Intracellular Expression of *Vitreoscilla* Hemoglobin Alters the Aerobic Metabolism of *Saccharomyces cerevisiae*

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Vitreoscilla hemoglobin (VHb) has been expressed in *Saccharomyces cerevisiae*, and its influence on yeast aerobic metabolism has been investigated. New expression vectors were constructed to express VHb constitutively under the control of the ADH-1 promoter. The presence of VHb was shown by Western blot analysis. VHb has been shown to localize predominantly in the cytoplasm. Batch fermentation results indicated that the wild-type strain expressing VHb exhibited a shift in the carbon flux toward ethanol production, with no significant alteration in the specific growth rate. This effect was not observed if cells were grown under respiration inhibition, indicating that the metabolic effect of VHb is likely linked to respiration. Expression of VHb in the *adh°* strain MC65-2A, which produces ethanol only via a respiration-coupled pathway, revealed that ethanol production was decreased and cells reached a higher final cell density in a culture of the VHb-expressing strain. Growth enhancement due to expression of VHb was observed only during the final stage of culture growth when the acetaldehyde produced during the first growth phase was used as a substrate. This metabolic effect of intracellular VHb was seen more clearly in an acetaldehyde fed-batch fermentation in which VHb-expressing cells grew to at least 3-fold higher final cell density. These results suggest that the action of VHb is likely linked to electron transfer.

Introduction

Recombinant DNA technology enables genetic engineering of industrial organisms in order to achieve some special tasks. Many such applications have focused on improved production of desirable foreign proteins or redirection of metabolic flux toward a particular end product (for example, amino acids or antibiotics). However, in recent years, researchers have moved toward the goal of genetically engineering cells to function as "designer biocatalysts", in which certain desirable heritable properties from different organisms are brought together in one single host with the aim of performing some specific task(s) (Brennard et al., 1986; Windass et al., 1980; Stanzak et al., 1986; Bailey, 1991).

Wakabayashi et al. (1986) reported the amino acid sequence of a soluble dimeric hemoglobin-like molecule found in the obligately aerobic bacterium *Vitreoscilla* and demonstrated significant sequence similarity of this protein, denoted *Vitreoscilla* hemoglobin (VHb), with known eukaryotic globins. *Vitreoscilla* is commonly found in oxygen-poor habitats (Pringsheim, 1951), and the cytoplasmic heme content is elevated in *Vitreoscilla* cultures grown under oxygen limitation, presumably a response to increased VHb expression under these conditions (Boerman and Webster, 1982). Therefore, it was conjectured that VHb somehow enhances growth metabolism under oxygen-limited conditions.

Khosla and co-workers first demonstrated that the expression of VHb enhances growth properties (Khosla and Bailey, 1988) and protein synthesis (Khosla et al., 1990) in recombinant *Escherichia coli*. It was proposed that these effects are possibly due to an improved overall efficiency of oxygen-limited ATP production. Enhancement in antibiotics production was also observed for VHb-expressing *Streptomyces* (Magnolo et al., 1991).

Although not as well-characterized as *E. coli*, *Saccharomyces cerevisiae* (baker's yeast) is one of the best understood eukaryotes. It has been used extensively as an industrial microorganism for the production of ethanol, cloned proteins (Valenzuela et al., 1982), and food-related products. The technology required to grow yeast under both aerobic and anaerobic conditions to high cell densities in large, process-scale bioreactors is well-established. Similar to aerobic fermentation with *E. coli*, maintaining an adequate supply of oxygen to high cell density yeast cultures remains a central problem in a wide variety of applications. In order to investigate whether VHb has an effect on overall metabolism or physiology in *Saccharomyces cerevisiae*, VHb has been expressed in this yeast and its effects on metabolism have been examined.

Materials and Methods

Strains and Media. *Saccharomyces cerevisiae* SEY-2101 (*ura 3-52, leu 2-3 112, suc 2-Δ9, ade 2-7, gal 2*) was utilized as the host strain in most experiments. Strain MC65-2A (*Matα, adh 1-Δ, adh 2, adh 3, adh 4:URA3, trp 1 (adh°)*) is an *adh°* strain provided by Dr. M. Ciriacy that carried mutations in all four known cytoplasmic alcohol dehydrogenases (Drewke et al., 1990). *E. coli* DH5α (F–, *endA1, hsdR17*($r_k^-m_k^+$), *supE44, thi-1*, λ–, *recA1, gryA96, relA1, φ80dlacAm15*) was used for all intermediate DNA cloning steps.

