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(54) A PROCESS FOR SEPARATING INTERFERONS FROM CRUDE PREPARATIONS CONTAINING THEM, AND PURIFIED INTERFERON PREPARATIONS OBTAINABLE THEREBY

(71) We, AGENCE NATIONALE DE VALORISATION DE LA RECHERCHE (ANVAR), a Public Corporation organized under the Laws of the Republic of France, of 13, rue Madeleine Michelis, 92522 Neuilly-sur-Seine, France, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:-

The invention is concerned with a process for separating interferons from crude preparations containing them, and with purified interferon preparations obtainable thereby.

It is known that interferons are therapeutic products of great value, specifically by reason of their remarkable anti-viral and immuno-depressive properties.

According to the usual methods of synthesis *in vitro*, interferons are obtained by the action of virus or of chemical substances upon tissue cultures. The resultant preparations however also contain contaminating substances. Specifically, such crude preparations contain proteins which have a pyrogenous action and besides display inconvenient ability to induce a specific sensitisation in the recipient.

Consequently the crude interferon preparations cannot be used directly, in particular for the applications envisaged hereinabove, and must be purified.

Various methods have been suggested, specifically methods for purification by filtration through gel or through ion exchangers.

The use of these methods for the purification of crude interferon preparations however runs up against difficulties specifically due to very small yields.

Methods of affinity chromatography have also been suggested.

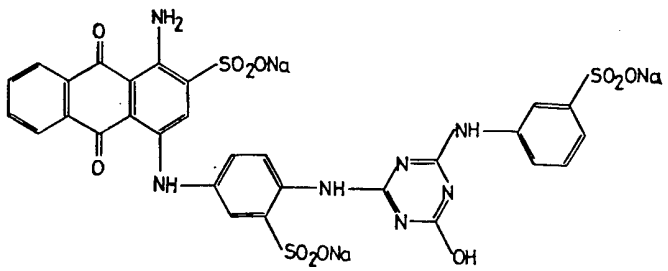
As is known these methods are based upon a selective affinity between an adsorbing phase constituted by a ligand bonded covalently to a support and the product which it is desired to prepare in purified form. The passage of a crude preparation containing the product through the adsorbing phase enables the product to be retained thereupon in a selective manner, and thus enables the product to be separated from the preparation containing it. The recovery of the product thus fixed can then be achieved with the help of an appropriate eluant.

The use of these particular techniques for the purification of crude interferon preparations however runs up against difficulties, and the attempts carried out up to the present time have not led to satisfactory results. In particular, methods of affinity chromatography using anti-interferon specific antibodies have not yet been developed upon a large scale due to the difficulties of manufacturing anti-interferon anti-sera.

The object of the present invention is to remedy these inconveniences and provide a process which will make it possible to obtain, in an extremely simple manner and moreover with excellent yields, relatively concentrated interferon preparations which are to a major extent freed from contaminating proteins, and consequently capable of use in thereapy.

Another object of the invention in one of its preferred aspects, is to provide such purified preparations as aqueous solutions, which are directly capable of use in therapy.

Accordingly, this invention provides a process of separating interferons from crude preparations containing them by selectively fixing the interferons on an adsorbent, which adsorbent comprises a ligand attached to a supporting gel porous to macromolecules in the preparation, the ligand being able to selectively fix interferons and being either the blue chromophore of the formula:



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or a compound having the same polycyclic structure and configuration, in which process the adsorbent, having first been contacted with an equilibrating buffer having a total salt concentration not exceeding 0.15M, is contacted with the crude preparation so as to selectively fix the interferons on the adsorbent.

Once separated from the preparations by being fixed upon the adsorbent, the interferons may be recovered by a desorption step in which the adsorbent is contacted with a desorbing buffer having a total salt concentration exceeding 0.15M. The interferons then pass into the desorbing buffer to form a purified interferon preparation.

