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Comparison of long R³ IGF-1 with insulin in the support of cell growth and recombinant protein expression in CHO cells

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Summary

Cells cultured in the absence of serum need other sources of mitogenic stimulation for growth. Insulin is the primary mitogen used in most current serum-free medium formulations. A modified insulin-like growth factor 1 (IGF-1) has been developed by GroPep Pty. Ltd. in Australia that exhibits better biological activity than non-modified IGF-1. We have compared insulin to this modified IGF-1 (long R³ IGF-1) and demonstrated equivalent or better performance using two recombinant CHO cell lines.

Keywords: Insulin, Insulin-like growth factor 1, CHO cells, mitogen, long R³ IGF-1

Introduction

Insulin is a critical component of most serum-free media used in cell culture production processes. Its mitogenic function is essential for obtaining the viable cell density needed for optimum production in most batch production formats. Insulin-like growth factors (IGFs) are similar to insulin in amino acid sequence and tertiary structure, and they also serve as mitogens and/or support the survival and differentiation of several cell types (1-3). Much of the mitogenic activity of serum may be in the form of IGFs for some cell types (4), and physiological activity of these molecules is generally found in the 10 to 100 ng/ml range.

Key factors that influence the response of cells to IGFs are the types of receptors expressed on the cell surface for signal transduction, and the presence of IGF binding proteins in the medium. The receptors for insulin and for IGFs share extensive homology, just as their respective cognates, and each transduces signal through tyrosine kinase-activity (5). IGFs and insulin will bind with high affinity to their own receptors, but they can also bind with low affinity to each others receptor (6, 7). It is possible that the large, non-physiological concentration of insulin required to promote the growth of many cell lines in serum-free media may be the result of low affinity binding of insulin to IGF receptors instead of insulin receptors.

Truncation of IGF-1 at the N-terminus has been shown to reduce binding of IGF-1 to IGF-1 binding proteins, thereby enhancing its biological activity. A group in Australia has extensively studied the biological activity of different truncated forms and found that des-(1-3)-IGF-1 provided the greatest half-maximal response in an L6 myoblast assay of protein synthesis (8, 9). Other modifications of IGF-1 that were found to contribute to potency were substitution of the Glu residue at position 3 with Arg, and the addition of a 13-residue N-terminal extension peptide. These last modifications provided the most active form of IGF-1, and is the form termed long R³ IGF-1 as shown in Figure 1. The GroPep group in Australia produces long R³ IGF-1 in *E. coli* and it is distributed by Hyclone Laboratories.

An initial experiment compared the performance of a culture that was not supplemented with mitogen with cultures supplemented with either long R³ IGF-1 (50 ng/ml, then 3 x 25 ng/ml) or insulin (4 x 10 µg/ml). The results of this comparison are found in Table 1.

Table 1 Experimental Case	Volumetric Production	Specific Production	Cumulative Viable Cell Days
Insulin *	100.00	100.00	100.00
long R ³ IGF-1	150.00	133.00	120.00
no mitogen	38.00	82.00	36.00

* The insulin control (4 x 10 µg/ml) represents 100%, and all other experimental cases are normalized as a percent of control values.

These data show the necessity of mitogenic factors like insulin or IGF-1 for performance of CHO cells in serum-free media. Volumetric production was only 38 % of control values and can be explained primarily by fewer cumulative viable cell days during the culture period. On the other hand, multiple additions of long R³ IGF-1 showed enhanced volumetric and specific production, and also supported more cumulative viable cell days than the insulin control. These results were followed by another bioreactor experiment in which single and multiple additions of long R³ IGF-1 were again compared to an insulin control case. A summary of these data are provided in Table 2.

Table 2 Experimental Case	Volumetric Production	Specific Production	Cumulative Viable Cell Days
Insulin *	100.00	100.00	100.00
long R ³ IGF-1 (multiple)**	127.00	107.00	127.00
100 ng/ml	102.00	95.00	104.00
50 ng/ml	98.00	107.00	102.00
25 ng/ml	107.00	100.00	96.00

* The insulin control (4 x 10 µg/ml) represents 100%, and all other experimental cases are normalized as a percent of control values.

** Multiple additions of long R³ IGF-1 (50 ng/ml, then 3 x 25 ng/ml)

Due to the nature of these calculations, where viable cell numbers and ELISA values are used, errors can be additive. Numbers that are within 20 % of control are probably no different than control. Using this as a guide, the only group that may have outperformed the control insulin case is the culture receiving multiple additions of long R³ IGF-1. Both the cumulative viable cell days and the volumetric production of IL-1R were greater in this case. When the glucose consumption was determined for these cultures, the multiple addition long R³ IGF-1 case also consumed about 20% more glucose than the other cultures. This supports the greater cumulative viable cell day observation for this culture and resulted in more IL-1R volumetric production.

An initial experiment with another CHO cell line expressing TNFR:Fc was also performed comparing a single addition of long R³ IGF-1 (100 ng/ml) with the previously described insulin control case. The results are summarized in Table 3.

Table 3

Experimental Case	Volumetric Production	Specific Production	Cumulative Viable Cell Days
Insulin *	100.00	100.00	100.00
long R ³ IGF-1 100 ng / ml	124.00	99.00	111.00

* The insulin control (4 x 10⁶ µg/ml) represents 100%, and all other experimental cases are normalized as a percent of control values.

This preliminary information suggests that the CHO cells expressing TNFR:Fc are just as responsive to long R³ IGF-1 as those expressing IL-1R. Volumetric production and cell growth expressed as cumulative viable cell days were at least equivalent, if not slightly better, than the insulin case.

Discussion

The ability of long R³ IGF-1 to support cell growth and expression has been established using two recombinant CHO cell lines. Experiments presented in this report show that performance of cultures supplemented with long R³ IGF-1 are at least equivalent to cultures receiving multiple additions of insulin. Multiple additions of long R³ IGF-1 may actually provide better performance than the insulin control case. As little as 25 ng/ml of long R³ IGF-1 provided equivalent performance compared to a total of 40 µg/ml of insulin. Since lower concentrations of long R³ IGF-1 were not tested, it is conceivable that concentrations lower than 25 ng/ml would also be as effective as insulin. No attempt was made to optimize insulin concentrations. Rather, the objective of these experiments were to provide a comparison of long R³ IGF1 to a control case that we believe is saturated with insulin. For a true comparison of biological activity per unit weight, a more rigorous optimization of both insulin and long R³ IGF-1 is needed. From experiments presented here we conclude that recombinant CHO cells will respond to long R³ IGF-1 at concentration that are from 100 to 1000 times lower than concentrations of insulin normally used in serum-free cell culture media.

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