Analysis and Purification of Human Lymphoblastoid (Namalwa) Interferon Using a Monoclonal Antibody

By G. ALLEN, 1 K. H. FANTES, 1 D. C. BURKE 2* AND J. MORSER 2
1 Wellcome Research Laboratories, Beckenham, Kent BR3 3BS, U.K. and 2Department of Biological Sciences, University of Warwick, Coventry CV4 7AL, U.K.

(Accepted 28 June 1982)

SUMMARY

Highly purified interferon-α (IFN-α) prepared from a human lymphoblastoid line (Namalwa) was analysed by gel filtration and polyacrylamide gel electrophoresis (PAGE). Gel filtration separated the IFN-α into two peaks (A and B). All the components of peak A were retained by a monoclonal antibody (NK2) column, but some of those from peak B were not retained. The IFN that was not bound was active on mouse cells and could be resolved into two major bands by PAGE. The bound fraction (about 75% of the interferon protein) was purified by means of the monoclonal antibody column, although complete purification of crude interferon was not achieved in one passage.

INTRODUCTION

Antibody-affinity chromatography is an extremely powerful technique for the purification of minor protein components from complex mixtures. The major factor limiting the application of this technique has been the unavailability of monospecific antisera. Until recently, these have been obtained only via immunization with already highly purified antigens or by the difficult and tedious purification of antibodies by absorbing out antibodies to likely contaminants of the immunogens. However, the availability of monoclonal antibodies, obtained from hybridoma cells (Kohler & Milstein, 1975), has revolutionized the technique. A monoclonal antibody to human interferon alpha (IFN-α) was first described by Secher & Burke (1980); since then, other monoclonal antibodies to IFN-α have been described (Montagnier et al., 1980; Staehelin et al., 1981; J. Ivanji, personal communication).

Namalwa IFN-α consists of at least eight different species separable by gel filtration and polyacrylamide gel electrophoresis in the presence of SDS (SDS–PAGE) (Allen & Fantes, 1980). Similar observations were made with IFN-α, separated by high-performance liquid chromatography, from primary leukocytes (Rubinstein et al., 1979, 1981). These results are consistent with the observations of multiple amino acid sequences (Allen & Fantes, 1980) and gene sequences (Streuli et al., 1980; Nagata et al., 1981; Goeddel et al., 1981) for human IFN-α. A single monoclonal antibody has affinity for a single epitope on the interferon molecule and it is likely that variations in structures of interferons will be associated with variations in affinity for the antibody. We describe here the analysis of different components of Namalwa IFN-α by using the monoclonal antibody, NK2, described by Secher & Burke (1980).

METHODS

IFN preparation and assay. Crude IFN was prepared from butyrate-treated Namalwa cells as previously described (Johnston, 1980) and had titres of 1 × 10^4 to 2 × 10^4 U/ml. IFN was normally assayed by the dye uptake method of Finter (1969) with V3 monkey cells (Christofinis, 1970) and Semliki Forest virus as the challenge virus. In some experiments interferon was assayed on bovine (EBTr), human (HFF) and on mouse (L929) cells by inhibition of nucleic acid synthesis as described by Atherton & Burke (1975). For the first three cell lines mentioned, results were expressed as human reference research units using MRC 69/19 as the standard but on mouse cells the results were expressed as mouse reference research units using NHG-002-904-511 as the standard. When MRC 69/19 was assayed on mouse cells, it had 1% of its activity on human cells.

Affinity chromatography. Monoclonal antibody, NK2, bound to Sepharose was obtained from Dr D. S. Secher.
Bovine polyclonal antibody to Namalwa IFN was prepared and was bound to Sepharose as previously described (Finter et al., 1978; Fantes et al., 1980). Chromatography of IFN on these columns was performed as described by Secher & Burke (1980), except that washing with ethanediol was not routinely carried out.

**Gel filtration and gel electrophoresis.** Sephadex G-75 chromatography and SDS-PAGE were carried out as described elsewhere (Fantes & Allen, 1981). Briefly, a 196 x 1 cm column of Sephadex G-75 (superfine) in 50 mM-NH₄HCO₃ pH 8.0 was eluted at 3.0 ml/h, and fractions of 1.5 ml were collected. Samples (0.5 ml) were added to 1.0 ml of a 1% solution of bovine serum albumin (Sigma; Cohn fraction V) in phosphate-buffered saline pH 7.2 and stored frozen until assayed for antiviral activity. Other samples (0.7 to 1.0 ml) of column fractions were freeze-dried, and then submitted to SDS-PAGE under reducing conditions (Laemmli, 1970) on 15% acrylamide slab gels. Protein bands were revealed after fixing in 15% trichloroacetic acid solution, by staining with Coomassie Brilliant Blue R-250.