The crude interferon preparations may be of human or of animal origin and are normally constituted specifically by the supernatant liquids of tissue cultures, either freshly transplanted (primary or secondary cultures) or continuously grown, where the cells have been induced to form interferon, advantageously in a culture medium freed from serum, by a virus or by a synthetic nucleic acid such as poly I.C.

The desorption step described above enables the interferons fixed to the adosrbent to be almost totally recovered in practice, without being denatured. Furthermore, it has been found that the adsorption capacity of the adsorbent employed is practically undamaged. Consequently, the adsorbent can be reused for numerous purification operations, which is extremely advantageous from the economic view point.

In a preferred embodiment of the invention the supporting gel comprises a polysaccharide such as agarose or a derivative thereof, specifically a modified agarose in which the polysaccharide chains are cross-linked in a three-dimensional sieve and in particular the modified agarose sold under the Trade Mark SEPHAROSE. Alternatively, the supporting gel may be a polyacrylamide or an acrylic resin.

As mentioned above, the ligand employed must either be the blue chromophore of general formula I or be a compound having the same polycyclic structure and configuration, for it is that polycylic structure and configuration which is responsible for the ligand's ability to fix interferons selectively. The kind and/or the position of substituents in the polycyclic structure may accordingly be varied without changing the polycyclic structure or configuration and destroying that ability - for example, a compound identical with the blue chromophore of general formula I in all respects save that a hydrogen atom replaces the $-SO_2ONa$ group substituted into the terminal phenyl ring has been found to operate effectively as a ligand in the process of the invention.

Both the blue chromophore of general formula I and the similar compound with a hydrogen atom replacing the $-SO_2ONa$ group on the terminal phenyl ring are available commercially, under the Trade Marks BLUE CIBACRON F3GA and BLUE CIBACRON 3GA respectively.

Whatever ligand is employed, it may be attached to the supporting gel either directly or through the intermediary of a bridging polysaccharide. In the latter case the ligand is attached directly to the bridging polysaccharide, which in turn is attached directly to the supporting gel.

The bridging polysaccharide, where employed, will generally be a polysaccharide of high molecular weight, such as one of those designated under the name "dextran". The preferred bridging polysaccharide is dextran 2000, which has a molecular weight of about 2 millions.

A product consisting of the aforesaid BLUE CIBACRON F3GA attached to dextran 2000 is sold under the Trade Mark BLUE DEXTRAN 2000, and is suitable for use in this invention. BLUE DEXTRAN 2000 will of course preferably be attached to an agarose-type supporting gel and in particular to SEPHAROSE; BLUE DEXTRAN 2000 attached to SEPHAROSE is sold under the Trade Mark BLUE DEXTRAN SEPHAROSE.

The reasons why BLUE CIBACRON F3GA and compounds having the same polycyclic structure and configurations have the ability to fix interferons selectively are not fully understood. Nevertheless, it is a phenomenon of great practical importance. Adhering to the conditions of buffer molarity set out above, preferably by using an equilibrating buffer having a molarity with respect to total salt content of from 0.01 to 0.15M, upon simple contact the interferons are selectively adsorbed, while the major part of the undesirable substances present in the preparations are not retained.

It is particularly convenient to bring the adsorbent and the crude interferon preparation into contact within a chromatography column, by making the preparation pass through such a column packed with the adsorbent. The column thus packed is beforehand contacted with the equilibrating buffer, whose pH should be of the order of 6 to 8, preferably around neutrality.

The crude preparations of interferon will normally have interferon titres of from 10^4 to 10^6 International Units of Interferon per 0.1 to 0.2 g of proteins, which gives a specific activity (International Units of Interferon, of I.U.I., per mg of protein) lying between 10^5 and 10^7. These preparations can be employed as such for passage through the column, or they can first be subjected to a preliminary dialysis against the equilibrating buffer.

It has been found that where the process of the invention is operated after having first contacted the adsorbent with an equilibrating buffer having a total salt concentration of 0.15M, the yield of interferon is from 80% to 90%, while the yield approaches 100% where a less concentrated buffer is employed. A particularly suitable equilibrating buffer is an aqueous solution of Tris-HCl, pH 7.5 containing 10 mM of Tris-HCl. "Tris" is the abbreviation currently adopted to denote tris-(hydroxymethylaminomethane).

