Isolated interferonα–receptor complexes stabilized in vitro

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We describe the extraction and stabilization in vitro of discrete complexes of interferon and cellular receptor proteins. A homogeneous complex of M_r 230000 was extracted at the time of peak receptor binding (30 min). Complex formation was specific for human interferon. At later times a second complex could also be extracted suggesting transfer of interferon to a second site.

Lymphoid cell *Membrane receptor* Interferonα *Labelled interferon* *Cellular receptor solubilization*

1. INTRODUCTION

Interferon (IFN) binds to cells to elicit a variety of effects that differ both with respect to kinetics and to the response upon dose [1–5]. Membrane-binding sites have been demonstrated with radiolabelled IFNs [9–11] and different forms of human IFN have been shown to compete for a common binding site [6–8]. However, differences in binding measured at 4°C and 37°C suggest that more than one site is active at the higher temperature [10,11]. To distinguish between different sites and to elucidate the steps following binding of IFN to receptor we have extracted and separated the cellular complexes appearing after the addition of IFN using ^{125}I-IFN as a marker.

2. MATERIALS AND METHODS

2.1. *Cell-bound radioactivity*

IFNα_2 was labelled to a slight molar excess of ^{125}I to IFN (150 μCi/mg IFN protein, M_r 20000) [1] and added to exponentially growing Daudi cells at 200000 cells/ml to a final IFN concentration of 300 units/ml. After incubation for various times at 37°C, 4 ml culture was placed on ice, centrifuged, and washed twice with medium (RPMI 1640) + 1% fetal calf serum, all at 4°C. Cells were counted for radioactivity (LKB-rackgamma, 80% counting efficiency) and for cell number. The binding characteristics of labelled IFNα_2 were identical with those of unlabelled IFNα_2 as determined by competition [13].

2.2. *Chromatography of digitonin extracts*

Daudi cells (10^7) were incubated with IFN as in section 2.1. The culture was harvested after 30 min treatment; 0.5 ml 1% digitonin (Calbiochem) in 0.1 M Tris–HCl (pH 7.4) containing 1 mM phenylmethylsulphonyl fluoride, was added to the washed cell pellet. After 25 min at 25°C, the suspension was centrifuged for 15 min at 11000 rev./min in an Eppendorf microfuge. The supernatant, containing 70% of the original cell bound radioactivity was applied to a column of Sephacryl S-400 (Pharmacia Ltd; 1.6 × 50 cm; flow 13 ml/h), equilibrated with 0.05% digitonin in the same Tris buffer. 0.9 ml fractions were counted for radioactivity. The following M_r markers (Pharmacia Ltd) were pre-equilibrated with digitonin and used to calibrate the column: aldolase, 158000; catalase, 232000; ferritin, 440000; thyroglobulin, 669000. K_{av} is the fractional elution volume for the markers.

3. RESULTS AND DISCUSSION

Fig.1 shows the kinetics of binding of labelled IFNα to exponentially growing Daudi cells. At 300 units/ml, IFN will completely inhibit the growth of

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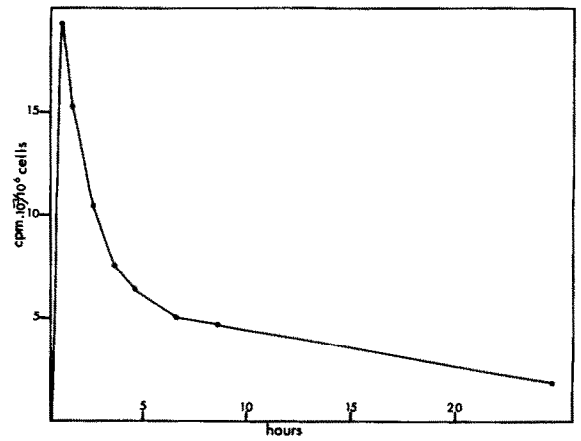

Fig.1. Cell bound radioactivity at times after the addition of ^{125}I-IFNα_2.

these cells after one generation (~20 h). On the basis that peak binding represents the first reaction of IFN with a cellular binding site, we extracted the complex formed after 30 min incubation. Fig.2 shows the gel filtration of radioactivity, solubilized by treatment of the cell pellet with 1% digitonin.

