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PURIFICATION AND PARTIAL CHARACTERIZATION OF MOUSE FIBROBLAST INTERFERON INDUCED BY SYNTHESIZED POLYRIBOADENYLIC ACID: POLYRIBO-5-AZIDOURIDYLIC ACID HYBRID.

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ABSTRACT: Interferon (IFN) induced in Swiss mouse embryo primary tissue culture by synthesized polyriboadenylic acid: polyribo-5-azidouridylic acid was purified by a procedure which involved salt precipitation by zinc acetate, ion-exchange chromatography and SDS-polyacrylamide gel electrophoresis. The final activity obtained was 2.86 x 10^5 U/mg protein (5.06 x 10^5 IRU/gm protein). The protein had an estimated molecular weight of 46,238 ± 694 daltons and 45,100 ± 710 daltons under denaturing and non-denaturing conditions respectively. An estimated molecular weight of 52,480 ± 707 daltons was however obtained by gel-filtration on Sephadex G-200. The IFN protected mouse embryo cells against Potiskum viral cytopathic effect and showed appreciable stability to the action of neuraminidase. However, activity was rapidly destroyed by trypsin and ultraviolet light. Analysis of sugar indicated that the IFN is a glycoprotein. It also contained one polypeptide chain.

Key Words: Interferon; Mouse fibroblast; Tissue culture; Polyriboadenylic acid; Polyribo-5-aziduric acid.

INTRODUCTION

Interferon (IFN) in the last few decades have been purified with the use of various combinations of many different conventional protein purification techniques (1-5). As a result of this, many investigators have reported pure IFN species especially for humans. The most commonly used sources of mouse α and β IFNs are the L, C 243, and Ehrlich ascites tumour cells with the L cell line producing as high as 0.1 - 0.2 I.U of IFN- α and β per cell when induction is carried out by Newcastle Disease Virus (NDV) (6). Mouse IFN thus obtained from this cell line have been purified completely in two steps using controlled pore glass and antibody columns (7). However, IFN obtained is a mixture of α and β types.

We report here the purifications and partial characterization of a single species of mouse IFN obtained by induction of mouse embryo primary tissue culture with synthesized polyriboadenylic acid: polyribo-5-azidouridylic acid (PolyA:Poly$^5{}_3$U) with a view to expanding available information on mouse IFN.

MATERIALS AND METHODS

Materials

Eagle minimal essential medium composed of Earle's salt with glutamine, penicillin, streptomycin, fungizone and foetal calf serum were obtained from Flow

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Laboratories, Irvine, Scotland. Trypsin from bovine pancreas and neuraminidase type II from *Vibrio cholera* were purchased from Sigma Chemical Company Ltd., St. Louis, Mo., U.S.A.. Trypsin (Difco 1:250) was obtained from Gibco Laboratories, Detroit, while various filters with various pore sizes were products of Millipore Corporation, Bedford, Massachusetts, U.S.A. Tissue culture flasks and tubes were products of Corning Glass Works, Corning, New York while Swiss mouse was obtained from the Virology Department, University College Hospital,, Ibadan, Nigeria. Polyriboadenylic acid: polyribo-5-azidouridylic acid hybrid (polyA:PolyN $^{5}_{3}$U) was synthesized in our laboratory and stored as lyophilized powder at 4^{O}C until required for use.

Preparation of Mouse Embryo Tissue Culture

Mouse embryo tissue culture was prepared as described by David-West (8). The cells were allowed to grow to confluency and were maintained in maintenance medium (minimal essential medium, Eagle, supplemented with 2% (v/v) foetal calf serum) and incubated at 37^{O}C until they were required for use.

Induction of Interferon

Interferon was induced in mouse embryo culture by a modification of the methods previously described (9, 10). The monolayers of confluent fibroblast were washed several times with phosphate buffered saline (PBS) and incubated with 10ml of serum-free minimal essential medium (Eagle) containing 100 µg/ml polyA:polyN $^{5}_{3}$U hybrid, 20mM $CaCl_2.2H_2O$ and 20 µM phenylmethyl-sulphonylfluoride (PMSF) at 37^{O}C for 24 hours after which the medium was decanted, pooled and centrifuged at 800 x g for 20 minutes at 4^{O}C to remove cell debris. The supernatant was stored frozen for purification. In the parallel control experiment, PolyA:PolyN U) was not added to the incubation medium.