The products initially yielded by the process of this invention - that is interferons fixed upon the adsorbent - are novel products. One preferred group of these products is constituted by interferons fixed upon the BLUE DEXTRAN SEPHAROSE complex.

The desorbing buffer employed in the desorption step may be formed simply by increasing the concentration of salts in the equilibrating buffer employed - specifically by the addition of acetates, phosphates, chlorides or other salts - in such manner as to create a total concentration of salts exceeding 0.15 M, preferably of 0.5 M and still more preferably of 1 M.

The desorption step by which the interferons are recovered is advantageously carried out by passing the desorbing buffer through the column already preferably employed to contact the crude preparation with the adsorbent.

It should be noted that the desorption step may be performed entirely in an aqueous medium. Tests done by the inventors have evidenced that interferons fixed on the adsorbent can be recovered without having to resort to organic solvents by using an aqueous desorbing buffer.

The results obtained are equally satisfactory whatever the origin of the interferons. However they may particularly originate either from fibroblasts or from leucocytes.

Upon recovery of the fractions eluted from the column, purified interferon preparations are obtained which may have an increased content of interferons, and which are practically wholly freed from contaminating proteins. Generally the purified preparations are free of at least 90% of the contaminating proteins initially present in the crude preparations.

The conditions employed for the elution make it possible to recover the major part of the interferons in a fraction of the eluate of small volume, which has the advantage of making available preparations having an increased concentration in interferon as compared with the concentration in the crude preparations.

Thus, even starting from the better crude preparations of interferon described hereinabove, having an already high specific activity of the order of 10^7 IUI per mg of protein, by using the process of this invention it is possible with an almost total yield to obtain interferon preparations, 30 to 40 times purer as compared with the crude preparations, and which have a specific activity lying between 1 and 5×10^8 IUI per mg of protein. This specific activity is amongst the highest which has been obtained by other, extremely complex and costly methods, which moreover have the disadvantage of giving mediocre yields.

The scope of the invention also includes the purified preparations obtainable using the process of the invention. More particularly the invention concerns the preparations obtained by purification of crude fibroblast or leucocyte interferon preparations.

The purified preparations have a most interesting anti-viral action, as evidenced by various tests which have been carried out.

Their anti-viral action against the hepatitis B virus is of particular note and this has been evidenced by administering to patients suffering from chronic hepatitis B purified interferon preparations obtained by the invention and originating from human fibroblast or leucocyte interferon. The tests have been carried out according to the techniques described by Desmyter et al. in The Lancet. p. 645 - 647, 1976 and Greenberg et al. in Med. 295 p. 517 - 522, 1976. A considerable improvement of the hepatic functions could be observed in these patients, due to the replacement of the infected cells by non infected cells protected by the interferon.

The purified interferon preparations have also proved effective against herpes simplex and particularly for preventing from the recurrence of herpetic keratitis. The tests carried out, using human interferon preparations purified as per the invention, according to the techniques described by Kaufman and al. in J. Infect. Dis 133 (suppt) A 165-168, 1976 have demonstrated the protective action of such preparations against infections caused by the virus and their inhibitive action against the recurrence of hepatic keratitis.

Moreover, said preparations may reduce the frequency and the duration of viral complications, more especially those resulting from herpes Zoster, as evidenced by carrying out the tests according to Jordan et al. in J. Infect. Dis 130, 56 - 62, 1974. On the other hand, they may increase the life duration of patients suffering from osteosarcome. Such properties may be evidenced by carrying out tests according to Strander et al. Acto Orthop. Scand. 45 p. 958-959, 1974.

All the above tests clearly demonstrate the perfect innocuousness of the purified interferon preparations of the invention, as well as the absence of noxious secondary effects resulting from their administration.

Moreover, in view of their high degree of purity, the purified interferon preparations of the invention may be utilized as active principles of drugs (medicines).

