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PRESENCE OF A POLYNUCLEOTIDE BINDING SITE ON MURINE IMMUNE INTERFERON (T-TYPE)

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SUMMARY - The T-type interferon induced by PHA has been shown to be quantitatively bound to poly I-sepharose. The binding is not impaired by the presence of 50% ethylene glycol. The interferon-Poly I complex could be only dissociated at high ionic strength. The homogenous elution profile suggests that almost the whole population of PHA-interferon molecules have the polynucleotide binding site. Comparison of crude and ammonium sulfate fractionated preparations shows that the elution of the interferon activity of the latter from poly I-sepharose column was shifted to a lower ionic strength. This suggests that the protein molecules might be modified by chemical or enzymatic action affecting the polynucleotide binding site. The presence of a polynucleotide binding site on immune interferon (Type T) is thus one of a few common properties to viral induced interferon. Furthermore, taking advantage of this property, a practical purification method could be set up.

INTRODUCTION

It has been recently shown that interferon induced by virus or poly I.poly C binds selectively to certain single stranded polynucleotides such as poly I or poly U (1) or to some species of tRNA (2). From this binding property, the presence of a polynucleotide binding site on the interferon molecule has been inferred. The rational of this assumption

Abbreviations :
poly I = polyinosinic acid
poly C = polycytidylic acid
poly U = polyuridylic acid
PHA = phytohemagglutinin

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was based on the conclusions drawn from studies on a series of nucleotidyltransferases which have a known RNA attachment site (3). The fact that several kinds of viral induced interferons, display this property led to the suggestion that the polynucleotide binding site might be related to a regulatory function of interferon, either in its induction or in its action. It appeared thus interesting to analyse in more details the behaviour of other classes of interferons as regard to its association with a polynucleotide ligand.

In particular, the immune interferon (T-type) induced by stimulating immunocompetent cells with mitogens, such as the T-mitogen PHA, which was shown to be antigenically unrelated to viral induced interferon (4). PHA-interferon differs also from viral interferon by its lability at pH 2 (4). T-type interferon shows properties similar to those of interferon induced by specific antigen when injected in sensitized mice (type II interferon) (5).

We have recently reported (6) that PHA-interferon preparations displayed significant molecular heterogeneity but show hydrophobic properties similar to viral induced interferon.

We described in this paper, the binding properties of the immune induced PHA-interferon to poly I-Sepharose and show that it has, as a common property to viral induced interferon a polynucleotide binding site. Furthermore, we show also that taking advantage of this polynucleotide binding capacity, a practical purification method could be set up.

METHODS

Murine PHA interferon was prepared using spleen cells from nude heterozygous mice (nu/+) (supplied by the Centre de Sélection et d'Elevage d'Animaux de Laboratoire CSEL,France)

essentially as described in (7). In short : spleen cells were suspended at the concentration of $1x10^7$ cell/ml in RPMI 1640 medium (Flow), supplemented with 5% fetal calf serum, 2 mM glutamine and 4 mg% gentamicin, and incubated with 3 µg/ml of purified PHA (Wellcome) in Petri dishes (Munclon 90 mm) for 24 hours at 37°C in a humidified incubator (5% CO_2). Cells were then spun off and the supernatant frozen at -70°C until used (crude interferon)- titers of interferon were in the range of 500-1000 µ/ml. For same experiments, crude interferon preparationswere precipitated with ammonium sulfate to a final saturation of 42%, the precipitated removed and the supernatant concentrated under vacuum and dialyzed against phosphate buffered physiological saline (ammonium sulfate fractionated PHA-interferon)

Titration of interferon was performed by a cytopathogenic inhibition test (8)i in L-cell monolayers using vesicular stomatitis virus as a challenge virus. All interferon titers are expressed in international reference units. Protein concentration was measured by a fluorometric assay (9) with bovine serumalbumin as the standard

Affinity chromatography was performed at room temperature at a flow rate of 1 ml/hr for adsorption and 4 ml/h for desorption. Fractions, 200 µl were collected into plastic tubes ; 100 µl aliquots were diluted immediately with 100 µl of 1% bovine serumalbumin and used for interferon titration.