Synthetic dextrose (SD) medium was used for all routine cultivations of strain SEY2101 carrying plasmids pEX-2 and pAAH5. This medium contained 6.7 g/L Difco yeast

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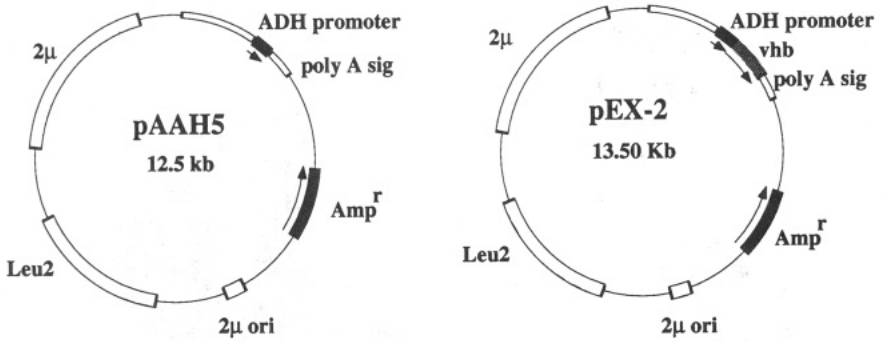

Figure 1. Plasmids for expressing *Vitreoscilla* hemoglobin (VHb) in yeast strain SEY2101. Plasmid pAAH5 is the parental shuttle vector containing the 2μ origin of replication and the *LEU2* selection marker. Plasmid pEX-2 is derived from pAAH5 expressing VHb constitutively under control of the ADH promoter.

nitrogen base without amino acids, 2 g/L amino acid powder mix (0.01 g of *p*-aminobenzoic acid, 0.1 g of uracil, adenine sulfate, inositol, and each amino acid except leucine or tryptophan), and the desired amount of glucose (1–4 g/L). Leucine was added to the culture medium for the plasmid-free host at 25–100 mg/L. Citrate buffer (4.93 g/L citric acid and 7.8 g/L sodium citrate, pH 4.5) was used to maintain pH at 5.0 during fermentation. For the cultivation of strain MC65-2A, SD medium was used, except that tryptophan (50–100 mg/L) instead of leucine was not included for the selection for plasmid-containing cultures.

Chemicals, Reagents, and DNA Manipulations. All restriction endonucleases and modifying enzymes (T4 DNA polymerase, Klenow fragment, T4 DNA ligase) were purchased from either New England BioLabs or Boehringer Mannheim Biochemicals. All DNA manipulations were performed according to standard methods (Sambrook et al., 1989). DNA fragments were eluted from agarose gels using the Geneclean Kit (Bio 101). Yeast transformation was performed using the LiAc method as described by Sherman et al. (1986).

Preparation of Cell Extracts and Immunoblot Analysis. Yeast extracts were prepared as follows. Cell pellets were incubated for at least 2 h in a solution containing 1.0 M sorbitol, 0.05 M potassium phosphate (pH 7.5), 14 mM β-mercaptoethanol, and 20 units/mL of lyticase (Sigma L-8012). After 2 h the spheroplasts were pelleted out and resuspended in a solution containing 100 mM Tris-HCl (pH 8.0), 50 mM NaCl, and 1 mM EDTA. The protoplasts were then sonicated using a microtip at 20% power for 10 × 30-s bursts interspersed with 30 s of cooling. The lysed cell debris was then removed by microfuging for 15 min. For the immunoblot analysis, an aliquot (3–5 μg of protein) was electrophoresed on a 15% polyacrylamide–SDS gel according to the method of Laemmli (1970). The proteins were then transferred electrophoretically to a nitrocellulose membrane, as described elsewhere (Ausubel et al., 1989). The proteins were screened with antiserum generated against *Vitreoscilla* hemoglobin as described elsewhere (Ausubel et al., 1989). The hemoglobin standard was produced in recombinant *E. coli*.

Analytical Methods. Glucose was analyzed by an enzymatic kit available from Sigma (cat. no. 510-A). Acetaldehyde was analyzed using an assay kit available from Boehringer Mannheim (cat. no. 668613). Ethanol was analyzed by gas chromatography with a Supelco Inc. Chromosorb 101 (80/100) column (2 m × 0.5 cm): column temperature, 1500 °C; carrier flow, 50 mL/min. Glucose-6-phosphate dehydrogenase and isocitrate dehydrogenase activities were assayed with Sigma kits (cat. nos. 400K-25 and 153-A).

Fermentation and Growth Conditions. Batch and fed-batch fermentations were performed at 30 °C, pH 5.0, and 250 rpm in a BiofloIII fermentor with a 2.5-L working volume. Cultures were started with a 1% (v/v) seed inoculum. Optical density was determined by a Spectronic 21 spectrophotometer (Milton Roy). For fed-batch fermentations, the feed medium consisted of 50% acetaldehyde and 2.67 g/L yeast nitrogen base without amino acids. Details of the feeding are described in the captions to the figures. Shake flask experiments were performed in a New Brunswick G1000 incubator shaker at 30 °C and 300 rpm in 1-L Erlenmeyer flasks containing 400 mL of medium.

Preparation of Cell Fractions. Cell fractionation was performed as described by Oeda et al. (1985). Cell lysates were centrifuged at 3000*g* for 10 min to precipitate cell debris. The mitochondrial fraction was recovered by further centrifugation at 10000*g* for 20 min. The supernatant obtained was assigned as the cytoplasmic fraction. Enzymatic activities of mitochondrial enzymes were determined by disruption of the membrane by lysis with 1% Triton X-100 according to Campbell et al. (1988).