Interferon Assay

Interferon was assayed in mouse culture by a modification of the method based on reduction of cytopathic effect described by Rubinstein *et al* (11). The assay was standardized using standard mouse α and ß fibroblast IFN (Lot 54 F-0598-1, total activity 2.2 x 10^5 IRU/mg protein). One unit of IFN activity is defined as the amount of IFN that protects 50% of mouse embryo cells from Potiskum virus cytopathic effect.

Protein Assay

Protein determination was done by the modification of the method of Lowry *et al*. (12).

Purification of Interferon

Precipitation of Interferon With Zinc Acetate: Precipitation of IFN with zinc acetate was done by the method of Lampson *et al*. (13). 2,500ml of 0.02M zinc acetate were added to 50ml of the thawed cultured brew with stirring and the pH was adjusted to 6.0 with a few drops of 1M HCl. The precipitate formed was collected by centrifugation at 2000 x g at 4^{O}C. The pellet obtained was carefully suspended in 0.01M HCl (1/20 volume of starting material) and the resulting turbid solution was centrifuged at 3000 x g for 30 minutes at 4^{O}C. The supernatant which contained IFN was saved and dialysed extensively against acetate buffer, pH 4.5 and then 0.1M PBS, pH 7.2 at 4^{O}C, before it was assayed for IFN activity or purified further. Culture brew obtained from the parallel control was also treated the same way.

Purification by Ion-Exchange Chromato-graphy:

For further purification of IFN, ion-exchange chromatography was performed on CM-Sephadex C-50 (1.5 x 40cm) column. The column was equilibrated by passing 5 bed volumes of 0.1M sodium phosphate buffer, pH 5.8 through the column at the flow-rate of 30ml/hr.

The IFN was dissolved in 0.01M HCl (125ml), the pH was adjusted to 5.8 with a few drops of 1M NaOH. It was dialysed extensively against 0.1M phosphate buffer pH 5.8 at 4^{O}C for 24 hours followed by centrifugation at 3000 x g for 10 minutes to remove any undissolved material. The supernatant was applied to the column which was thoroughly washed with 0.1M sodium phosphate, pH 5.8 at the rate of 30ml/hour to remove all unabsorbed protein. The proteins adsorbed to the

column were eluted with a linear pH gradient and 5-ml fractions were collected.

The absorbance of the fractions was measured at 280nm to monitor protein elution and each fraction was assayed for IFN activity and the peak with highest IFN activity was pooled and assayed for total activity. The pooled fraction was concentrated by dialysing against dry Sephadex G-50 powder at 4^{O}C.

Purification by Sodium Dodecyl Sulphate-Polyacrylamide Gel

Electrophoresis (SDS-PAGE): A modification of the method previously described by Van der Meide *et al*. (14) was used. The concentrated IFN obtained by ion-exchange chromatography was made up to 4ml in 0.1% (w/v) SDS and dialysed against 0.02% (w/v) SDS for 4 hours at 4^{O}C, After dialysis, the IFN was concentrated by vacuum evaporation and then dried at 4^{O}C. The residue was dissolved in 5ml of 0.1M Tris-HCl buffer pH 6.8 containing 10% (v/v) glycerol and the resulting solution warmed for 10-15 minutes at 37^{O}C.

Electrophoresis was carried out with a current of 1mA/gel during stacking and was increased to 3mA/gel during electrophoresis until the tracking dye was about 1cm from the bottom of the gel.

The gels were removed, and cut into two-millimetres slices starting at the top of the gel. Each slice was put into 1ml of 0.20M sodium phosphate buffer pH 7.2 containing 0.05% (w/v) SDS and incubated at 25^{O}C for 24 hours to elute the IFN. Each tube was assayed for IFN activity and activity profile constructed. The area corresponding to active IFN was cut on the other gels, pooled and cut into smaller pieces and interferon eluted as described above. The gels were then centrifuged and the clear solution withdrawn, dialysed against 0.2% (w/v) SDS before the total IFN activity was determined.

