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PRODUCTION OF HIGH ACTIVITY FIBRINOLYTIC AGENTS

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This invention relates to the production of high activity fibrinolytic agents of microbiological origin substantially free of blood pressure lowering agents and to the fibrinolytic agents so produced.

It is now known that certain micro-organisms are the source of a product which has a lytic action on fibrin clots of human origin and that for example *Aspergillus oryzae* is a source of such product. The growing of certain strains of *Aspergillus oryzae* by deep culture techniques to provide an important source of such fibrinolytic product forms the subject matter of one co-pending application Serial No. 156,142, now abandoned. Another co-pending application Serial No. 156,144, now abandoned, relates to the separation of fibrinolytic material of microbiological origin from the medium in which it is produced and to the purification of such separated fibrinolytic material.

While such fibrinolytic material produced up to the present has been found to have the above-referred-to beneficial lytic action on fibrin clots, it has also been found to contain blood pressure lowering agents and it will be appreciated the value of such material would be materially enhanced if such blood pressure lowering agents were removed.

It is therefore the object of this invention to enable the production of fibrinolytic material substantially free from blood pressure lowering agents. Further, it is the object to enable the removal of such pressure lowering agents to be effected from fibrinolytic material whether of relative crude form or of highly purified form.

Another important object is to provide not only a fibrinolytic material free from such blood pressure reducing material but a material having high fibrinolytic activity.

Another important object is to enable the removal of such blood pressure lowering agents with a relatively simple procedure so that such enhanced fibrinolytic material may be produced in substantial quantities.

The principal feature of the invention resides in filtering the fibrinolytic material through a cross-linked dextran known in the trade as "Sephadex G50," which has a cross linked dextran having a water regain value of 5.7 as defined in the Journal Acta histochemica Bd. 11, 1961, page 306.

According to the invention, the starting fibrinolytic material employed is a relatively crude preparation of a substance produced by growing strains of the mould *Aspergillus oryzae*, strain B1273, for example, by the deep culture techniques as set out in said co-pending application Serial No. 156,142. This strain B1273 of *Aspergillus oryzae* is available from the Quartermaster Research and Development Center, Dept. of Army, Natick, Mass. (see Proc. Soc. Exper. Biol. & Med. 99, 505, 1958 and 102, 203, 1959). Alternatively, the starting material may be a more purified material such as, for example, the purified fibrinolytic materials disclosed in said co-pending application Serial No. 156,144.

According to the invention such fibrinolytic material in either the crude or more purified form is filtered through a column of cross-linked dextran described under the trademark "Sephadex G50." The filter is then developed with an eluting agent and the eluate collected in fractions and the fractions of high fibrinolytic activity may then be pooled and dried in vacuo from the frozen state to produce fibrinolytic product which not only has a high potency but also is substantially free from blood pressure lowering agents.

Preferably, according to the invention, the filtering of the starting fibrinolytic material through the cross-linked dextran, Sephadex G50, is carried out at low temperatures, for example, at temperatures slightly above the freezing point of water to provide increased yields of the desired blood-pressure-lowering-agent-free fibrinolytic material.

The invention will be more fully understood from the following examples:

Example I

1.0 gram of fibrinolytic material having an activity of approximately 5000 units per mg. (as determined by a fibrin plate method of assay as is well understood in the art) (see proceedings of the Society for Experimental Biology and Medicine, volume 102, 1959, pages 201 to 203) but also having the characteristic of reducing the blood pressure when administered was dissolved in 15 ml. of water and the acidity adjusted to pH 4.8 with hydrochloric acid. This solution was filtered through cross-linked dextran (medium "Sephadex G50") in a column approximately 2" in diameter and 22" in height and having a temperature slightly above the freezing point of water, about 3° C. to 6° C. The filter was developed with an eluting agent consisting of distilled water adjusted to pH 4.8 by the careful addition of hydrochloric acid. The eluate was collected in 15-ml fractions. The fractions with high activity (fractions 33 to 41) were pooled and dried in vacuo from the frozen state. The yield was 190 mg. of material having a potency of approximately 12,000 units per mg. This product was tested and found to be substantially completely free of any blood pressure lowering agents.

While in the specific example, the eluting agent was adjusted to pH 4.8, in similar examples the pH varied from about ph 3.5 to about pH 5.5 and similar yields obtained.

Example II

Although the material obtained from Example I has remarkably high activity in addition to being free from blood pressure lowering agents and is suitable for clinical use, its activity may be further increased as follows according to these examples.

A quantity of the material of Example I having a potency of 12,000 units per mg. was dialysed against running tap water yielding a fibrinolytic product containing about 18,000 units per mg.

Example III

50 mg. of a relatively cruder fibrinolytic material having a potency of between 3,000 and 4,000 units per mg. were dissolved in 10 ml. of 8 molar urea solution and the solution filtered through a column of cross-linked dextran, Sephadex G50, as in Example I. Water was then run through the column and approximately thirty 3 ml. fractions collected. Four of the fractions (fractions 13–16 inclusive) containing most of the activity were freeze-dried to give 11 mg. of a product having fibrinolytic activity of approximately 8,000 units per mg. Again, the use of the cross-linked dextran, Sephadex G50, was found to not only materially increase the potency of the fibrinolytic material but also to remove the blood pressure lowering agents present in the starting material.

It is to be pointed out that the filtering the material through the cross-linked dextran is best accomplished by using the column in a refrigerator as it appears increased yields are obtained when work is carried out at low temperature, that is, a temperature slightly above the freezing point of water.

The process of using cross-linked dextran, Sephadex G50, in accordance with the invention is thus shown to be a convenient and useful method for preparing a product having high fibrinolytic activity and a product from which all blood pressure lowering agents have been substantially completely eliminated. It has been found that for efficient operation of these columns on a repetitive basis such as repeating Examples I and III, it is useful to wash the columns between successive uses with dilute acid, such for example, as 0.1 N hydrochloric acid where hydrochloric acid was used as the eluate. Subsequently, this acid may be removed with the eluant which is used for eluting the fibrinolytic material.

While the above examples are typical illustrations of the invention, it is not intended the invention be limited thereto and variations in such examples, as will be apparent to those skilled in the art, may be made without departing from the spirit of the invention or scope of the appended claims.

What we claim is:

1. A process for purifying fibrinolytic material produced from *Aspergillus oryzae* B1273 and removing blood pressure lowering agents therefrom consisting in filtering an aqueous solution of said fibrinolytic material through cross-linked dextran known as Sephadex G50, developing the filter with an eluting agent and collecting the eluate which contains the desired resulting fibrinolytic material.

2. A process as claimed in claim 1 in which the solution is filtered through the cross-linked dextran in a column.

3. A process as claimed in claim 1 in which the solution is filtered through the cross-linked dextran while maintaining the temperature thereof at about from 3° C. to 6° C.

4. A process as claimed in claim 1 in which the eluting agent consists of distilled water adjusted to have a pH of about 3.5 to about 5.5.

5. A process as claimed in claim 1 in which said collected eluate is dried in vacuo from the frozen state.

6. A process as claimed in claim 5 in which the product resulting from said drying is dialysed.

References Cited in the file of this patent

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