Results

Plasmids for Expressing *Vitreoscilla* Hemoglobin (VHb) in *Saccharomyces cerevisiae*. In order to express VHb in strain SEY2101 (obtained from Dr. S. Emr, Caltech), plasmid pEX-2 was constructed from plasmid pAAH5 by inserting the *vhb* gene after the ADH-1 promoter (Figure 1). A *Bam*HI–*Sph*I fragment from pRED302 (provided by C. Khosla) was subcloned into the *Bam*HI/*Sph*I sites of the vector pUC18. From this new construct, a *Hin*dIII–*Bam*HI fragment was isolated and cloned into the *Hin*dIII site of pAAH5 with blunt-end ligation at the *Bam*HI end. The resulting construct expresses VHb constitutively under control of the ADH-1 promoter.

Plasmid pVHb-trp1 was constructed to express VHb in strain MC65-2A with *trp1* as the selection marker (Figure 2). First, a 3-kb *Bam*HI fragment containing the entire VHb-expressing cassette from pEX-2 was inserted into plasmid pYE (Tschumper and Carbon, 1980) to yield plasmid pVHb-tm1. To construct plasmid pVHb-trp1, a 4.7-kb *Nhe*I–*Eag*I fragment containing the *trp1* marker and the VHb expression cassette was inserted into the corresponding sites of pEX-2.

Transformed Cells Expressed the VHb Protein. Immunoblot analysis was performed to demonstrate the expression of VHb in *S. cerevisiae* transformed with plasmids pEX-2 and pVHb-trp1. Whole cell extracts were electrophoresed on a 15% SDS–PAGE gel. The proteins were then transferred to a nitrocellulose membrane and

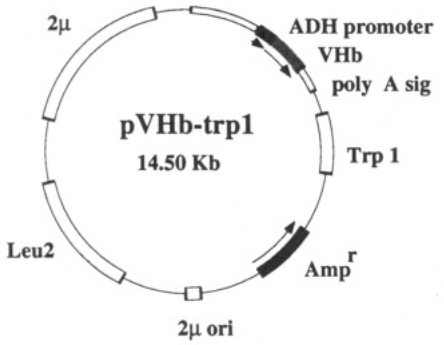

Figure 2. Plasmid pVHb-trp1 for expressing VHb in strain MC65-2A. This plasmid is essentially the same as pEX-2, except that it also contains a *trp1* selection marker isolated from plasmid pYE.

incubated with VHb antiserum. As shown in Figure 3, a band that comigrates with the VHb standard is present in SEY2101:pEX-2 (Figure 3A, lane 1) and MC65-2A: pVHb-trp1 (Figure 3B, lane 2). This band, however, does not appear for cells carrying control plasmids pAAH5 and pYE. These results indicate that VHb protein of the correct molecular weight is being produced by cells harboring VHb expression plasmids.

Subcellular Localization of VHb Synthesized in Yeast. Previous results with recombinant *E. coli* indicated that the VHb protein expressed in that organism was distributed equally between the cytoplasm and the periplasm (Khosla and Bailey, 1989), thus suggesting that this protein can translocate across the cytoplasmic membrane. Since the location of VHb may be important in determining its function in yeast, we examined the subcellular localization of VHb. In this experiment, the cytoplasmic fraction was separated from the mitochondrial fraction, as described in Materials and Methods. These samples were analyzed by Western blotting, and results reveal that most of the VHb is present in the cytoplasmic fraction as indicated by a much higher intensity of the VHb band (see Figure 4). Only a small amount of VHb is observed in the mitochondrial fraction.

In order to verify that the fractions obtained are actually from the cytoplasm and the mitochondria, glucose-6-phosphate dehydrogenase (G-6-PDH) and isocitrate dehydrogenase (IDC) were assayed as cytoplasmic and mitochondrial control markers, respectively. Table 1 shows the percentage of control markers in each fraction. It can be seen that 96% of the cytoplasmic marker G-6-PDH activity is present in the cytoplasmic fraction. In contrast, about 30% of the IDC activity is found in the cytoplasmic fraction. The data indicate some breakage of the mitochondria during the fractionation procedure. However, the great majority of VHb is found in the cytoplasmic fraction with almost none in the mitochondrial fraction, thus indicating that VHb in yeast is localized primarily in the cytoplasm.