Gel-Filtraion on Sephadex G-200: A separate IFN fraction obtained after CM-Sephadex C-50 chromatography and having a specific activity of 1.11 x 10 U/mg protein (1.95 x 10 IRU/mg protein) and total protein of 922 ug was concentrated by vacuum evaporation and dried at 4^{O}C. The residue was dissolved in 1ml of 0.02M PBS, pH 7.2 and dialysed against 0.02M PBS at 4^{O}C.

The sample was gently applied on a column (1.5 x 100cm) of Sephadex G-200 packed to a bed height of 85.5 cm without disturbing the gel bed. Elution was carried out with 0.02M PBS, pH 7.2 in fractions of 3ml each at an approximate rate of 18ml/hour. The IFN activity of each fraction was determined and the IFN activity profile constructed. Fractions that were active were pooled, concentrated and were found pure. The molecular weight by gel-filtration method was estimated alongside purification and the same sample was used to determine molecular weight under non-denaturing conditions.

Characterization of Interferon

Molecular Weight Studies: The molecular weight of IFN was estimated by gel-filtration on a column (1.5 x 100cm) of Sephadex G-200 by the modified method of Andrews (15). The column was packed to a bed height of 85.5cm. Phosphorylase b from rabbit muscle (94,000), bovine serum albumin (67,000), egg white ovalbumin (43,000), soybean trypsin inhibitor (20,100) and cytochrome c (12,270) were the protein standards ued to calibrate the column. Each protein standard (2ml) and PolyA:PolyN $^{5}{}_{3}$U-induced mouse IFN were separately chromatographed on the column and they were eluted with 0.02M PBS, pH 7.2 at a flow-rate of 18ml/hour while 3ml fractions were collected. Elution was monitored at 280nm except for cytochrome c which was monitored at 410nm.

The modification of the method described by Hedrick and Smith (16) was employed to estimate molecular weight under non-denaturing condition. The protein standards used (1mg/ml) were rabbit muscle phosphorylase b (94,000), bovine serum albumin (67,100), egg white ovalbumin (43,0000) and trypsin inhibitor (20,100). They were prepared in 0.5M sodium phosphate buffer, pH 7.2. IFN preparation was dried at 4^{O}C and later dissolved in 0.5M sodium phosphate buffer, pH 7.2 to a final protein concentration of 345.8 ug/ml. Aliquots (50 ul) of each standard and IFN were used. They were separately mixed with 5 ul of tracking dye (0.50% (w/v) bromophenol

blue in 0.5M sodium phosphate buffer, pH 7.2), 10% (v/v) glycerol and layered separately on phosphate gels of 3.5%, 7.5%, 10.0% and 15% concentration. Electrophoresis was performed at room temperature using 0.5M sodium phophate buffer, pH 7.2 as the reservoir buffer. The run was carried out at low current density of 4mA/tube.

The molecular weight of IFN was estimated under denaturing condition in 10% separating tris gel by a modification of the method described by Weber and Osborne (17). A mixture of protein standards comprising rabbit muscle phosphorylase b (94,000), bovine serum albumin (67,0000), egg white ovalbumin (43,000), bovine erythrocyte carbonic anhydrase (30,000), trypsin inhibitor from soybean (20,100) and α-lactalbumin from bovine milk (14,400) were used.

Enzyme Sensitivity Studies:

The modification of the method described by Bose and Hickman (18) was employed to study the effect of enzymes on IFN activity. To 0.1ml of IFN (512 units, 50 µl of neuraminidase (5.0 x 10 unit) in McIlvane's phosphate-citrate buffer, pH 6.0 was added. Both the control and the experimental tubes were incubated at 37^{O}C for 4 hours before they were assayed for IFN activity.

The effect of trypsin was studied by a modification of the method described by lampson *et al*. (13). To 0.1ml of IFN (256 units), trypsin (20 units) in 0.006M phosphate buffer, pH 7.4 containing 0.15M NaCl was added. Both the control and the experimental tubes were incubated at 37^{O}C for 4 hours. The reaction was stopped by adding 10 µl to 20 µM PMSF before IFN activity was assayed for.

