Recombinant Human Serum Albumin Expressed in Plants Improves the Productivity and Growth Kinetics of CHO

Steve Pettit, Mary Ann Santos, Tanya Tanner, and Ning Huang
Email: Info@InVitria.com • Phone: 1-800-916-8311

Abstract

A recombinant human serum albumin (rHSA) derived from a plant-based expression system was evaluated for its ability to improve the growth kinetics and productivity of CHO cells in serum-free production media. A CHO K1 line expressing a humanized monoclonal antibody was adapted to 6 different commercial serum-free CHO media. rHSA and plasma derived HSA (pHSA) was compared at 1g/L concentration. rHSA outperformed pHSA and resulted in an average 50% increase in the Integrated Viable Cell numbers (IVCN) and volumetric productivity across media formulations. rHSA improved productivity up to 75% in the two chemically-defined (CD) media examined. Dose response studies indicated that increasing rHSA concentration increased productivity and that rHSA outperformed both BSA and pHSA.

CHO passaged in a CD medium with rHSA for 46 days showed stable growth kinetics and had more population doublings. Similar increases in productivity and IVCN were seen after extended passage. The data indicate that rHSA derived from a plant-based expression system is a robust supplement for CHO culture and can improve both the growth kinetics and productivity of CHO.

Introduction and Experimental Approach

Human Serum Albumin (HSA) is a media supplement that improves the growth and productivity of cells in serum-free culture. Albumin has several activities that make it desirable in cell culture: lipid binding, waste and toxic contaminant removal, antioxidant activities, metal carrying, and membrane stabilization. However beneficial, plasma-derived HSA (pHSA) and bovine serum albumin (BSA) are undesirable in bioproduction due to the increased risk of transmitting adventitious infectious agents. Albumin has been reduced or eliminated from the majority of modern production media formulations due to these regulatory concerns.

We evaluated a recombinant albumin produced via expression in plants (Cellastim™ - InVitria) for its ability to enhance the growth and productivity of a model CHO cell line in commercially available CHO production media.

The parameters we evaluated were initial growth, the Integrated Viable Cell Number (IVCN), and volumetric productivity. rHSA was examined in a series of four experiments. First, as a broad test of applicability, we compared the effectiveness of rHSA to pHSA in a variety of commercial serum-free, protein-free and chemically defined (CD) media. Second, we performed a dose response to confirm the effectiveness and applicability of rHSA in two different CD media. Third, we compared the growth kinetics of CHO during extended passage (46 days) in a CD media supplemented with either pHSA or rHSA. Fourth, we determined whether increases in IVCN and productivity were maintained after extended passage.

Cellastim increased the growth, IVCN, and productivity in a variety of serum-free formulations better than pHSA. Volumetric productivity increased up to 75% in two CD media. CHO cells grown in either rHSA or pHSA supplemented CD media showed stable growth during extended passage. However, CHO cells grown in rHSA-supplemented media produced more cell doublings. Finally, similar increases in productivity (75%) and IVCN were found after extended passage in an rHSA-supplemented CD medium. These data indicate that plant-derived rHSA albumin has a unique performance profile that typically outperforms pHSA and BSA.

Material and Methods

HSA, cells, media, and adaptation. pHSA was obtained from Seracare or Baxter. BSA fraction V (Probumin) was obtained from Celliance/Millipore. rHSA was Cellastim™ rHSA from InVitria. Adherent dhfr- CHO line DP-12 clone 1934 producing a humanized monoclonal antibody was adapted to shake culture in 6 different commercial serum- free formulations supplemented with 0.5% dFBS as shown in Table 1. During the adaptation methotrexate (MTX) was increased, stepwise, from 200nM to 5 µM. Individual cell subclones were not selected during the adaptation procedure in avoid skewing results to a particular cell growth characteristic.

Cells were maintained in media, 0.5% dFBS, MTX 5 µM in 125 ml shake flasks. Growth curves were performed in duplicate 4 ml shake-batch cultures with washed cells seeded at 1.0 x10^5 viable cells/ml and subsequently cultured for 14 days.

Viable cell determination, IVCN, and volumetric productivity. The viable cell concentration was determined daily by a Guava PCA cell counter. IVCN was calculated as the sum of viable cell counts over the 14 day culture. The concentration of antibody produced was determined by quantitative ELISA (Bethyl).

Table1. CHO 1934 Adaptation Time Line

A. Serum Reduction in plate culture:
- Adherent CHO 1934 arrives in DMEM/5% FBS
- Media changed to a mixture of RPMI/SAFC CHO DHFR 5% dFBS, 200 nM MTX 1 passage
- 2% dFBS, 200 nM MTX 1 passage
- 1% dFBS, 200 nM MTX 1 passage
- 0.5% dFBS, 200 nM MTX 1 passage

B. Shaker adaptation
- Seeded to 14 ml shake culture at 3.0x10^5 viable cells/ml each passage until able to grow to > 1x10^6cells/ml 4 days; this took 3 months

C. MTX increase
- MTX increased to 600 nM, 1 month
- MTX increased to 800 nM, 1 week
- MTX increased to 1000 nM, 1 week

D. Medium adaptation
- 6 CHO media with 0.5% dFBS, 1 µM MTX, 1 month
- MTX increase to 2 µM, 1 month
- MTX increase to 5 µM, 1 month

Activity of rHSA in a Variety of Serum-free Media

The effect of rHSA supplementation was determined in a variety of serum-free formulations. Six media were chosen to represent a diverse collection of popular CHO production media. The media chosen include serum-free, protein-free, and chemically-defined formulations. In addition, two of the media contain hydrolysates in their formulations.

