METHODS FOR PRODUCING AFLIBERCEPT IN CHEMICALLY DEFINED MEDIA HAVING REDUCED AFLIBERCEPT VARIANTS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to and the benefit of U.S. Provisional Patent Application No. 63/065,012, filed on Aug. 13, 2020, the content of which is incorporated herein by reference in its entirety. This application also claims priority to and the benefit of Provisional Patent Application No. 62/944,635, filed on Dec. 6, 2019.

SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been filed electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on Jan. 18, 2021, is named 070816-02250_SL.txt and is 148,897 bytes in size.

FIELD

[0003] The present invention generally pertains to anti-VEGF compositions and methods for producing the same.

BACKGROUND

[0004] Protein-based biopharmaceutical compositions have emerged as important products for research, the treatment of ophthalmological diseases, cancer, autoimmune disease, and infection, as well as other diseases and disorders. Biopharmaceuticals represent one of the fastest growing product segments of the pharmaceutical industry.

[0005] A class of cell-derived dimeric mitogens with selectivity for vascular endothelial cells has been identified and designated vascular endothelial cell growth factor (VEGF).

[0006] Persistent angiogenesis may cause or exacerbate certain diseases such as psoriasis, rheumatoid arthritis, hemangiomas, angiofibromas, diabetic retinopathy and neovascular glaucoma. An inhibitor of VEGF activity would be useful as a treatment for such diseases and other VEGF-induced pathological angiogenesis and vascular permeability conditions, such as tumor vascularization. The angiopoietins and members of the vascular endothelial growth factor (VEGF) family are the only growth factors thought to be largely specific for vascular endothelial cells.

[0007] Several eye disorders are associated with pathological angiogenesis. For example, the development of age-related macular degeneration (AMD) is associated with a process called choroidal neovascularization (CNV). Leakage from the CNV causes macular edema and collection of fluid beneath the macula resulting in vision loss. Diabetic macular edema (DME) is another eye disorder with an angiogenic component. DME is the most prevalent cause of moderate vision loss in patients with diabetes and is a common complication of diabetic retinopathy, a disease affecting the blood vessels of the retina. Clinically significant DME occurs when fluid leaks into the center of the macula, the light-sensitive part of the retina responsible for sharp, direct vision. Fluid in the macula can cause severe vision loss or blindness.

[0008] Various VEGF inhibitors, such as the VEGF trap EYLEA® (aflibercept), have been approved to treat such eye disorders.

SUMMARY

[0009] The present invention relates to anti-VEGF proteins including the VEGF trap protein aflibercept, which is a fusion protein. The instant invention also pertains to a new anti-VEGF protein, the aflibercept MiniTrap or VEGF MiniTrap (collectively referred to as MiniTrap unless otherwise noted). Disclosed herein are methods of making these anti-VEGF proteins, including production modalities that provide efficient and effective means to produce the proteins of interest. In one aspect, the instant invention is directed towards the use of chemically defined media (CDM) to produce anti-VEGF proteins. In a particular aspect, the CDMs of interest are those that, when used, produce a protein sample wherein the sample has a yellow-brown color and may comprise oxidized species. Still further in the present application, protein variants of aflibercept and VEGF MiniTrap are disclosed together with attendant production methods.

Production of Aflibercept

[0010] The present disclosure describes the production of aflibercept using a cell culture medium. In one embodiment, the cell culture medium is a chemically defined medium ("CDM"). CDM is often used because it is a protein-free, chemically-defined formula using no animal-derived components and there is certainty as to the composition of the medium. In another embodiment, the cell culture medium is a soy hydrolysate medium.

[0011] In one embodiment, a method of producing a recombinant protein comprises: (a) providing a host cell genetically engineered to express a recombinant protein of interest; (b) culturing the host cell in a CDM under suitable conditions in which the cell expresses the recombinant protein of interest; and (c) harvesting a preparation of the recombinant protein of interest produced by the cell. In one aspect, the recombinant protein of interest is an anti-VEGF protein. In a particular aspect, the anti-VEGF protein is selected from the group consisting of aflibercept and recombinant MiniTrap (examples of which are disclosed in U.S. Pat. No. 7,279,159), an aflibercept scFv and other anti-VEGF proteins. In a preferred aspect, the recombinant protein of interest is aflibercept.

[0012] In one aspect of the present embodiment, aflibercept is expressed in a suitable host cell. Non-limiting examples of such host cells include, but are not limited to, CHO, CHO K1, EESYR®, NICE®, NS0, Sp2/0, embryonic kidney cells and BHK.

[0013] Suitable CDMs include Dulbecco's Modified Eagle's (DME) medium, Ham's Nutrient Mixture, Excell medium, and IS CHO-CD medium. Other CDMs known to those skilled in the art are also contemplated to be within the scope of the present invention. In a particular aspect, a suitable CDM is CDM1B (Regeneron) or Excell Advanced Medium (SAFC).

[0014] In one embodiment, a clarified harvest sample from a CDM culture comprising aflibercept is subjected to a capture chromatography procedure. In one aspect, the capture step is an affinity chromatography procedure using, for example, Protein A. In a further aspect, the eluate of the