Effect of Ultraviolet Light on Interferon:

1000 units of IFN in 0.02M sodium phosphate buffer, pH 7.2 containing 0.05% SDS was put in a sterile petri-dish and covered. This was placed 10cm from a uv lamp (220-380nm) for 2 hours at 4^{O}C before it was assayed for IFN activity.

Determination of Total Sugar in Interferon:

Total sugar in IFN was determined by a modification of the method described by Spiro (19) using glucose (100 µg/ml) as the standard.

RESULTS

Purification of Interferon:

IFN was induced in serum-free medium to make purification less cumbersome. Consequent upon this, IFN was purified using zinc acetate precipitation, ion-exchange chromatography and sodium dodecyl sulphate-polyacrylamide gel electrophoresis. A summary of a typical purification results is presented in Table 1.

Zinc acetate precipitated 57% of the total IFN activity of the crude sample. This step resulted in the selective precipitation of IFN while leaving most other protein impurities in solution. Hence, this step resulted in a 25--fold purification but did not produce a homogenous preparation as three distinct bands with relative mobilities of 0.26, 0.36 and 0.54 respectively were observed in 7.5% polyacrylamide gel when subjected to electrophoresis under non-denaturing conditions (Fig. 1).

Additional purification was carried out on an ion-exchange column using CM-Sephadex C-50. While the bulk of inactive proteins passed through the column at pH 5.8, peak II, (Fig. 2) containing fractions with IFN activity was pooled and saved. A third peak (designated III in Fig. 2) containing approximately 2.2% of protein introduced into the column showed no IFN activity. When the pooled fraction (Peak II) was concentrated and subjected to electrophoresis under non-denaturing conditions, three bands with relative mobilities of 0.27, 0.36 and 0.55 were observed (Fig. 3). The bands with relative mobilities of 0.27 and 0.36 were faint compared with the one with relative mobility of 0.55 suggesting that this purification step removed substantial part of the proteins in the two former bands. In this step, a 4-fold purification was achieved over the preceding purification step.

Table 1: Summary of Purification of Mouse Interferon Induced by PolyA:PolyN $^{5}_{3}$U.

Purification step	Volume (ml)	Total Protein (mg)	Total Interferon Units		Specific Activity		Yield (%)	Purification Factor
			U	IRU	U/mg protein	IRU/mg protein		
1. Crude	3500	262.5	375375	663663	1.43×10^{3}	2.53×10^{3}	100	1.0
2. Zinc acetate precipitation	125	5.9	213639	377714	3.64×10^{4}	6.43×10^{4}	57	25.5
3 Ion-exchange chromatography on CM-Sephadex C-50	40	0.71	102950	182016	1.44×10^{5}	2.55×10^{5}	27.4	100.7
4 Sodium dodecyl sulphate polyacrylamide gel electrophoresis	10	0.18	51480	91016	2.86×10^{5}	5.06×10^{5}	13.7	200.0

Table 2: Effect of trypsin and neuraminidase on interferon activity.

Enzyme	Interferon Units in 0.1ml		Final activity recovery (% of control)
	Control (U)	Treated (U)	
Trypsin	256	0	0
Neuraminidase	512	409 ± 125	80.0

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The active CM-Sephadex C-50 fraction was further purified by subjecting it to polyacrylamide gel electrophoresis in the presence of SDS at 10% gel concentration and IFN activity eluted as earlier described. When all gel fractions eluted as described under methods were pooled, concentrated and subjected to electrophoresis on a 7.5% SDS-Polyacrylamide gel, a single band with anodic relative mobility of 0.54 was obtained (Fig. 4). These procedures produced a homogenous mouse IFN as adjudged by SDS-Polyacrylamide gel electrophoresis. The preparation has a specific activity of 2.86 x 10^5 units/mg (5.6 x 10^5 IRU/mg) protein.

In another final purification attempt, CM-Sephadex C-50 IFN was subjected to gel-filtration on Sephadex G-200 column (Fig. 5). This resulted in IFN with the lower final specific activity of 1.563 x 10^5 units/mg protein (2.763 x 10^5 IRU/mg protein) and a final yield of 10.7%).

Characterization of Interferon

Molecular Weight Studies: The molecular weight of purified IFN was determined by three different methods. Non-SDS-PAGE gave a molecular weight of 45,100 ± 710 daltons. (Fig. 6). While SDS-PAGE gave 46,238 ± 694 daltons (Fig. 7). Gel-filtration on Sephadex G-200 gave a molecular weight of 52,480 ± 707 daltons (Fig. 8).