Interferons are specific of the species, so that for example it is necessary for therapeutic applications in man to employ interferon originating from human cells.

The medicaments of the invention, which contain said purified preparations as active ingredients, can be administered, more especially by the intramuscular or sub-cutaneous route, in the form of an aqueous solution. A unit posological dose of 10^6 to 10^8 IUI can be employed.

The treatment of chronical hepatitis is indicated as an example : patients are administered a 10^7 IUI unit dose of fibroblast interferon on alternate days, during a period of two weeks.

Osteosarcomes may be treated by administering a 3×10^6 IUI unit dose of leucocyte interferon three times a week for periods which may range from 6 months to 1 year.

In order that the invention and its advantages may be well understood it will now be described in further detail, though only by way of illustration, in the following Examples :

Example 1

Preparation of a BLUE DEXTRAN SEPHAROSE affinity column for purification of interferon of animal and human origin

1. *Preparation of BLUE DEXTRAN SEPHAROSE gel*

The process for manufacture of BLUE DEXTRAN SEPHAROSE gels has been described by L.D. Ryan and C.S. Vestling (*Arch. Biochem. Biophys.*, (1974), *160*, 279-284). Since that publication, it has become no longer necessary to undertake the activation of the SEPHAROSE by cyanogen bromide according to Cuatrecasas (P. Cuatrecasas, *J. Biol. Chem.*, [1970], 3059-3065) because there are ready lyophilised commercial preparations for the employment of already activate SEPHAROSE.

One employs SEPHAROSE 4B activated with cyanogen bromide and BLUE DEXTRAN 2000, such as those sold by Pharmacia (Uppsala, Sweden).

a) *Preparation of the SEPHAROSE gel (CNBr SEPHAROSE 4B).*

In order to swell the gel and to eliminate the batericidal agents present in the lyophilised preparation, the desired quantity of CNBr-SEPHAROSE is washed on a ground glass filter with a 1 mM solution of HCl, for about a quarter of an hour. The desired quantity of CNBr-SEPHAROSE is worked out upon the basis that one gram of dry product gives about 3.5 ml of gel. For washing one uses 200 ml of HCl mM per gram of dry weight of CNBr-SEPHAROSE. The gel thus formed is immediately coupled with BLUE DEXTRAN 2000.

b) *Coupling of the SEPHAROSE gel with BLUE DEXTRAN 2000.*

The quantity of BLUE DEXTRAN necessary for the coupling is worked out upon the basis that one gram of dry weight of SEPHAROSE is capable of fixing from 80 to 100 mg of BLUE DEXTRAN.

This quantity is dissolved in an alkaline buffer having a pH of the order of 8 to 10, especially a 0.4 M carbonate buffer of pH 10 (20 mg of BLUE DEXTRAN 2000 while easily dissolved in one ml of carbonate buffer).

The solution of BLUE DEXTRAN is poured onto the SEPHAROSE gel previously rinsed with 3 or 4 times its volume of 0.4 M carbonate buffer pH 10. The mixture of the gel and the BLUE DEXTRAN is poured into a deep glass flask and subjected to slight agitation, for example with a revolving paddle wheel, for about 18 to 24 hours, at a temperature of the order of +4°C, in order to promote the coupling.

After the coupling stage, the BLUE DEXTRAN SEPHAROSE product obtained is rinsed, on a ground glass filter, with an excess of 0.4 M carbonate buffer pH 10, in order to eliminate any non-fixed BLUE DEXTRAN, until the buffer remains colourless (optical density equal to or less than 0.02).

In order to eliminate the active sites which have not been fixed to the BLUE DEXTRAN, the product of the coupling is then put in contact with a solution of an alkanolamine, particularly 1 M ethanolamine at pH 8, for about two hours at a temperature of +4°C. For this operation, the product of the coupling is preferably transferred from the flask into another container.

To make the gel resistant to variations in pH and in molarity, it is then subjected to three rinsing cycles, each cycle consisting of alternate rinsing firstly with an acid solution consisting

of a 0.1 M acetate buffer at pH 4.0 to which 1 M NaCl has been added, and secondly with an alkaline solution which consists of a 0.1 M borate buffer at pH 8.5, to which 1 M NaCl has been added.