Electrophoresis. Electrophoresis was performed in polyacrylamide slab gels as described by Laemmli (11), with Tris-glycine buffer in the presence of sodium dodecyl sulfate (SDS). The slab gel was 0.5 mm thick allowing to visualize protein band at 0.1 µg level. The proteins were stained with coomassie blue.

Polyinosinic acid Sepharose was prepared according to Wagner et al (10). Samples of poly I- agarose were also obtained from Choay Laboratory, Paris. Essentially the same results were obtained.

RESULTS

When crude PHA-interferon was chromatographed on a poly I-Sepharose column in low ionic strength buffer (Tris-HCl 10 mM, pH 7.5), no significant interferon activity was found in the break through fractions (figure 1). During washing a slight activity, if any, was detected in the first fractions suggesting that a very small portion of interferon molecules was merely retarded on the column. On the contrary, the totality of interferon activity was almost recovered from the columns by high concentrations of KCl. In fact, one major peak of interferon activity was eluted around 0.4 to 0.6 M KCl and a minor peak of activity at 1,0 M elution. Such elution profile with two fractions having interferon activity suggests the presence of distinct-molecular species

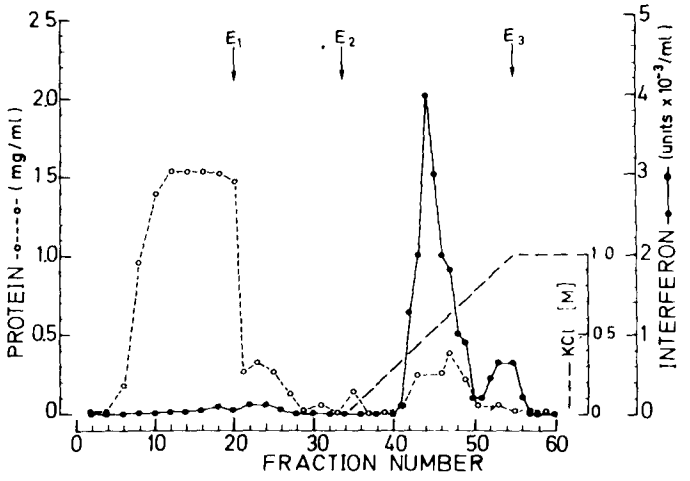

Figure 1.- Chromatography of murine PHA-interferon on poly I-Sepharose column. A sample of 4000 units of crude PHA-interferon (in 4 ml containing 2.2 mg protein/ml) was dialyzed overnight against 10 mM Tris-HCl buffer, pH 7.5. The dialyzed sample was loaded onto a polyI-Sepharose column (1 ml bed volume) equilibrated with the same Tris buffer. After the washing step with 3 volumes of the Tris buffer (E_1), a linear concentrations gradient of KCl (E_2) formed by mixing 2 ml of E_1 and 2 ml of 1 M KCl in E_1, was applied to the column. The overall recovery of interferon activity was complete. 92% of the activity was recovered with the gradient .

of interferon as it has been observed with other types of affinity chromatography (6).

To ascertain that this behaviour is not due to the presence of a large amount of serum and other proteins in the PHA-interferon preparation, another sample of PHA-interferon, obtained after ammonium sulfate fractionation, was used for the chromatography. The results of such filtration are illustrated in figure 2. The elution profile showed that the main interferon activity peak was eluted around 0.5 M as previously observed. However, significant amounts of interferon activity were found in the break through and washing fractions. In addition, the elution profile revealed a broad distribution of activity along the gradient from 0.2 M to 0.8 M, albeit the activity on both sides of the major peak was rather low. Furthermore, no activity was eluted by 1.0 M KCl. The slightly different behaviourof these