The model CHO cell line used in this study was adapted to each medium for 3 months as described. Following adaptation, we compared the effect of supplementing each medium with plasma derived HSA (pHSA) or recombinant HSA (rHSA) on cell growth and productivity. Cells were seeded with 1.0 x 10^5 cells/ml in 14-day shake-batch cultures. pHSA and rHSA were tested at 1 g/l concentration. The titer of viable cells was determined daily. At the end of the 14day culture, the IVCN (Integral of Viable Cell Number) and antibody concentration was determined. IVCN is the integrated area under the growth curve and is considered a measure of the total mass of cells generated in a culture system.

Figure 1

Medium	Characteristic
A	Serum-free, <100ug/ml undisclosed protein
B	Protein-free, animal-free, has plant hydrolysates
C	Protein-free, no bovine components
D	Chemically defined, animal-free, no protein
E	Protein-free, animal free, has plant hydrolysates
F	Chemically defined, animal-free, protein free

Figure. 1. Serum-free CHO formulations. In order to test the broad applicability of rHSA in a variety of media formulations, CHO cells were adapted to 6 (A-F) different serum-free commercial formulations. The formulations include serum-free (A), protein free (B,C,E) and chemically defined (D, F) media. Media B and E contain hydrolysates. Adaptation was performed in each media supplemented with 0.5% FBS (without HSA).

Figure 2

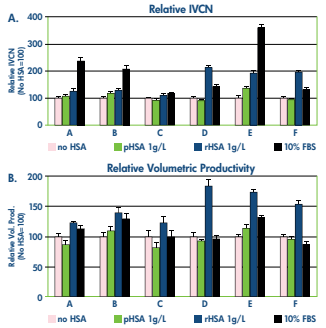

Figure 2. Increased IVCN and productivity of CHO cultured in 6 commercial media supplemented with either 1g/l pHSA (green), 1g/l rHSA (blue), or 10% FBS (black). Unsupplemented media with no HSA is shown in yellow and set to a value of 100. Shake cultures were seeded on day 0 with 1.0 x 10^6 cells/ml. A) Comparison of IVCN. B) Comparison of volumetric productivity. Error bars denote SD.

Table 2

Average increase in the growth and productivity of CHO in 6 serum-free commercial CHO media supplemented with 1g/L pHSA or rHSA (no HSA=100).

	No HSA	pHSA	rHSA
5 day growth	100	131	182
IVCN	100	107	161
Vol. Prod.	100	97	149

Dose response of rHSA chemically-defined media.

The activity of BSA, pHSA, and rHSA at different concentrations in chemically-defined media was determined. The dose response of 2 different pHSAs, BSA and 2 lots of rHSA were compared at 0.5, 1, 2, and 3 g/L concentration. The experiment was performed twice to evaluate the dose response in both CD media D and F.

Stock CHO cells were washed in medium and seeded at 1.0 x 10^5 cells/ml in 14 day shake-batch cultures. The number of viable cells was determined daily. IVCN and volumetric productivity was compared.

Figure 3

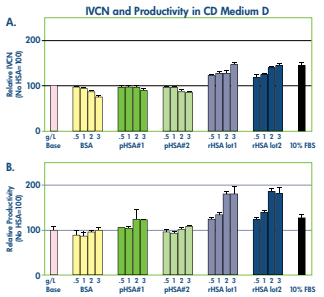

Figure 3. The dose response of pHSA and rHSA on the IVCN and volumetric productivity of CHO in chemically-defined medium D. BSA, 2 pHSAs and 2 lots of rHSA were compared at 0.5, 1, 2, 3 g/L concentration (Sequential bars). Unsupplemented base medium (pink) and medium/10% FBS (Black) are controls. A) Relative 14 day IVCN B) Relative Productivity. The relative values are normalized where unsupplemented base medium (pink) is set to a value of 100. Error bars denote SD.

Figure 4.

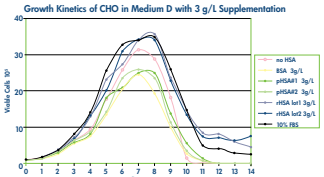

Figure 4. A representative growth profile from the dose response study of CHO in chemically-defined medium D supplemented with BSA, 2 pHSAs, 2 lots of rHSA or 10% FBS. Shown are growth curves at 3 g/L albumin supplementation. yellow, BSA; dark green, pHSA #1; light green, pHSA #2; light Blue, Lot 1 rHSA; dark Blue, Lot2 rHSA, Black, 10% FBS. Cells were seeded at 1.0 x 10^5 cells/ml in shake-batch culture. Cells grew faster, reached higher peak density, and survived longer when supplemented with rHSA. Also, the growth kinetics of CHO in rHSA supplemented medium was similar to that seen with 10% FBS.