After these rinsing cycles, the gel is contacted with a 10 mM Tris-HCl buffer pH 7.5 (the equilibrating buffer) to which a bactericidal and anti-septic agent has been added, in particular 0.02% sodium azide.

2. *Preparation of the affinity chromotrography column:*

The BLUE DEXTRAN SEPHAROSE is poured into a chromatography column of the appropriate dimensions (it is estimated that one ml is capable of fixing about 8 million International Units of Interferon).

Before the very first use of the column, it is rinsed with 20 times its empty volume of the 10 mM Tris buffer pH 7.5 to which 0.02% of sodium azide has been added.

To eliminate the molecules of BLUE DEXTRAN which might tend to detach themselves, the desorbing buffer, consisting of the 10 mM Tris-HCl pH 7.5 to which 1 M NaCl has been added, is passed through until the optical density at 254 nm remains at 0.

Where columns of large dimensions are employed, it is recommended to undertake several successive rinsing cycles with the Tris buffer with and without NaCl in order to strengthen the adaptation of the column to variations in pH and in molarity.

A BLUE DEXTRAN SEPHAROSE column can be used for several months and for numerous cycles of interferon purification provided that it does not become contaminated by bacteria. It is therefore desirable to keep it, outside periods of use, under sodium azide and in the cold, at about + 4°C.

Since however sodium azide is toxic for cells, it is then necessary before carrying out fixation of interferon to pass through the column at least three times its empty volume of the Tris-HCl buffer without azide in order to eliminate any trace of that antiseptic.

Example 2

Purification of a human interferon preparation by chromatography on a column according to example 1

The interferon preparation to be purified consists of 32 ml of a supernatent liquid from a freshly-transplanted culture of human fibroblasts, induced to produce interferon by poly I.C. The titre is 6250 IUI per ml, the content of proteins is 1 mg per ml (the high content of proteins in this preparation is due to the fact that it has been enriched with human albumin for its preservation). The specific activity of the said preparation is thus 6.2×10^4 IUI per mg of protein.

The preparation is dialysed, at a temperature of about +4°C, for fifteen hours, against the equilibrating buffer for the column, in this particular case the 0.01 M Tris-HCl pH 7.5.

32 ml of the preparation are passed, at a rate of 1 ml per minute, through a column 1.6 cm in diameter, containing a BLUE DEXTRAN SEPHAROSE gel according to Example 1 up to a height of 10.5 cm. The column is rinsed as a preliminary matter with at least three times its empty volume of equilibrating buffer without azide. The sorption phase is monitored on a recorder of optical densities or OD at 280 nm.

As soon as all the preparation has permeated into the gel, it is washed by passing through the column from five to eight times the empty volume of the 10 mM Tris-HCl buffer of pH 7.5 in aqueous solution so as to ensure the elimination of non-adsorbed contaminating substances.

A fall in OD is observed, which finally comes to rest at zero.

To recover the interferon, an aqueous solution of 10 mM Tris-HCl of pH 7.5, to which this time there has been added 1 M of NaCl (the desorbtion buffer) is made to pass through the column, with an output of 0.5 to 0.7 ml/mn.

Fractions of 6 ml are collected in tubes, whose OD is measured to monitor the adsorption phase. The results obtained with the fractions in tubes numbered 8 to 11 are given hereinafter, in terms of their interferon titre and their content of proteins.

Tube No.	*IUI/ml*	*Proteins (μg/ml)*	*Specific activity*
8	6 250	250	
9	61 900	278	2.2×10^6
10	830	160	
11	660	80	

It is found that the products with interferon-type activity are mainly recovered in tube No. 9. The interferon titre (U.I.I/ml) is ten times greater than that found in the starting material, while the content of proteins is about 25% of that of the crude preparation; the specific activity has been increased by a factor of 36, or other words the preparation which emerges from the BLUE DEXTRAN SEPHAROSE column has been purified 36 times as compared

with the crude preparation.

