serum-free medium. Franek et al., 2002, Biotechnol. Prog. 18, 155-158. They reported that while single amino acid or dipeptide did not significantly alter the culture parameters, tri-, tetra-, and penta-glycine as well as tri- and tetra-alanine enhanced viable cell density and viability. Id. Certain tri-peptides enhanced production of monoclonal antibody from this mouse monoclonal cell line. Id. In subsequent experiments on CHO cells recombinantly engineered to produce SEAP, this group reported that tetra-glycine increased viable cell density, and that the tri-peptide Gly-Lys-Gly enhanced SEAP production. Franek and Fussengger 2005, Biotechnol. Prog. 21, 96-98.

[0006] There remains a need in the art to develop methods for recombinant production of protein in completely defined – media or chemically defined media. While protein hydrolysates can improve survival, they remain a source of heterogeneity. Although certain tri- and tetra-peptides have been reported to improve culture parameters, it is preferable to have as simple and as defined a cell culture process as possible which maximizes recombinant protein titer. Any improvements to recombinant polypeptide expression, titer, cell growth and/or cell viability can lead to higher production levels, thereby reducing costs associated with the manufacture of protein therapeutics. The invention fulfills these needs by providing simple, easy and novel methods of increasing cell growth and protein production.

SUMMARY ~~OF THE INVENTION~~

[0007] Described herein is~~The invention provides~~ a method of culturing animal cells that have been recombinantly engineered to express a protein of interest, the method comprising, growing the CHO cells in a serum-free medium during a growth phase, and growing the CHO cells in a serum-free defined production medium during a production phase, wherein during the production phase the serum-free medium is supplemented with at least one dipeptide selected from Tyr-His, Tyr-Lys, Tyr-Ala, Tyr-Val, His-Gly, and Ala-His, and wherein the titer of the protein is improved in the presence of the dipeptide or dipeptides as compared to the absence of the dipeptide or dipeptides. A~~The invention also~~ described~~provides~~ further is supplementing the serum-free defined production medium with at least one dipeptide selected from Thr-Phe, His-Glu, Glu-His, His-Ser, and His-Gln. The addition of such dipeptides can further improve titer, and/or can improve the viability and viable cell density of the resulting production culture.

[0008] ~~F~~The invention further described herein is~~provides~~ a method of culturing Chinese hamster ovary (CHO) cells that have been recombinantly engineered to express a protein, the method comprising growing the CHO cells in a serum-free medium during a growth phase, and

growing the CHO cells in a serum-free defined production medium during a production phase, wherein during the production phase the serum-free medium is supplemented with at least one dipeptide selected from His-Glu, Glu-His, His-Ser, His-Gln, Tyr-His, Tyr-Lys, Tyr-Ala, Tyr-Val, His-Gly, and Ala-His, and wherein the viability of the cell culture is improved in the presence of the dipeptide or dipeptides as compared to the absence of the dipeptide or dipeptides.

[0008a] In a first aspect, the present invention refers to a method of culturing Chinese hamster ovary (CHO) cells that have been recombinantly engineered to express a protein, the method comprising growing the CHO cells in a serum-free medium during a growth phase, and growing the CHO cells in a serum-free defined production medium during a production phase, wherein during the production phase the serum-free medium is supplemented with at least one dipeptide selected from Tyr-His, Tyr-Lys, Tyr-Ala, Tyr-Val, His-Gly, and Ala-His, and wherein the titer of the protein is improved in the presence of the dipeptide or dipeptides as compared to the absence of the dipeptide or dipeptides.

[0009] Accordingly, using the methods described herein~~of the invention~~, cell viability, viable cell density and expression of the protein of interest are improved relative to cells grown in chemically defined medium without dipeptides.

[0010] A~~The present invention also~~ described herein is~~provides~~ an animal cell culture recombinantly engineered to express a protein, in a serum-free defined production medium supplemented with at least one dipeptide selected from Tyr-His, Tyr-Lys, Tyr-Ala, Tyr-Val, His-Gly, and Ala-His. A~~The invention also~~ described herein is~~provides~~ an animal cell line in a serum-free defined production medium supplemented with at least one dipeptide selected from Thr-Phe, His-Glu, Glu-His, His-Ser and His-Gln.

[0010a] In a second aspect, the present invention refers to a cell culture comprising a Chinese hamster ovary (CHO) cell line recombinantly engineered to express a protein, a serum-free defined production medium supplemented with at least one dipeptide selected from Tyr-His, Tyr-Lys, Tyr-Ala, Tyr-Val, His-Gly, and Ala-His.

[0010b] In a third aspect, the present invention refers to a method of culturing Chinese hamster ovary (CHO) cells that have been recombinantly engineered to express a protein, the method comprising growing the CHO cells in a serum-free medium during a growth phase, and growing the CHO cells in a serum-free defined production medium during a production phase, wherein during the production phase the serum-free medium is supplemented with at least one dipeptide selected from His-Glu, Glu-His, His-Ser, His-Gln, Tyr-His, Tyr-Lys, Tyr-Ala, Tyr-Val, His-Gly, and Ala-His, and wherein the viability of the cell culture is improved in the presence of the dipeptide or dipeptides as compared to the absence of the dipeptide or dipeptides.

[0011] Within the ~~embodiments~~examples of the ~~invention~~present disclosure, the dipeptide can be added at a final concentration in the serum-free defined production medium from about 0.1 g/L to about 5 g/L. The dipeptide can also be added in a feed medium to the production phase. Also encompassed within the ~~embodiments~~examples of the ~~invention~~present disclosure is the addition of two or more dipeptides. One ~~embodiment~~example is the addition of both dipeptides Tyr-His and Thr-Phe. The serum-free defined production medium can also contain putrescine and/or spermine and/or insulin-like growth factor type 1 (IGF-1).

[0012] Within the ~~embodiments~~examples of the ~~invention~~present disclosure, the protein of interest can be a human antibody, a humanized antibody, a chimeric antibody, a recombinant protein, a recombinant fusion protein, growth factor, enzyme, or a cytokine.

BRIEF DESCRIPTION OF THE DRAWINGS

[0013] Figure 1 shows the effect on titer (figure 1A) and culture viability (figure 1B) with the addition of various dipeptides as indicated to a production culture of cell line A.

[0014] Figure 2 shows the effect on titer (figure 2A) and culture viability (figure 2B) with the addition of various dipeptides as indicated to a production culture of cell line B.

[0015] Figure 3 shows the effect on titer (figure 3A) and culture viability (figure 3B) with the addition of various dipeptides as indicated to a production culture of cell line C.