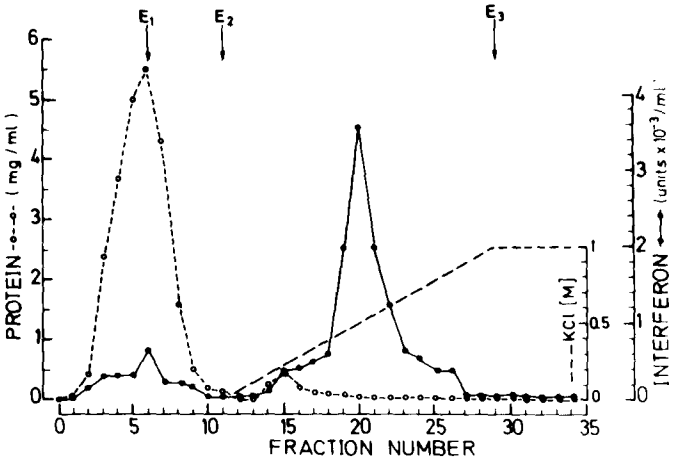

Figure 2.- Elution profile of ammonium sulfate fractionned PHA-interferon from th poly I- agarose by a KCl gradient.

An interferon preparation, 1 ml, containing 2,500 units of interferon activity and 5.7 mg of protein per ml, was dialyzed against 10 mM Tris-HCl buffer pH 7.5 (E_1) an applied on a column of poly I- agarose (0.5 ml bed volume). The column was washed with 1 ml of E_1 and then, a linear gradient of KCl (E_2) was developed by mixing 1.8 ml of E_1 and 1.8 ml of 1 M KCl in E_1. The column was finally eluted with 1 ml of 1 M KCl (E_3). The overall recovery of interferons activity was 95%.

two types of PHA-interferon samples might be due either to modifications of the interferon molecules introduced during the ammonium sulfate fractionation or to elimination of some interferring protein by the same procedure. The essential feature remains that the majority of the PHA-interferon activity was retained on poly I-Sepharose and eluted from this ligand at 0.5 M KCl. Such high concentration of KCl needed for desorption indicated that PHA-interferon bound tightly to the polynucleotide ligand, as just the case observed with mouse viral interferon(1).

Since it has been shown recently that immune interferon has a potential for hydrophobic interactions (6), a chromatography was developped in the presence of 50% ethylene glycol to minimize such interactions. The results of such experiments are shown in Figure 3. A PHA-interferon sample (ammonium sulfate fractionated)

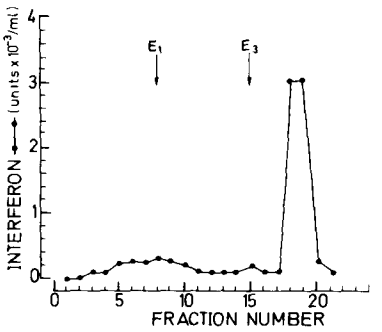

Figure 3.- Binding of murine PHA-interferon to poly I- Sepharose in the presence of 50% ethylene glycol.

0.5 ml of an interferon preparation identical to that used under Fig.2, and diluted with 0,5 ml of ethylene glycol was applied to a poly I- Sepharose column (0.5 ml bed volume), equilibrated with the same buffer. After charging the column was washed with 10 mM Tris-HCl buffer pH 7.5 (E_1) and finally eluted with 1 M KCl in mM Tris HCl buffer pH 7.5 (E_3). The overall recovery of the interferon activity was complete. About 90% of the activity was recovered by elution with E_3.

similar to that used in experiment illustrated in Figure 2, was made in Tris-HCl buffer 10 mM pH 7.5 containing 50% ethylene glycol and chromatographed through a poly I-Sepharose column equilibrated with the same buffer. It is clear that leakage of interferon activity in both pass-through and washing fractions was similar to that observed in the absence of ethylene glycol. About 90% of the initial activity were retained on poly I-Sepharose even in the presence of 50% ethylene glycol and could be recovered by 1 M KCl elution. These results strongly suggested that hydrophobic interactions are not primarily involved in the binding of PHA-interferon to poly I ligand. The binding property leads us to postulate that immune interferon could have a polynucleotide binding site as we have previously postulated for mouse viral interferon (1). This similarity between viral and immune interferon (type T) may have important significance.

