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# Enhancement of human serum albumin production in *Saccharomyces cerevisiae*

Seo, Dong Sik[1], Byung Hyun Choi[1], Whan Koo Kang[2], In ho Kim[3], Eock Kee Hong[1]
[1]Division of Environmental and Biological Engineering, Kangwon National University
[2]Department of Chemical Engineering, Hannam University
[3]Department of Chemical Engineering, Chungnam National University
TEL (033) 250-6275, FAX (033) 243-6350

## Abstract

Experiments were accomplished to reduce the extent of proteolysis by simply controlling the culture conditions instead of the gene manipulation techniques. L-arginine and L-lysine were chosen as a protease inhibitor analogue with the assumption that they might act as the potential inhibitors against proteases involved in the rHSA proteolysis. The addition of arginine and lysine resulted in a considerable positive effect on the secreted rHSA production level.

## Introduction

Human serum albumin is a single chain nonglycosylated polypeptide composed of 585 amino acids with a molecular weight of 66.5 kD. It functions as a soluble carrier for the transport of fatty acids, amino acids, steroid hormones, and metal ions. It is clinically used in the treatment of patients with severe burns, shock or blood loss, and at present is produced commercially by the extraction from human blood. Approximately 400 tons are used each year. Presently the only source of human serum albumin for clinical usage is from a donated human bood or placentae. In recent years, yeast species have been widely used as the host organisms for the production of rHSA.[1 2] When the HSA is expressed as a secretory product from *S. cerevisiae*, most of the secreted rHSA is internally cleaved by endoproteolytic processing. The unwanted proteolytic cleavage takes place and consequently the yield of rHSA production is significantly reduced. The yeast endoproteases such as Kex2 and Yap3 are involved in the endoproteolytic processing of rHSA from S. cerevisiae.[3]

Therefore, in this work, the experiments were accomplished to reduce the extent of proteolysis by simply controlling the culture conditions instead of the gene

manipulation techniques. L-arginine and L-lysine were chosen as a protease inhibitor analogue with the assumption that they might act as the potential inhibitors against proteases involved in the rHSA proteolysis.

## Materials and Methods

*Saccharomyces cerevisiae* strain used in this study was Y2805 which was mutated by autoselection for the plasmid stability. The plasmid pYHSA5-1(8.5kb) was a vector containing the *URA3* selection marker and the yeast *GAL10* promoter which regulated the gene expression of human serum albumin.

For the seed culture, one colony on SD(glucose 2%, yeast nitrogen base without amino acids 6.7 g/L, adenine 100 mg/L, histidine 80 mg/L, lysine 150 mg/L, methionine 100 mg/L) agar plate was inoculated into 250 mL flask containing 50 mL SD medium and incubated for 20 hr at 250 rpm and 30℃ with the initial pH 5.5. This seed culture was inoculated into YDG (yeast extract 1%, glucose 2%, galactose 3%) medium in 250 mL flask with the working volume of 50 mL. The main culture was accomplished at shaking incubator. Inoculum size was 2%.

Biomass concentration was determined from the optical density measurement at 600 nm. Protein assay was carried out using SDS PAGE. The proteins separated by SDS-PAGE were stained with silver staining and gel images were captured by digital camera. The amount of rHSA was quantified by the gel analysis program(Gelscope 1.5, IMAGELINE, Inc.).

## Results

Effects of arginine and lysine.

L-arginine and L-lysine were chosen as a protease inhibitor analogue with the assumption that they might act as the potential inhibitors against proteases involved in the rHSA proteolysis like YAP3.

Arginine and Lysine were added with the variation up to 0.5M into the YDG medium. The results were shown in Figure 1 and 2. To investigate the effect of arginine and lysine combinations, 0.3M of arginine with the variation up to 0.5M of lysine and 0.4M of lysine with the variation up to 0.3M of arginine were added to YDG medium. The results were shown in Figure 3 and 4.

Flask cultivation

To verify the determined optimum concentration, a flask culture was conducted in medium containing yeast extract 4%, glucose 2%, galactose 3%, 0.3M of arginine and 0.1M of lysine, and compared with the results from YDG medium. The results were shown in Figure 5 and 6. The HSA production was proportionally increased with the cell mass proportionally and stably maintained at the final level. It was presumed that these results were caused by the addition of arginine and lysine as a protease inhibitor analogue such as YAP3 or some other proteases.