Figure 5.

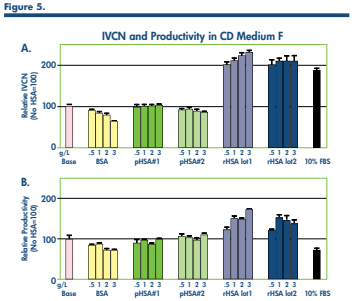

Figure 5. The dose response of pHSA and rHSA on the IVCN and volumetric productivity of CHO in chemically-defined medium F. BSA, 2 pHSAs and 2 lots of rHSA were compared at 0.5, 1, 2, 3 g/L (Sequential bars). Unsupplemented base medium (pink) and medium/ 10% FBS (Black) are controls. A) Relative 14 day IVCN B) Relative Productivity. The relative values are normalized where unsupplemented base medium (pink) is set to a value of 100. Error bars denote SD.

46 Day Passage of CHO in rHSA Supplemented Media.

CHO was passaged for 46 days in chemically-defined medium D supplemented with two lots of pHSA or rHSA at 1g/L concentration. Comparative cultures were grown in unsupplemented CD medium D or medium D supplemented with 1g/L pHSA or 10% FBS. Cells were seeded at 4.0 x10^5 cells/ml and passaged twice a week. The number of cumulative populations doublings was calculated with each passage.

The purpose of the extended passage to examine the stability of growth with supplementation. Another purpose was to generate stocks of cells adapted to media supplemented with pHSA or rHSA.

Figure 6.

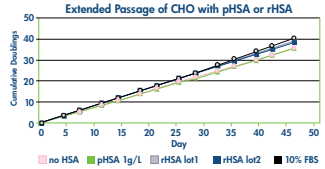

Figure 6. 46 day passage of CHO in chemically-defined medium D supplemented with 1g/L pHSA or 1g/L rHSA. The cumulative population doublings over 46 days of culture is shown. Pink, unsupplemented; green, pHSA; light blue, rHSA lot1; blue, rHSA lot2; black, 10% FBS. The rate of growth was relatively stable during the 46 days. Cultures supplemented with rHSA produced more doublings. Doubling time was reduced ~ 2h compared to unsupplemented medium and was more similar to 10% FBS than to pHSA (P<0.01).

Growth Kinetics and Productivity of CHO after Extended Passage with rHSA.

CHO cells that had been passaged for 46 days in chemically-defined medium D supplemented with either pHSA or rHSA were compared in a dose response study. The dose response was determined at 0.5,1,2, or 3 g/L pHSA or rHSA. Cells were washed and reseeded at 1.0 x10^5 cells/ml. The number of viable cells was determined daily. After the run, the IVCN was calculated and the antibody titer was determined.

The results indicate that rHSA supplementation post-passage increased IVCN and productivity. The magnitude of the increase was similar to that observed before the extended passage. Productivity increased up to 75% when CD medium D was supplemented with rHSA.

Figure 7.

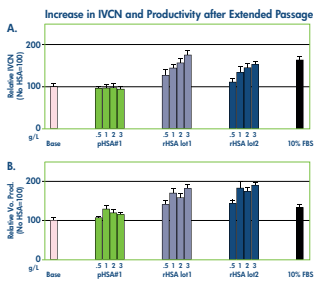

Figure 7. The IVCN and volumetric Productivity of CHO cultures after 46 day passage in chemically-defined medium D. CHO cultures were passaged for 46 days as shown in Figure 6 with 1g/l pHSA or 2 lots of rHSA. After the passage, a dose response study was conducted on each culture at 0.5, 1, 2, 3 g/L supplementation (sequential bars). The effect of rHSA on IVCN is similar before and after long term passage (Compare to Figure 4) Also, the corresponding increases in IVCN and productivity are of similar magnitude.

Figure 8.

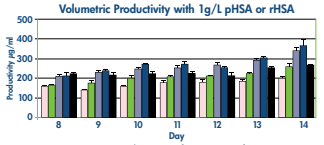

Figure 8. Antibody concentration of CHO cultures supplemented with 1g/L pHSA or rHSA. The concentration of antibody was determined for days 8 - 14. The concentration of antibody in rHSA supplementation cultures increased and was higher than that seen with pHSA supplementation.

Conclusions

Animal-free Cellastim™ rHSA from a plant-based expression system was evaluated for its ability to improve CHO growth kinetics and productivity. When tested in a variety of media formulations rHSA improved IVCN and productivity by 50%. The performance profile of rHSA was better than pHSA in each of the formulations.

A dose response study in two chemically defined media showed that productivity increased with increasing rHSA concentration. Productivity increased up 75% compared to the unsupplemented or pHSA-supplemented media.

CHO cells passaged in media supplemented with pHSA or rHSA showed stable growth kinetics during 46 days of extended passage. After extended passage, CHO cells showed similar increases in IVCN and productivity upon rHSA supplementation to that observed before the extended passage.

rHSA derived from a plant based expression system was found to be a robust supplement for CHO culture.