Effect of VHb Expression on Cell Growth and Ethanol Formation. Previous fermentation results with recombinant *E. coli* expressing VHb illustrated that VHb-expressing *E. coli* have higher specific growth rates, final cell densities, and oxygen consumption rates compared to a control strain without VHb when both are cultivated under microaerobic conditions (Khosla and Bailey, 1988; Khosla et al., 1990). In this work, batch fermentations were performed under poorly aerated conditions in order to explore the effect of VHb on yeast cell growth and carbon metabolism. Yeast strains SEY2101, SEY2101:pAAH5, and SEY2101:pEX-2 were grown at 30 °C in SD medium

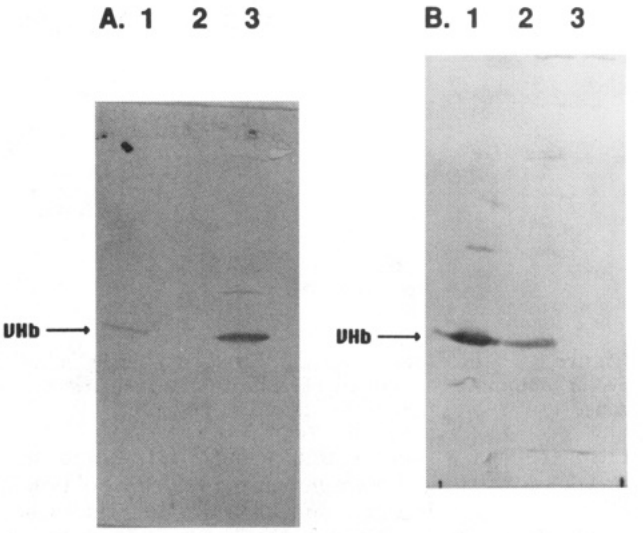

Figure 3. Western blot analysis of whole-cell extracts from *S. cerevisiae*: (A) lane 1, SEY2101:pEX-2; lane, 2, SEY2101:pAAH5; lane 3, VHb standard; (B) lane 1, VHb standard; lane 2, MC65-2A:pVHb-trp1; lane 3, MC65-2A:pYE.

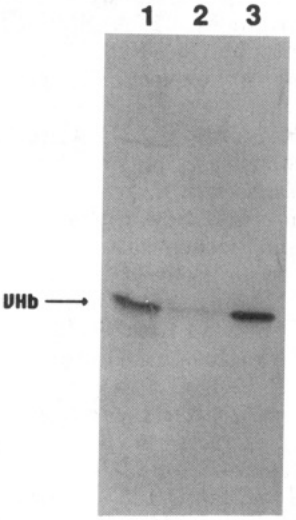

Figure 4. Western blot analysis of extracts from the cytoplasmic and mitochondrial fractions: lane 1, cytoplasmic fraction; lane 2, mitochondrial fraction; lane 3, VHb standard.

Table 1. Percentage of Cytoplasmic (G-6-PDH) and Mitochondrial (IDC) Marker Enzymes in the Two Different Fractions

fraction	G-6-PDH (%)	IDC (%)
cytoplasm	96	27
mitochondria	4	73

supplemented with 1 g/L glucose as the carbon source. Figures 5 and 6 show the results of the experiment under microaerobic conditions in which the agitation rate was adjusted manually such that the dissolved oxygen (DO) was lower than 10%. Strain SEY2101:pEX-2 exhibits a shorter lag time but grows to a lower cell density than the other two strains (Figure 5). Furthermore, no significant differences in the exponential-phase specific growth rates are observed (average of two batch fermentations). Glucose consumption and ethanol production were monitored as shown in Figure 6. Examination of the data at 10 h postinoculation when all cultures have about the same density shows that more ethanol has been produced by SEY2101:pEX-2 than by the other two strains. VHb was stably expressed throughout the cultivation.

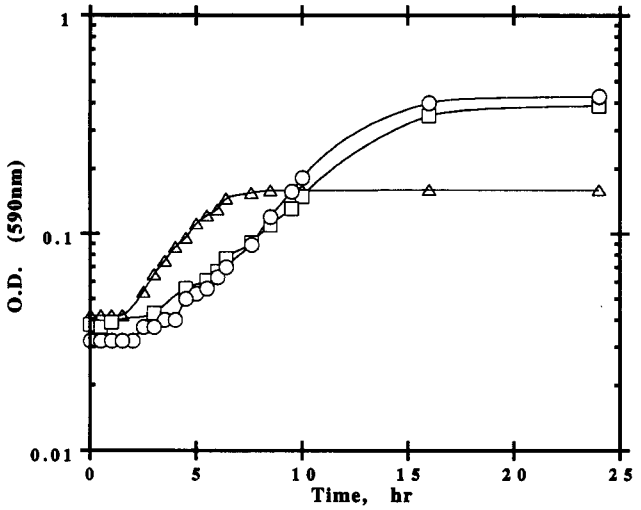

Figure 5. Growth trajectories (OD_{600}) of SEY2101 (○), SEY2101:pAAH5 (□), and SEY2101:pEX-2 (△) under poorly aerated conditions. The batch fermentations were carried out at 30 °C, pH 5.0, and 250 rpm. Dissolved oxygen (DO) was adjusted manually to be lower than 10%. The growth curves are measured in terms of OD_{600}.