A practical aspect could be immediately drawn from this binding property. Indeed, from experiments described under figure 2,

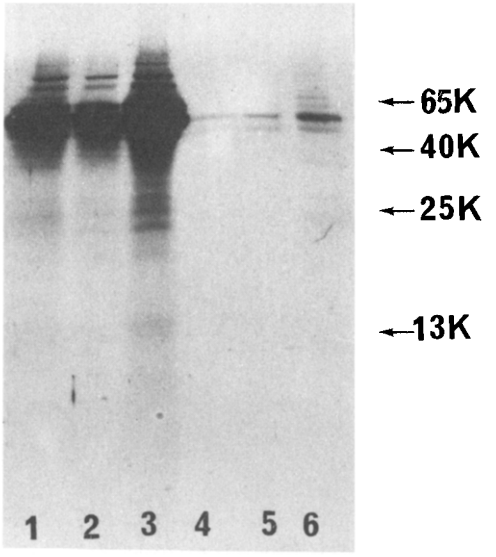

Figure 4.- Electrophoretic patterns of fractions of crude PHA-interferon after chromatography on poly I- Sepharose.

Polyacrylamide-SDS gel (15%) was performed as described in Methods. Chromatography was described in Fig. 1. Lane 1 (dialyzed starting sample 5 µl) ; lane 2 (Fraction 10, 5 µl) ; lane 3 (Fraction 25, 20 µl) ; lane 4 (Fraction 44 30 µl) ; lane 5 (Fraction 45, 30 µl) ; lane 6 (Fraction 46, 10 µl). The positions of the molecumar weights markers are indicated by arrows.

the KCl peak fraction had a specific activity of $1x10^5$ u/mg protein with a 200 fold purification from the initial material in one step. Even with a crude preparation described in figure 1, a specific activity of $1.6x10^4$u/mg protein could be obtained in the 0.5 M KCl elution peak. Analysis of different fractions from such affinity chromatography by electrophoresis on polyacrylamide-SDS gels showed clearly that the main contaminant in the purified fractions is the serum albumin (Figure 4). It is thus rather easy to eliminate this main protein by appropriate procedure.

DISCUSSION

The T-type interferon induced by PHA has been shown to be quantitatively bound to poly I ligand immobilized on Sepharose. The binding is not at all impaired by the presence of 50% ethy-

lene glycol, minimizing the involvement of hydrophobic interactions. The interferon-poly I complex could be only dissociated at high ionic strength (around 0.5 M KCl). With the crude PHA-interferon preparation, the elution profile of the antiviral activity from the affinity chromatographic column and developped with a salt concentrations gradient showed a relative homogeneity albeit a small portion eluted only at 1 M KCl. Even the ammonium sulfate concentrated fraction, display a rather homogenous elution profile. Such chromatographic behavior suggested that almost the whole population of the PHA-interferon molecules have a polynucleotide binding site with a similar affinity to poly I. This particular property contrasted from the behavior of the same interferon on other affinity sorbents such as Concavalin A-Sepharose,Affi-gel 202, and blue-sepharose on which PHA-interferon preparations display a significant degree of molecular heterogeneity (6). This heterogeneity might mainly result from the different degrees of glycosylation while the interaction of the protein with the polynucleotide might be related to a constant domain of the protein moiety. However, this particular domain assigned for nucleic-acid-protein interaction could be impaired by chemical or enzymatic modifications. Indeed, we did find, in some cases, ammonium sulfate fractionated preparations which showed an elution profile from the poly I-Sepharose column shifted on a lower ionic strength region. More dramatic was the profile derived from a purified fraction obtained with phenyl-Sepharose. Part of the antiviral activity was no more bound to the poly I ligand (data not shown). We infer this as a result of the degradation of the polynucleotide attachment site during purification procedure. This eventual modification of the protein moiety is under study.

The property of binding to polynucleotide could reveal a structural or functional homology between different classes of interferons. Comparative study performed in human leucocyte interferon showed that the antiviral activity could be dissociated from the property of polynucleotide binding, while the species specificity seemed to be closedly related to the presence or the absence of the polynucleotide binding site (Thang et al. unpublished data).
The present study on the interaction between PHA-interferon and polyinosinic acid does not allow to draw any conclusion from the point of view of the structure and the function relationship. However, viral interferon (12) as well as T-interferon (13) or Type II interferon (14) display immune regulation properties. One cannot preclude the possibility that the polynucleotide binding domain could be the site interacting with a cell receptor for immune response. To this regard, one raises the question whether other lymphokins produced at the same time that immune interferon would have also the polynucleotide binding property.

ACKNOWLEDGEMENTS

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