ_2AR remained in the cell (Fig. 6). The expected MW of hβ_2AR is 43 kDa, but about 66 kDa of hβ_2AR was produced. hβ_2AR is reported as being a glycoprotein of which the MW is 64 kDa (Lefkowitz et al., 1983). In yeast this modification also occurred.

In the case of glucanase, only high MW forms were detected in the cell and low MW forms, from 34 kDa to 40 kDa, were detected as major forms in the culture medium, while in the case of preS1+S2, the high MW form was in the culture medium and the low MW form was in the cell. When large HBsAg was expressed in yeast no modification occurred even though it has four potential N-glycosylation sites (Hong et al., submitted). But when a region of large HBsAg (preS1+S2 region that has two potential N-glycosylation sites) was expressed, severe modifications occurred. All of these results suggest that the nature of the protein has a critical role in the secretion and post-translational modifications. The expressed, secreted, and modified glucanase retained its activity, but it remains to be examined whether the modified proteins — hβ_2AR, preS1+S2, and bGH — maintain specific functions.

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