**RESULTS**

Crude interferon (1 x 10⁶ to 2 x 10⁴ U/ml) was passed through columns of monoclonal or polyclonal antibody, which were then washed. Elution with 0.1 M-sodium citrate pH 2.2 yielded interferon which still contained impurities detectable by Sephadex G-75 chromatography and SDS-PAGE. These impurities could be proteins either non-specifically retained by the columns or leached from the Sepharose matrices. The specific activities of monoclonal and polyclonal eluates in one experiment were respectively, 1.9 x 10¹⁸ and 1.7 x 10¹⁷ U/mg. Washing the columns with 9 i-ethanediol (Secher & Burke, 1980) did not lead to substantial improvements.

In other experiments eluates from a polyclonal antibody column were further purified (Allen & Fantes, 1980) to give material with a specific activity greater than 10⁸ U/mg. Some of this material was chromatographed in Sephadex. The protein elution profile together with antiviral activities (in V3 cells) of one such experiment is shown in Fig. 1 (a). Some of the purified polyclonal eluate was applied to the monoclonal antibody column and the non-retained and the retained fractions were analysed in the Sephadex column. The protein profiles with antiviral activities are shown in Fig. 1 (b, c). The non-retained fraction (Fig. 1 b) gave a single peak at the position of peak B. The retained fraction (Fig. 1 c), in comparison with the input material (Fig. 1 a), had peak B substantially reduced, both in the amount of protein and in the antiviral activity. SDS-PAGE (Fig. 2) showed that all peak A material, but only two (lane 13) of the four major peak B bands of the input (lane 11) were retained by the NK2 columns. The non-retained fraction (lane 12) showed two major and two minor bands. Faint bands of lower molecular weight were also seen in the retained material (lanes 10, 16 to 19); these were absent from the input material and may indicate partial degradation.

Other experiments were performed in which crude interferon was chromatographed on the monoclonal antibody column and the retained and non-retained fractions were subsequently further purified (Fantes et al., 1980) before Sephadex chromatography. This had to be done in order to eliminate non-interferon proteins which otherwise obscured the interferon peaks of the Sephadex profile. The elution profiles and SDS-PAGE results were very similar to those in Fig. 1 (b, c) consisting of one and two (roughly equal) peaks respectively.

By measuring the area under the A₂₅₆ peaks of the Sephadex G-75 column eluates (Fantes & Allen, 1981) approximately 75% (mean of three experiments) of the total interferon protein bound by the polyclonal column was found to be bound to the monoclonal antibody column, including nearly all of the first peak (A) but only 55% of the second peak (B).

We next wanted to find out whether material separated by Sephadex and also by monoclonal antibody affinity chromatography differed in their biological activities. Therefore, the fractions were assayed on human (HFF), bovine (EBTr) and mouse (L929) cells; the results are shown in Fig. 1 (d to f). The distribution of antiviral activity on bovine and human cells was similar to that on monkey (V3) cells, i.e. it showed the typical double peak, but activity on murine cells was confined to peak B. Separation of IFN-α components by Sephadex chromatography thus showed that they differed in their respective biological activities. The differences between the activities on V3 and on HFF cells may not reflect a species difference, since activities on other human cells, Hep2c and GM2504, follow those on V3 cells more closely (results not shown). It is therefore important in general to state the nature of the cell that is used when referring to the antiviral activity of a sample.
Fig. 1. Chromatography on Sephadex G-75 of interferon samples before and after fractionation on NK2-Sepharose. Highly purified interferon (1.49 × 10⁸ U/mg) was passed through a column of NK2 monoclonal antibody bound to Sepharose. Samples of (a, d) input material (72 µg IFN protein), (b, e) non-retained material (41 µg IFN protein) and (c, f) retained and subsequently eluted material (71 µg IFN protein) were subjected to chromatography on Sephadex as described in the text.