Enzyme Sensitivity Studies:

Table 2 shows that IFN activity was totally destroyed by trypsin. Neuramidase on the other hand reduced IFN activity to 80% of initial activity.

Effect of Ultraviolet Light on Interferon Activity:

Results show that after 2 hours of exposure to ultraviolet irradiation, 106.7 ± 37.71 units was recovered representing 83.33% loss of activity.

Determination of Total Sugars:

Total sugars in purified mouse IFN was estimated using anthrone reagent. Results show that 1mg of IFN contains 0.049 ± 0.002mg of sugar expressed as glucose. This result indicate that carbohydrate is 4.9% of the total mass of the molecule. On the basis of the estimated molecular weight of 46,238 daltons in 10% SDS-Polyacrylamide gels, it is estimated that one mole of this protein contains approximately 13 moles of glucose.

DISCUSSION

Interferon (IFN) induced by POlyA: PolyN $^5{}_3$U in mouse embryo primary culture has been purified by a procedure which include zinc acetate precipitation, ion-exchange chromatography on CM-Sephadex C-50 and SDS-Polyacrylamide gel electrophoresis. A pure IFN preparation with a final specific activity of 2.86 x 105 units/mg protein (5.06 x 105 IRU/mg protein) was obtained. This preparation represents a 200-fold purification based on activity-protein ratios for the crude and final materials. In the final reparation, one unit of IFN activity is equivalent to 0.0003 µg of protein. This falls within the potency of pure mouse IFN obtained from murine leukemia L 1210 (20), mouse L cells (21),, mouse cells induced by MM virus (1) and mouse Ehrlich ascites tumour cells (22).

It was observed that the yield and purity obtained in this study compares favourably with previous reports (9, 13) in which similar method of purification as employed in this study has been used. In one study (9), a 15-fold increase in specific activity with yields varying between 315% and 52% after a single cycle of zinc acetate precipitation was obtained when it was employed for the purification of chick IFN. In this study, a 57% yield was obtained with a purification factor of 25.5. The higher purification factor obtained may be due to the fact that induction was carried out in a serum-free medium.

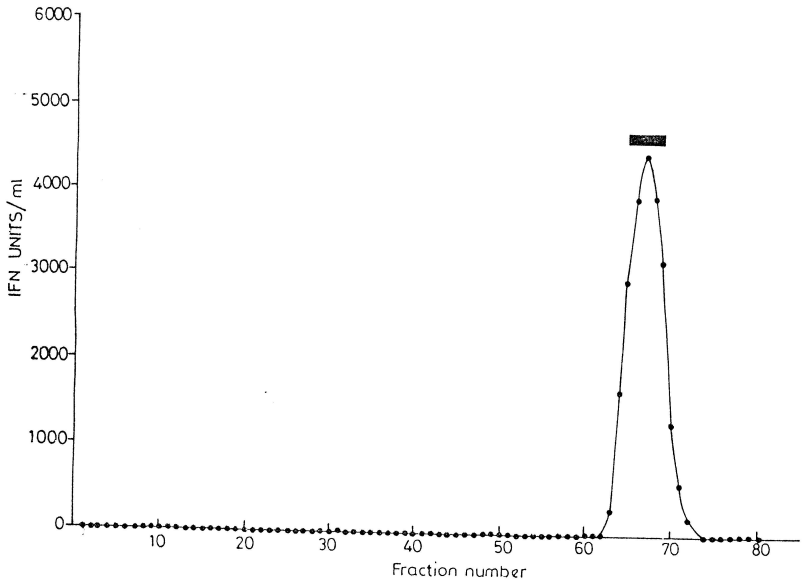

Fig. 5: Elution profile of CM-Sephadex interferon on a column of Sephadex G-200

Elution was carried out with 0.02M PBS, pH 7.2 on a column (1.5 x 100 cm) of Sephadex G-200 packed to a bed height of 85.5 cm. A flow rate of 18 ml/hr was maintained while 5-ml fractions were collected.

