

CELL CULTIVATION METHOD AND MEDIUM

BACKGROUND OF THE INVENTION

Factor VIII is a blood protein essential for the blood coagulation process. Lack of Factor VIII will reduce or prevent the coagulating ability of the blood, thereby causing a disease called hemophilia A. Patients suffering from this disease are treated with therapeutical preparations containing Factor VIII.

Factor VIII has conventionally been recovered from human blood plasma. Such Factor VIII concentrates contain several fragmented fully active Factor VIII forms (Andersson et al., Proc. Natl. Acad. Sci. U.S.A., Vol. 83, 2979-83, May 1986). The smallest active form has a molecular mass of 170 kDa and consists of two chains of 90 kDa and 80 kDa held together by a metal ion bridge, as disclosed in EP-A-197 901.

Factor VIII can, however, now also be produced as a recombinant protein expressed in mammalian cells, as described, for example, in J. Gitschier et al., Nature 312, 330-37, 1984 and EP-A-160 457. A truncated recombinant Factor VIII product which corresponds to the 170 kDa plasma Factor VIII is disclosed in WO 91/09122.

Media used for the cultivation of mammalian cells include a variety of nutrients and growth factors, many of which are traditionally supplied by serum. Serum is usually derived from either foetal calf, newborn calf or horse and added to the medium in concentrations from 0.5 to 20 % v/v. In addition to supplying growth enhancing components, serum also functions as a carrier/buffer/chelator for labile or water insoluble molecules, toxin neutraliser, protease inhibitor, cell attachment enhancer and as a protective agent in agitated suspension cultures.

The use of serum in cell culture media, however, has several disadvantages. It is comparatively expensive, it is not a defined component, and different lots of serum may vary in the concentration of compounds present and thus result in unpredictable culture growth and productivity. Serum may also be the source of contaminants such as mycoplasma, bacteriophages, virus and toxins. Additionally, the protein in serum may complicate the purification of cell products from cell culture media.

In efforts to overcome the disadvantages of serum containing medium, serum-free media have been developed in which serum is substituted with better defined or more characterised components. Due to the complexity of serum and the different growth requirements of cells, this has resulted in a variety of different media compositions. (For reviews it may be referred to Rizzino et al., Nutrition Reviews 37: 369-378 (1979); Barnes and Sato, Cell 22: 649-655 (1980); and Bodeker et al., Develop. Biol. Standard. 60: 93-100 (1985)). In most such serum-free media the serum is substituted by "cocktails" of trace elements, lipids, hormones, growth factors and purified proteins, for example serum albumin, and are therefore often only partially defined.

Media for the serum-free culture of Chinese hamster ovary (CHO) cells have been reported by Gasser et al., In-vitro Cellular Developmental Biology 21: 588-592 (1985), and Mendiaz et al., In-vitro Cellular Developmental Biology 22: 66-74 (1986). These media compositions, however, contain non-defined components derived from either human or animal source.

Albumin is considered a multifunctional transport protein for a broad spectrum of ligands including inorganic cations,

organic anions, amino acids and hydrophobic molecules such as fatty acids (Meucci et al., Journal of Biological Chemistry 266: No. 8, 4692-4699 (1991)). Due to the nature of human serum albumin (HSA), it is an important component in serum-free media used for the culture of cells expressing recombinant Factor VIII both in the context of cell growth and production. It is envisaged that it also acts as a stabiliser/protection factor for example against proteases and shear forces (van der Pol and Tramer, Effect of reducing the serum or albumin concentrations on the shear sensitivity of two hybridoma cell lines in sparged cultures. ESACT 11th Symposium, 1991).

The supplementation of cell culture media with HSA, however, potentially has similar disadvantages to the use of serum in media. Thus, it is expensive and periodically scarce, and HSA from different suppliers and even different lots may vary in the concentration of compounds present and therefore result in unpredictable culture growth and productivity. Serum albumin may also be the source of unknown contaminants and virus. Consequently, the replacement of HSA in the serum-free cell culture media used for the recombinant Factor VIII cell culture process by a non-human or non-animal derived substituent would be highly desirable.

WO 92/05246 generally discloses a serum-free mammalian cell culture medium for inter alia cells transformed to produce recombinant products, which medium comprises a synthetic basal medium, hydrolyzed yeast, albumin or dextran (specifically having a molecular weight of 500,000), insulin, transferrin or a transferrin substitute, and a fatty acid component.

EP-A-0441695 discloses a process for the preparation of Factor VIII or an analogue thereof in a cell culture medium containing a derivative of a polycationic or polyanionic polymer, such as dextran sulfate.

US-A-4,786,599 discloses a serum-free cell culture medium containing a mixture of fatty acids and albumin or dextran.

DE-A-3709282 discloses a lipid additive for cell culture media, which additive consists of lipids covalently bound to a water-soluble polymer such as starch, agarose, dextran or proteins.

SUMMARY OF THE INVENTION

The object of the present invention is to provide for the culture of recombinant Factor VIII, hereinafter for brevity referred to as "rVIII", in a serum-free medium that does not contain human serum albumin (HSA), and more particularly to replace HSA in a serum-free medium formulation used for the cultivation of mammalian cells expressing rVIII with a non-human or -animal derived component(s), without causing any diverse effects on cell growth characteristics or the production of rVIII.

In accordance with the present invention it has been found that HSA may be replaced by particular polysaccharide components to be defined below in the serum-free medium formulation used for the rVIII cell culture process. The resulting medium, which thus contains no human or animal derived products, permits the cell-line expressing the rVIII gene to be thawed directly into the medium and subsequently cultivated without any noticeable effects to cell growth characteristics. Further, the medium will support "normal" cell growth in terms of viability, growth rate and morphology over extended culture periods, and it will also enable the cultivation of the rVIII-producing cell-line in a stirred tank bioreactor.