It is found that the recovery of the chromatographed interferon carried on in aqueous medium is complete. In fact, in tube No. 9 some 6 ml were recovered of an interferon whose titre is 61900 IUI per ml, thus in total 371,400 IUI. This compares with the starting material consisting of 32 ml of a preparation containing 6250 IUI per ml, thus 200,000 IUI in total.

This example moreover only seeks to show the effectiveness of the method even in the case of a crude preparation of human interferon which was mediocre at the outset, as much because of its slight content of interferon as also because of its artifical contamination with albumin.

After the desorption of the interferons, the column is regenerated by causing about ten times its empty volume of the 10 mM Tris-HCl buffer pH 7.5 to which sodium azide has been added at a rate of 0.02% to pass through the column. In this way the column is made ready for re-use and can meanwhile be preserved at low temperature.

After several cycles of use, the gel can be regenerated to remove any traces of proteins which have become fixed thereon, and to do this the column is rinsed with five times its empty volume of 3 M KCl.

The effectiveness of the method for purification of interferon upon BLUE DEXTRAN SEPHAROSE gel is properly illustrated by Example 3, where chromatography is carried out upon a preparation of mouse interferon not previously enriched artifically with further proteins.

Example 3

Purification of a mouse interferon preparation by chromatography on a column according to Example 1

The interferon preparation to be purified consists of the supernatent liquid of a tissue culture (cells C 243) induced to produce interferon by the virus of Newcastle disease. After inactivation of the virus at pH 2, the supernatent liquid is dialysed against 0.01 M Tris-HCl pH 7.5, for about 14 hours, at a temperature of about + 4°C.

The interferon titre of the preparation is initially 1.5×10^6 IUI per ml, its content in proteins is 0.1 mg/ml and its specific activity is 1.5×10^7 IUI/mg of proteins.

100 ml of the preparation containing the interferon is made to pass through a BLUE DEXTRAN SEPHAROSE column according to Example 1, of 1.6 cm diameter, and containing the gel to a height of 10. cm, operating as in Example 2. After the column has been permeated by the preparation and rinsed, desorption is carried out with an aqueous solution of Tris-HCl, 0,0,1M enriched with 1M NaCl, recovering 6 ml fractions. The interferon is principally recovered in tubes 15 and 16 as shown by the results set out in the table below.

Tube No.	*U.I.I./ml*	*Proteins (μg/ml)*	*Specific activity IUI per mg of proteins)*
14	2.4×10^5	70	
15	2.3×10^7	111	2.1×10^8
16	5.8×10^6	12	4.8×10^8
17	2.4×10^5	11	
18	1.2×10^5	10	

As indicated above, the interferons are recovered principally in two of the tubes, mainly tubes No. 15 and No. 16.

As compared with the crude preparation, the increase in the specific activity, that is to say the degree of purification, is respectively 15 times for tube No. 15 and 35 times for tube No. 16. The specific activity of tube 16 in particular (4.8×10^8 IUI per mg of proteins) is up amongst the highest which have ever been published up to the present time.

Furthermore, the recovery of the interferon present in the crude preparation is complete.

In the 100 ml of the starting preparation, there were *in toto* some $1.5 \times 10^6 \times 100$, and thus 1.5×10^8, IUI.

Now, having recovered all the interferons in the two 6 ml fractions the amount in each tube will be:

tube No. 15: $2.3 \times 10^7 \times 6$ totalling 13.8×10^7 IUI

tube No. 16: $5.8 \times 10^6 \times 6$ totalling 3.5×10^7 IUI

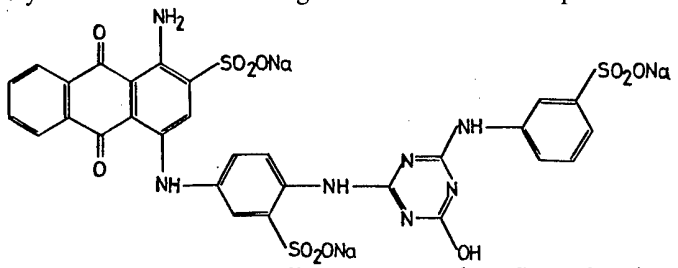
which is to say, on adding together the values for interferon contained in tubes 15 and 16, there are *in toto* 1.73×10^8 IUI.