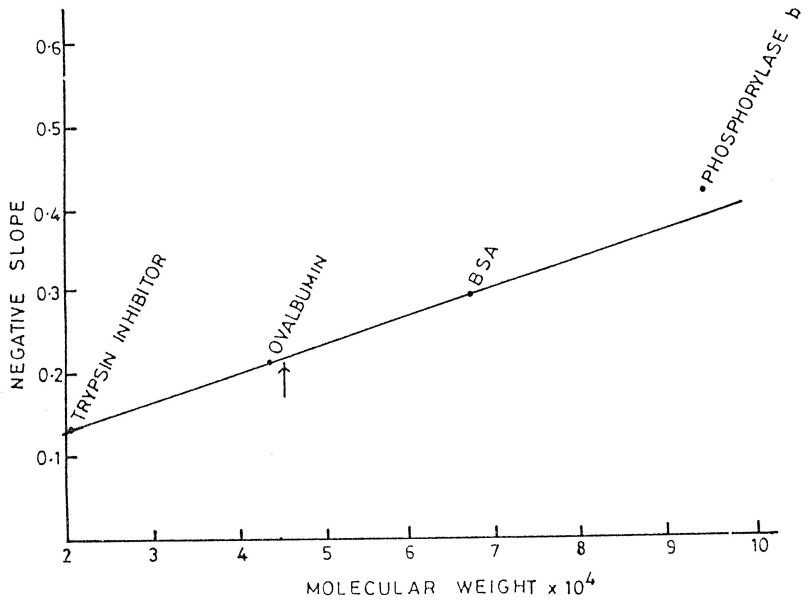

Fig. 6: The determination of molecular weight of mouse interferon induced by Poly A: Poly N $^{5}{}_{3}$U in mouse embryo primary tissue culture by polyacrylamide gel electrophoresis under non-denaturating conditions.

Ferguson plots were constructed for phosphorylase b (94,000), bovine serum albumin (67,000), ovalbumin (43,000) and trypsin inhibitor from soyabean (20,000) and Poly A: Poly N $^{5}{}_{3}$U-induced interferon having run them in gels of different concentrations (i.e. 3.5%, 5%, 7.5%, 10%, 15%). From the Ferguson plots of each protein standard and interferon, negative slopes were determined and from a plot of negative slope against molecular weight was constructed. Arrow indicates the negative slope of interferon.

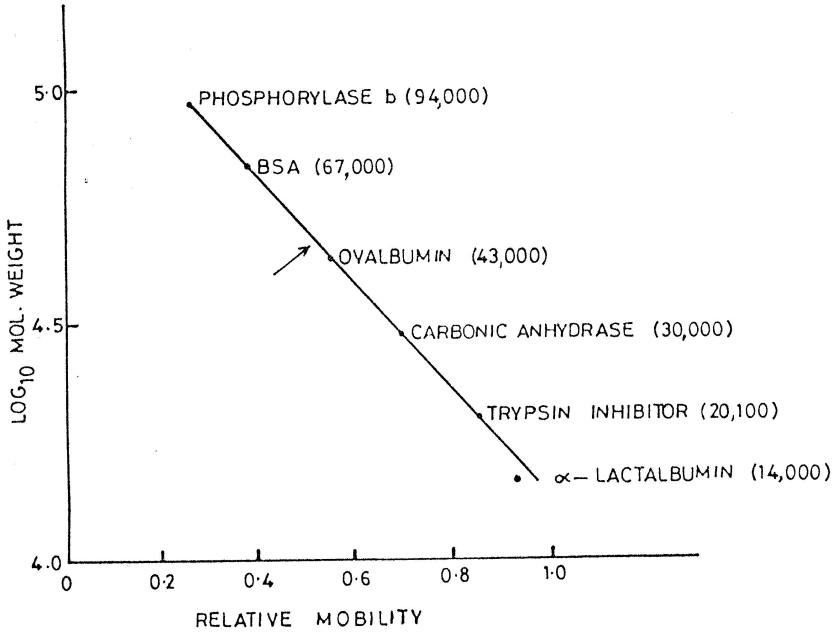

Fig. 7: The determination of molecular weight of mouse interferon induced by Poly A: Poly N $^{5}_{3}$U in mouse embryo primary tissue culture by polyacrylamide gel electrophoresis in 10% Tris gel. Arrow indicates the relative mobility of interferon.