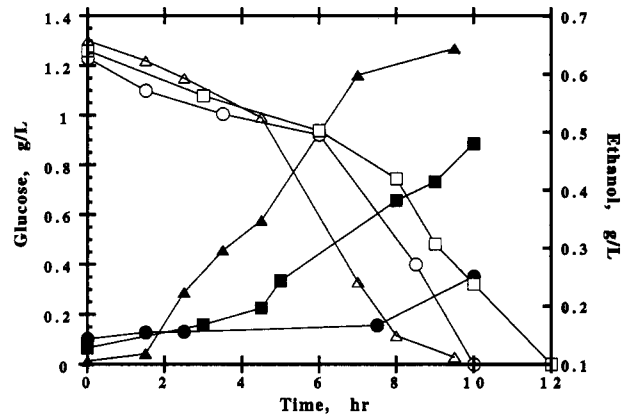

Figure 6. Profiles of glucose (open symbols) and ethanol (filled symbols) profiles for the batch cultivations shown in Figure 5: SEY2101 (circles), SEY2101:pAAH5 (squares), and SEY2101:pEX-2 (triangles).

One of the main hypotheses for VHb action in recombinant *E. coli* is the influence of VHb (or oxygenated VHb) on the respiratory apparatus leading to an improved overall proton translocation efficiency (Khosla et al., 1990). To test whether the action of VHb in yeast is also linked to respiration, we carried out experiments in which cells were grown under respiration inhibition, which was accomplished by the addition of the respiration inhibitor, antimycin A. The reported action of antimycin A is to inhibit electron flow to the cytochrome reductase; CO-binding activity assays showed that antimycin A does not interfere with VHb activity (data not shown). In this growth study, cells were grown in SD medium for 4 h before the addition of antimycin A. Results from this experiment are shown in Figure 7. Under this condition, the previously observed VHb effects no longer exist. As indicated, no difference in growth is observed between strains SEY2101:pEX-2 and SEY2101:pAAH5, both in specific growth rate and final cell density (Figure 7A). Furthermore, no difference in ethanol production is observed between the two strains under this condition (Figure 7B). These results indicate a possible link between respiration and the effect of VHb on yeast metabolism.

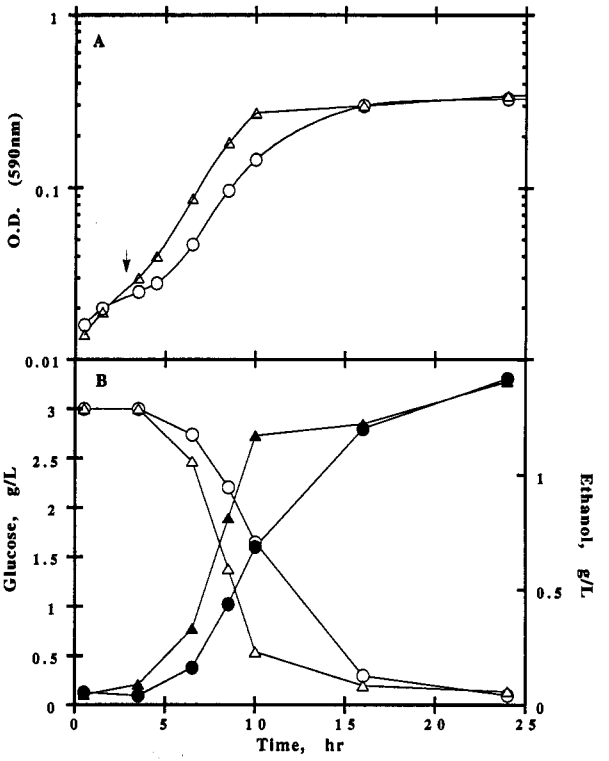

Figure 7. Comparison of the growth properties of SEY2101:pAAH5 (triangles) and SEY2101:pEX-2 (circles) with respiration inhibition by added antimycin A. Cells were grown under the same conditions as described in Figure 5. (A) The growth curves as measured in terms of OD_{600} are shown here for these three strains. The arrow indicates the time of antimycin A addition. (B) Glucose (open symbols) and ethanol (filled symbols) profiles for these two strains.

Investigation of the Interaction of VHb with Respiration Using the Cytoplasmic *adh*° Strain MC65-2A. Our fermentation results suggest that the action of VHb in yeast may be linked to respiration. How does this propagate into a redirection of carbon flux as indicated by an increase in ethanol production? Recently, a novel mitochondrial acetaldehyde-reducing pathway was identified to be responsible for the production of approximately one-third of the ethanol under aerobic conditions (Drewke et al., 1990). This pathway is highly coupled to the respiratory chain. Therefore, if the presence of VHb alters the production of ethanol, this pathway is a potential candidate for investigation.