The remarkable selectivity of the process of the invention should again here be emphasised, since it makes it possible simultaneously to enrich the interferon activity while eliminating the major part of the contaminating proteins.

WHAT WE CLAIM IS:

1. A process of separating interferons from crude preparations containing them by

selectively fixing the interferons on an adsorbent, which adsorbent comprises a ligand attached to a supporting gel porous to macromolecules in the preparation, the ligand being able to selectively fix interferons and being either the blue chromophore of the formula:



(1)

or a compound having the same polycyclic structure and configuration, in which process the adsorbent, having first been contacted with an equilibrating buffer having a total salt concentration not exceeding 0.15 M, is contacted with the crude preparation so as to selectively fix the interferons on the adsorbent.

2. A process as claimed in Claim 1 including the supplementary step of recovering the interferons by contacting the adsorbent with a desorbing buffer having a total salt concentration exceeding 0.15 M, thereby to form a purified interferon preparation.

3. A process as claimed in Claim 1 or Claim 2, in which the supporting gel comprises a modified agarose polysaccharide in which the polysaccharide chains are cross-linked into a three-dimensional sieve, a polyacrylamide or an acrylic resin.

4. A process as claimed in any of the preceding claims, in which the ligand is the blue chromophore of general formula I or a compound identical with the blue chromophore of general formula I in all respects save that a hydrogen atom replaces the $-SO_2ONa$ group substituted into the terminal phenyl ring.

5. A process as claimed in any of the preceding claims, in which the ligand is attached to the supporting gel through the intermediary of a polysaccharide having a molecular weight of about 2 millions, designated as "dextran 2000".

6. A process as claimed in Claim 1 or Claim 2, in which the adsorbent comprises the modified agarose polysaccharide defined in Claim 3 to which is attached the blue chromophore of general formula I through the intermediary of a polysaccharide having a molecular weight of about 2 millions, designated as "dextran 2000".

7. A process as claimed in any of the preceding claims in which the equilibrating buffer has a total concentration in salts of at least 0.01 M.

8. A process as claimed in Claim 2, in which the total concentration in salts of the desorbing buffer is at least 0.5 M.

9. A process as claimed in Claim 2 or Claim 8, in which the desorbing buffer is an aqueous solution.

10. A process as claimed in Claim 2, in which the contact of the adsorbent with the crude preparation and the subsequent step of contacting the adsorbent with the desorbing buffer are carried out in a chromatography column.

11. A process as claimed in any of the preceding claims, in which the crude preparations contain fibroblast interferons.

12. A process as claimed in any of Claims 1 to 10, in which the crude preparations contain leucocyte interferons.

13. A purified interferon preparation having a specific activity ranging between 1×10^8 and 5×10^8 IUI per mg of protein and being devoid of at least 90% of the initial contaminating proteins.

14. A purified preparation as claimed in claim 13 in the form of an aqueous solution.

15. A purified fibroblast interferon preparation in the form of an aqueous solution having a specific activity ranging between 1×10^8 and 5×10^8 IUI per mg of protein and being devoid of at least 90% of the initial contaminating protein.

16. A purified leucocyte interferon preparation in the form of an aqueous solution having a specific activity ranging between 1×10^8 and 5×10^8 per mg of protein and being devoid of at least 90% of the initial contaminating protein.

17. A purified interferon preparation when prepared by a method including the process of Claim 1.

18. A purified interferon preparation when prepared by the process claimed in Claim 9.

19. A process as claimed in Claim 2 and substantially as described hereinbefore with reference to Example 2 or Example 3.

20. A purified interferon preparation when prepared by a process as claimed in Claim 18.

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