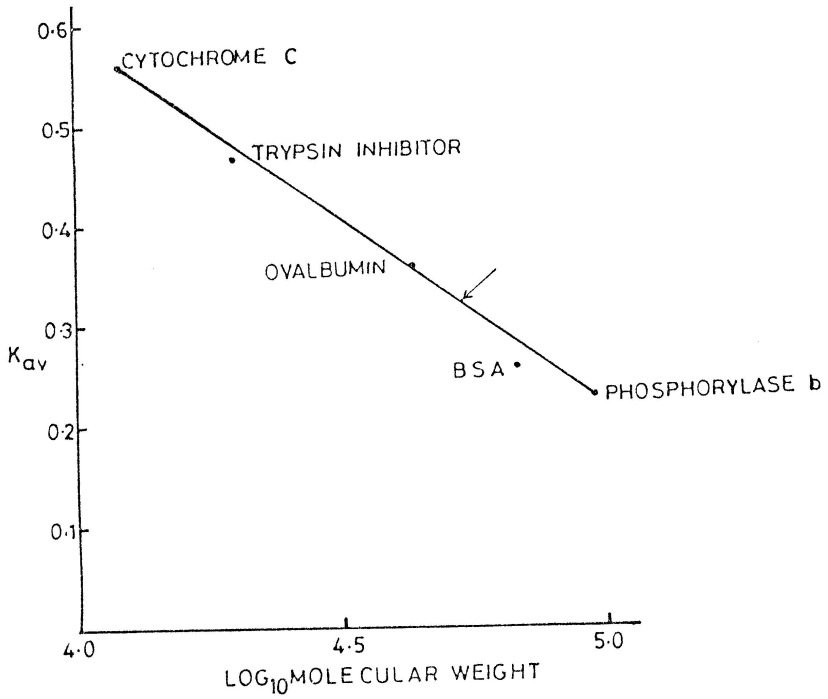

Fig. 8: The determination of the molecular weight of interferon by gel filtration on Sephadex G-200 column. The arrow indicates K_{av} for interferon.

The advantage of this is that crude IFN from which serum proteins do not have to be removed during purification is produced. A purification factor of 16 was reported by Lampson *et al*. (13) when the same procedure was employed for chick IFN. The IFN activity recovery was also lower in their study. A 49% recovery was reported. This lower recovery may be attributed to the fact that zinc acetate precipitation followed treatment of allantoic fluid with perchloric acid. Perchloric acid is found to inactivate chick and mouse IFNs (9). Though zinc acetate precipitation has been found to be effective in the concentration and partial purification of mouse IFN with lower yields usually 20-40% (9), a higher yield of 57% with a purification factor of 25.5 under the conditions of this experiment appears an improvement.

Ion-exchange chromatography on CM-Sephadex C-50 produced an IFN preparation four times purer than was obtained in zinc acetate precipitation stage. Also, 27.4% of IFN activity was recovered during this purification step. This appears lower than was obtained for the purification of chick embryo IFN whose physicochemical similarities with mouse IFN have been clearly shown (9). The lower purification factor obtained in this step may be due to the fact that many contaminating and inactive proteins have been removed during the preceding purification stage. However, a purer IFN preparation was obtained after ion-exchange chromatography.

Purification of IFN by gel electrophoresis followed by elution of IFN activity has earlier been tried with considerable success (10, 23). A 20-fold purification after ion-exchange of human fibroblast IFN was reported when the sample was subjected to gel electrophoresis (23) whereas, a 7-fold purification was reported when gel electrophoresis was employed as a last step of purification of human fibroblast IFN induced in a serum-free medium (10). All IFN preparations obtained by this method were found pure and homogenous. In this study,, a 2-fold purification was achieved over the preceding step which was higher than earlier obtained for human fibroblast IFN in which similar methods were used (10, 13) but a consistently low final activity recovery was observed in this study.