Slight variations in flow rate and fraction size (a, d, 1.35 ml; b, e, 1.5 ml; c, f, 1.4 ml) were allowed for in assigning the elution positions of peak A and peak B. The protein concentration in the eluate was monitored by u.v. absorbance (Δ). Samples of each fraction were assayed for antiviral activity on V3 cells (○) (in a, b and c) and on mouse L929 cells (□) on a scale 100-fold less than the activities on the other cell lines. The absorbance traces of the pairs (a) and (d), (b) and (e) and (c) and (f) are the same.
Fig. 2. SDS–PAGE of interferon samples separated by NK2 monoclonal antibody affinity chromatography and Sephadex G-75 chromatography. Samples of alternate fractions from the Sephadex G-75 column eluates (Fig. 1) were freeze-dried and subjected to SDS–PAGE as described in the text. Lanes 2 to 7 show fractions 62, 64, 66, 68, 70 and 72 respectively, of the input material (Fig. 1a). Lanes 16 to 20 show fractions 59, 61, 63, 65 and 67 respectively, of the retained material (Fig. 1c). Lanes 21 to 24 show fractions 63, 65, 67 and 69 respectively, of the non-retained material (Fig. 1b). Pooled samples from peak A of the input material (see Fig. 1a) (lane 9) and peak A of the retained material (Fig. 1c) (lane 10) and from peak B of each of the input material (Fig. 1a) (lane 11), the non-retained material (Fig. 1b) (lane 12) and the retained material (Fig. 1c) (lane 13) were also subjected to SDS–PAGE. The molecular weight markers (lanes 1, 8, 14, 15 and 25) were 5 μg each of bovine serum albumin (Mr 67000), hen ovalbumin (Mr 43000), soybean trypsin inhibitor (Mr 20000) and lysozyme (Mr 14300).

In a separate experiment, interferon purified in a polyclonal column was fractionated by gel filtration. Each fraction with antiviral activity (in V3 cells) was passed through the monoclonal column and the retained and non-retained materials were assayed on the four types of cells. The results showed that some of the peak B material that was not retained had activity on heterologous cells. This is in agreement with the results shown in Fig. 1.
DISCUSSION

The results presented here demonstrate that the monoclonal antibody NK2 (Secher & Burke, 1980), bound to Sepharose, may be used to purify human IFN-α, but not all components of Namalwa IFN-α are bound. Specifically, two of the four major bands seen after SDS-PAGE of the second peak (B) eluted from the Sephadex G-75 column are not bound under the conditions employed here. As was shown previously, amino acid sequences in peptides derived from a mixture containing these four bands all conformed to IFN-α sequences (Allen & Fantes, 1980), showing that the interferon components not bound by the NK2 antibody are indeed of the IFN-α type. The molecules that are active on mouse cells do not bind to NK2 antibody.

Other workers have observed that different molecular species of IFN-α, whether natural or produced in bacteria by genetic engineering, have different relative activities in a range of different cells and even against different viruses in the same type of cell (Streuli et al., 1981; Yelverton et al., 1981; Rubinstein et al., 1981; Evinger et al., 1981; Weck et al., 1981; Traub et al., 1981). In view of this it is possible that clinical results may differ according to which and how many components are present in the interferon preparation used.

We were unable to purify IFN-α completely by one passage through the monoclonal antibody column. A proportion of material eluted at pH 2.2 may have been immunoglobulin, as judged by SDS-PAGE, but other impurities are present. Following purification by other methods (Allen & Fantes, 1980), the eluted interferon gave multiple bands on SDS-PAGE, in contrast to the result reported by Secher & Burke (1980), who also found essentially quantitative recovery of the interferon activity. However, they passed their material twice through the column, and they detected the IFN-α after SDS-PAGE by autoradiography following radioiodination, and differences in extent of iodination might be relevant. Their starting material may also have had a different composition (Havell et al., 1978).

These results may be compared with the studies on monoclonal antibodies against IFN-α reported by Staehelin et al. (1981). Some of the 13 monoclonal antibodies described by these workers bind several, but not all, components of leukocyte interferon separated by high-performance liquid chromatography. NK2 antibody recognizes a determinant present on the majority, but not all, of Namalwa IFN-α species: all of peak A but only about 55% of peak B material binds to it.

In order to use monoclonal antibody affinity chromatography for the purification of the complete spectrum of IFN-α components, it will be necessary to find another antibody which binds all components, or to use a combination of two or more antibodies with complementary specificities.

We thank Ms P. Shockley, Mrs J. Oliver, Ms E. Wallis, R. King and Mrs S. Simpson for performing antiviral assays and for expert technical assistance, and the MRC for support of two of us (J.M. and D.C.B.).

REFERENCES


(Received 22 March 1982)