To investigate this possible connection, VHb was expressed in strain MC65-2A. This strain carries irreversible mutations in the genes ADH1, ADH3, and ADH4 and a point mutation in ADH2. The only way to produce ethanol in this mutant is via the mitochondrial acetaldehyde-reducing pathway. Construction of the plasmid pVHb-trp1 to express VHb was described earlier. Production of the VHb protein in MC65-2A:pVHb-trp1 has been demonstrated by Western blotting (see Figure 3B). Shake flask experiments were conducted using SD medium supplemented with 1 g/L glucose as the carbon source. No difference in cell growth between strains with and without VHb is observed for the glucose growth phase (Figure 8). After glucose exhaustion, the strain expressing VHb grows to a higher cell density. Analyses of ethanol and acetaldehyde reveal that the same amount of acetaldehyde is produced during the glucose growth phase in which no growth difference was observed (Figure 9). Only when the acetaldehyde was used as the carbon source was an advantage in growth observed for the VHb-expressing cells.

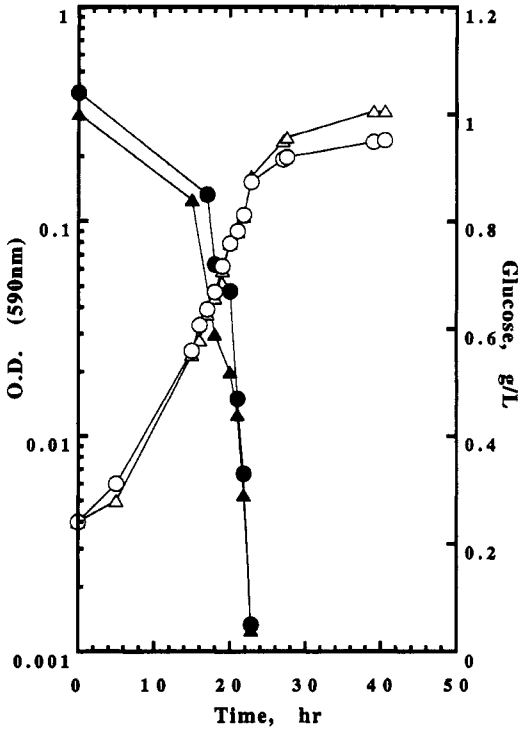

Figure 8. Comparison of the growth properties of MC65-2A (circles) and MC65-2A:pVHb-trp1 (triangles). Experiments were conducted in shake flasks at 30 °C, pH 5.0, and 300 rpm and measured culture density (OD_{600}) (open symbols) and medium glucose concentration (filled symbols).

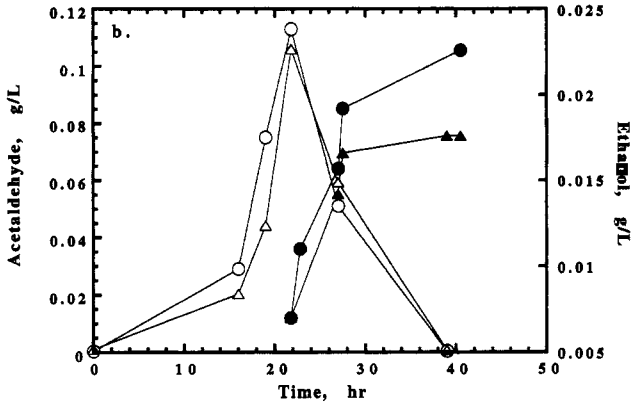

Figure 9. Acetaldehyde (open symbols) and ethanol (filled symbols) profiles for the cultivations described in Figure 8: MC65-2A (circles) and MC65-2A:pVHb-trp1 (triangles).

These data are again consistent with the hypothesis of VHb interaction with respiration.

Fed-batch fermentations were then conducted using acetaldehyde as the feeding substrate. The goal of this experiment was to operate under conditions for which the effect of VHb is more apparent. In this experiment, fermentation was started with cells using glucose as the main carbon source. After glucose exhaustion, acetaldehyde was fed to maintain cell growth. As depicted in Figure 10, cells expressing VHb grow to a much higher cell density. This difference is larger than that observed from the shake flask culture, emphasizing the effect of VHb with acetaldehyde as the carbon source. Similarly, more ethanol is produced from the strain not expressing VHb (data not shown). Possible mechanisms will be discussed later.

Discussion

We have investigated in this study the effect of intracellular VHb expression on *S. cerevisiae* aerobic

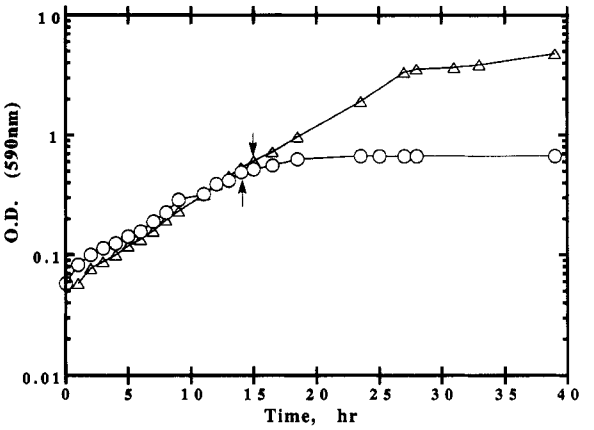

Figure 10. Comparison of growth properties of MC65-2A (○) and MC65-2A:pVHb-trp1 (△). The batch phase of the fermentation was carried out at 30 °C, pH 5.0, 250 rpm, and a constant air flow rate of 0.4 L/min. Arrows indicate the times of feeding. The feed, consisting of 50% acetaldehyde, was commenced at 2.5 mL/h. Growth was measured in terms of OD_{590}.