One of the physical properties that has been well-studied is the molecular weight. For nearly all types of IFN studied, size heterogeneity has been reported. Pure mouse IFN show considerable size heterogeneity (24, 25). For mouse induced by viruses, molecular weights range from 22,000 to 38,000 daltons (20-22, 24-26). The molecular weight of mouse IFN obtained by non-viral inducers has also been studied. Escerichia - coli endotoxin-induced mouse IFN obtained from the serum of intravenously injected mice was 85,000 daltons, while 54,000 and 77,000 daltons were obtained for Brucella abortus-injected mice (26). Merigan and Kleinschmidt (27) studied the molecular weights of IFN produce in mouse embryo cell cultures and intact mice. The tissue culture product has a molecular weight of 34,000 daltons whereas IFN isolated from the serum proved to be a 85,000 daltons molecular weight protein. Fragments of spleen isolated from mice injected with statolon produced IFN whose molecular weight was 33,000 daltons (28).

Other non-viral inducers have also produced various molecular species of mouse IFN. Cycloheximide injected intravenously into mice produced IFN with a molecular weight of 33,000 daltons (29). Trachoma-inclusion conjuctivitis agent induced IFN in both L cell and mouse serum. The molecular weight of IFNs induced by these agents was 50,000 daltons (30, 31). Circulating IFN induced as a result of injecting mice with synthetic anionic polymers has a molecular weight of 70,000 daltons (32). Two distinct molecular forms of mouse IFN have been produced by polynucleotides. They are of 24,000 and 36,000 daltons molecular weights (33-35). It is clear from above, that several factors may be involved in the molecular response of the mouse tissue to induction by different viral and non-viral stimuli. Molecular weight of IFN have been observed to be cell-specific, inducer-specific and also depends on period of stimulation (20, 36, 37).

In this study, the molecular weight of IFN induced by PolyA: PolyN $^{5}_{3}$U was determined by three different methods. The molecular weight obtained from gel-filtration studies appears higher than either of the two while values obtained when molecular weight was estimated under denaturing and non-denaturing conditions in polyacrylamide gels were comparable. The reason for this observation may be due to retardation of migration during elution from Sephadex G-200 column. This is expected to increase the elution volume of the IFN sample, its partition coefficient (Ka) and consequently its molecular weight. It is believed therefore that a more accurate estimate of the molecular weight is supplied by gel electrophoresis.

Although the values obtained (i.e. 45,100 and 46,238) appear slightly higher than earlier reported for polynecleotide-induced mouse IFN in mouse L cell (33-35), it is clear from previous reports that the molecular weight obtained for the IFN induced by PolyA; PolyN $^{5}_{3}$U is well within the range already established for mouse IFN. Mouse IFN activity has been shown to spread over a wide range of molecular weights (22,000 - 90,000) depending on the inducer and the cell system (29).

There is the evidence that the IFN produced in this study is a single polypeptide chain. This is supported by the fact that the molecular weight obtained by polyacrylamide gel electrophoresis under denaturing and non-=denaturing conditions are comparable. If there are more subunits in the protein, it is expected that lower molecular weight may be obtained under denaturing conditions as a result of dissociation into various subunits. This observation is in keeping with earlier reports (7) that mouse B-IFN consist of a single polypeptide chain. Similar observations have been reported for human IFN - B (38).

There is also the evidence that the IFN obtained in this study is a glycoprotein. Estimation of total sugar indicates that the molecule contains carbohydrate. This indicates that the molecule is a glycoprotein. The observations are consistent with earlier reports on mouse IFN. Both α and β mouse IFNs have been shown to contain glucosamine (39, 40) and L cell IFN obtained in the presence of tunicamycin, an inhibitor of protein glycosylation was reduced in molecular size to 18000 daltons from 24,000 daltons for α-type and to 36,000 from 42,000 for B-type (41-43).

The activity of the IFN obtained in this study was destroyed by ultraviolet irradiation and treatment with trypsin. Appreciable activity (80%) was however retained when treated with neuraminidase. Although previous reports (13, 16) indicate complete resistance to the action of nucleases and glycosidases, the reduction in activity recovery following neuraminidase treatment may be due to inactivation of the IFN preparation by other factors other than enzymatic degradation. However, appreciable stability to neuraminidase is demonstrated by this preparation. The results obtained in this study are consistent with earlier reports of α and β-mouse interferons induced by various stimulating agents (13,21,43-46).

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