## Conclusion

To optimize the medium concentration, L-arginine and L-lysine were chosen as a protease inhibitor analogue with the assumption that they might act as the potential inhibitors against proteases involved in the rHSA proteolysis. The optimum concentrations of arginine and lysine were determined as 0.3M and 0.1M, respectively. The addition of arginine and lysine into the culture medium resulted in a considerable positive effect on the secreted HSA production level with showing approximately 1.8 fold increase in comparison with the control, while there was little change in the cell mass. It was obtained that cell mass was about 11g/L and the secreted HSA production was 66mg/L in the optimum medium from the flask culture.

## References

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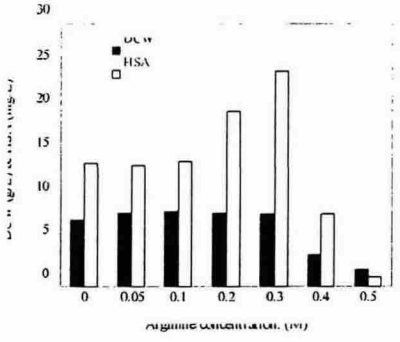


Fig 1. Arginine effect on HSA(48hr)

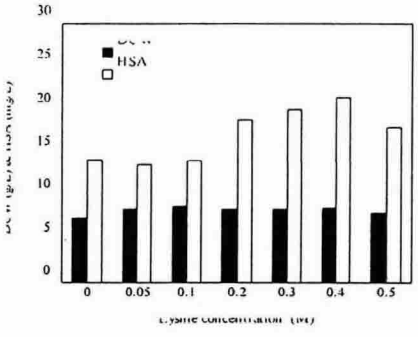


Fig 2. Lysine effect on HSA(48hr)

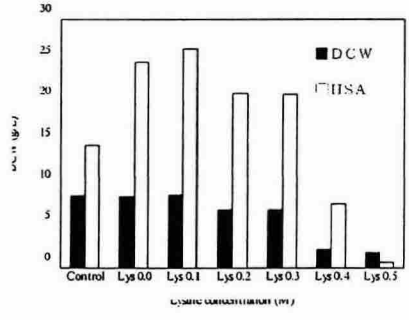


Fig 3. Lysine effect on HSA production with 0.3M arginine(48hr)

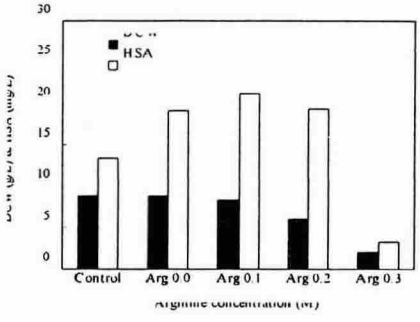


Fig 3. Arginine effect on HSA production with 0.4M lysine(48hr)

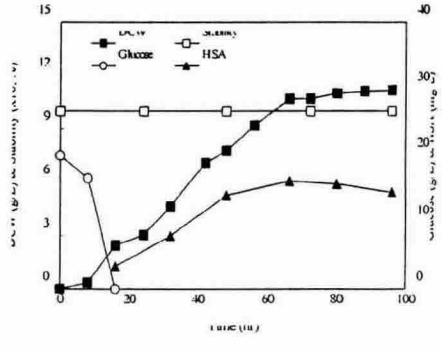


Fig 5. Culture behavior in YPG medium.

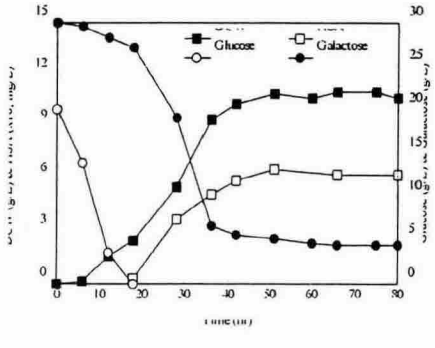


Fig 6. Culture behavior in modified medium.