metabolism. Production of the VHb protein has been shown by Western blot analysis. This protein does not appear to be glycosylated in yeast because its molecular weight is identical to the standard obtained from *E. coli*. When VHb is expressed in *S. cerevisiae*, more ethanol is produced during glucose utilization. Similar to the VHb effects observed in *E. coli*, the metabolic changes caused by VHb in *S. cerevisiae* appear to be closely linked to respiration. This is demonstrated by the absence of any VHb effect in cultivations that include the respiration inhibitor antimycin A. Although VHb may be interacting with another pathway that is altered significantly by respiration inhibition, it is more likely that the direct effect of VHb is to interact with an oxygen-related pathway such as respiration.

How could such a respiration-linked interaction influence the carbon flux distribution? There are two possible explanations for this question. First, previous reports have indicated that an increase in the glycolytic flux can lead to an increase in ethanol production very similar to what we have observed here (Van der Aar et al., 1990). However, it is unlikely that the action of VHb is to directly influence the enzymatic activities of the glycolytic pathway since we have shown that is it linked to respiration.

A more likely model can be found by the recent discovery of a novel mitochondrial acetaldehyde dismutation pathway. The production of ethanol and acetate from this pathway inside the mitochondria is dependent on electron transport (Thielen and Ciriacy, 1991). Therefore, if the effect of VHb is to influence some key steps involved in electron transport, the net result would be an alteration in ethanol production. Such a result is observed here, as indicated by a decrease in ethanol production from the *adh*° strain expressing VHb. Since none of the cytoplasmic ADHs are active in this mutant, the excess acetaldehyde must be processed by other pathways. One possibility is the conversion of the excess acetaldehyde into acetate via the cytoplasmic acetaldehyde dehydrogenase. It has been suggested before that acetate produced from acetaldehyde could be further converted into acetyl-CoA and could then enter the TCA cycle for energy (Thielen and Ciriacy, 1991). Such a pathway will be consistent with our results in which VHb-expressing cells exhibit greater final cell density. A more direct demonstration of this hypothesis is illustrated by using an acetaldehyde fed-batch fermentation in which the differences in cell growth and ethanol production are much more profound. This is indicative of the important

link between this mitochondrial acetaldehyde dismutation pathway and the effect of VHb. Although the consumption of numerous other metabolites may contribute to cell growth during the fed-batch fermentation, we believe that those metabolites play only a supporting role relative to acetaldehyde, which is the major significant carbon source.

What explains the differences in VHb responses between cultures of SEY2101 and the *adh°* mutant? We believe that, since the amount of acetaldehyde consumed through this mitochondrial acetaldehyde dismutation pathway is less for the VHb-expressing strain, the excess acetaldehyde may be processed by the cytoplasmic ADHs (ADH has a higher affinity for acetaldehyde than ALDH) into ethanol instead of acetate. The amount of ethanol produced via this pathway should be higher because, unlike the dismutation pathway, only ethanol will be produced.

The mechanism of possible interaction of VHb with the yeast electron-transport system is still unknown. However, it is possible that the presence of VHb decreases the coupling between the electron-transport chain and the dismutation pathway, leading to a reduction in ethanol production. Recent experimental results have suggested that one of the possible roles of VHb in *E. coli* is to increase the effective intracellular oxygen concentration, thereby affecting the activities of the terminal oxidases (Kallio et al., 1994). Similar influences on the yeast cytochrome oxidases may also occur. It has been shown that the rate of electron transfer can be reduced by 30% by influencing the activity of the cytochrome oxidases (Casey et al., 1980). To explain the VHb mechanism in *S. cerevisiae*, detailed investigations must be performed to identify the specific component(s) of the electron-transport chain that may interact with VHb.

Results from the physiology studies clearly suggest a change in mitochondrial function in the presence of VHb, although it is difficult to envision this as a direct interaction because the VHb has been shown to be localized primarily in the cytoplasm. However, we cannot rule out the presence of some VHb in the mitochondria or associated with mitochondrial membranes. Since no studies have yet determined the amount of VHb necessary to achieve a significant change in overall physiology, even a small quantity may have a large effect. Also, an indirect mechanism cannot be excluded; the molecular mechanism of the influence of VHb on aerobic metabolism is not known in *Vitreoscilla* or in any other organism.

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Literature Cited

Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., Struhl, K., Eds. In *Short protocols in molecular biology*; Greene Publishing Associates and Wiley-Interscience: New York, 1989.

Bailey, J. E. Toward a science of metabolic engineering. *Science* **1991**, *252*, 1668–1675.

Boerman, S. J.; Webster, D. A. Control of heme content in *Vitreoscilla* by oxygen. *J. Gen. Appl. Microbiol.* **1982**, *28*, 35–43.

Brennand, J.; Margison G. P. Reduction of the toxicity and mutagenicity of alkylating agents in mammalian cells harboring the *Escherichia coli* alkylatransferase gene. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 6292–6296.

Campbell, I., Duffus, J. H., Eds. In *Yeast, a practical approach*; IRL Press: Oxford, 1988.

Casey, R. P.; Thelen, M.; Azzi, A. Dicyclohexylcarbodiimide binds specifically and covalently to cytochrome oxidase while inhibiting its H^+ translocating activity. *J. Biol. Chem.* **1980**, *255*, 3994–4000.

Drewke, C.; Thielen, J.; Ciriacy, M. Ethanol formation in *adh°* mutants reveals the existence of a novel acetaldehyde-reducing activity in *Saccharomyces cerevisiae*. *J. Bacteriol.* **1990**, *172*, 3909–3917.

Kallio, P. T.; Kim, D. J.; Tsai, P. S.; Bailey, J. E. *Intracellular* expression of *Vitreoscilla* hemoglobin alters *Escherichia coli* energy metabolism under oxygen-limited conditions. *Eur. J. Biochem.* **1994**, in press.

Khosla, C.; Bailey, J. E. Heterologous expression of a bacterial haemoglobin improves properties of recombinant *Escherichia coli*. *Nature* **1988**, *332*, 633–635.

Khosla, C.; Bailey, J. E. Evidence for partial export of *Vitreoscilla* hemoglobin into the periplasmic space in *Escherichia coli*. *J. Mol. Biol.* **1989**, *210*, 79–89.

Khosla, C.; Curtis, J. E.; DeModena, J.; Rinas U.; Bailey, J. E. Expression of intracellular hemoglobin improves protein synthesis in oxygen-limited *Escherichia coli*. *Bio/Technology* **1990**, *8*, 849–853.

Laemmli, U. K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **1970**, *227*, 680–685.

Magnolo, S. K.; Leenutaphong, D. L.; DeModena, J. A.; Curtis, J. E.; Bailey, J. E.; Galazzo, J. L.; Hughes, D. A. Actinorhodin production by *Streptomyces coelicolor* and growth of *Streptomyces lividans* are improved by the expression of a bacterial hemoglobin. *Bio/Technology* **1991**, *9*, 473–476.

Oeda, K.; Sakaki, T.; Ohkawa, H. Expression of rat liver cytochrome P-450 cDNA in *Saccharomyces cerevisiae*. *DNA* **1985**, *4*, 203–210.

Pringsheim, E. C. The *Vitreoscillaceae*: a family of colorless, gliding, filamentous organism. *J. Gen. Microbiol.* **1951**, *5*, 124–149.

Sambrook, J., Fritsch, E. F., Maniatis, T., Eds. *Molecular cloning*: *A laboratory manual*; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, NY, 1989.

Sherman, F., Fink, G. R., Hicks, J., Eds. *Laboratory course manual for method in yeast genetics*; Cold Srping Harbor Laboratory Press: Cold Spring Harbor, NY, 1986.

Stanzak, R.; Matsushima, P.; Baltz, D. H.; Roa, R. N. Cloning and expression in *Streptomyces lividans* of clustered erythromycin biosynthesis genes from *Streptomyces erythreus*. *Bio/Technology* **1986**, *4*, 229–232.

Thielen, J.; Ciriacy, M. Biochemical basis of mitochondrial acetaldehyde dismutation in *Saccharomyces cerevisiae*. *J. Bacteriol.* **1991**, *173*, 7012–7017.

Tschumper, G.; Carbon, J. Sequence of a yeast DNA fragment containing a chromosomal replicator and the *Trp1* gene. *Gene* **1980**, *10*, 157–166.

Valenzuela, P.; Medina, A.; Rutter, W. J.; Ammerer, G.; Hall, B. D. Synthesis and assembly of hepatitis B virus surface antigen particles in yeast. *Nature* **1982**, *298*, 347–350.

Van der Aar, P. C.; Lopes, T. S.; Klootwijk, J.; Groneveld, P.; van Verseveld, H. W.; Stouthamer, A. H. Consequence of phosphoglycerate kinase overproduction for the growth and physiology of *Saccharomyces cerevisiae*. *Appl. Microbiol. Biotechnol.* **1990**, *32*, 577–587.

Wakabayashi, S.; Matsubara, H.; Webster, D. A. Primary sequence of a dimeric bacterial hemoglobin from *Vitreoscilla*. *Nature* **1986**, *332*, 481–483.

Windass, J. D.; Worsey, M. J.; Pioli, Z. M.; Barth, P. T.; Atherton, K. T.; Dart, E. C.; Byrom, D.; Powell, E.; Senior R. J. Improved conversion of methanol to single-cell protein by *Methylophilus methylotrophus*. *Nature* **1980**, *287*, 396–401.

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