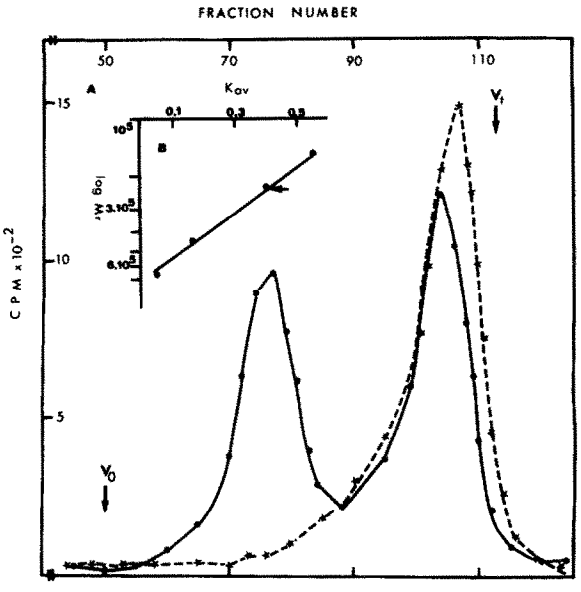

Fig.2. (A) Chromatography of digitonin extracts of Daudi cells treated with ^{125}I-IFNα_2: (★---★) ^{125}I-IFNα_2 alone in digitonin; (●—●) digitonin extracts of IFN-treated cells. (B) Standards for molecular ratio estimation of the solubilized complex; (←——) K_{av} for the labelled complex extracted from Daudi cells.

Two homogeneous peaks of radioactivity were separated. One corresponds to free IFN (M_r 20000); the other to a complex of M_r 230000. A similar separation was seen when the isolates were applied to sucrose density gradient (5–20% in 0.1 M Tris–HCl (pH 7.4)) and centrifuged for 20 h (Beckman SW-41, 40000 rev./min).

IFN accounts for >95% of the total cell-bound radioactivity (as estimated by binding in the presence of a 200-fold excess of unlabelled IFN). In all cases, the amounts of radioactivity found in the isolated complexes far exceeded the background amounts of non-specifically bound radioactivity. Identical complexes could be isolated from other lymphoblastoid cells using both ^{125}I-IFNα from Namalwa cells [11], as well as the cloned IFNα_2 described here [12].

The free IFN represents, for the most part, IFN liberated during extraction rather than IFN which dissociated from the complex after extraction; e.g., rapid separations on small gel filtration columns that retain IFN but exclude the complex

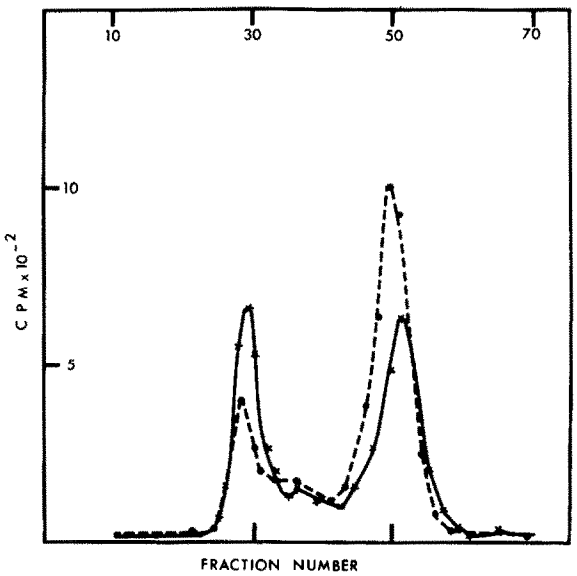

Fig.3. Dissociation of ^{125}I-IFNα_2 from the extracted digitonin complex after addition of an excess of unlabelled IFNα_2. Extraction of the complex was as in fig.1 and 2. Labelled extract (0.5 ml) was incubated overnight at 25°C either with (●---●) or without (★—★) 10 μl unlabelled IFNα_2 containing 200000 units. Complexes were separated on Sephacryl S-200 (Pharmacia Ltd.; 1.6 × 30 cm, flow 13 ml/h) in 0.05% digitonin in 0.1 M Tris–HCl (pH 7.4).

(G-75, Pharmacia Ltd.), show a similar proportion of free to complexed IFN as do the slower separations in fig.2 and 3.

Fig.3 shows the in vitro displacement of complexed ^{125}I-IFNα_2 following overnight incubation at 4°C, with an excess of unlabelled IFN. In a similar experiment, incubation with large amounts of unlabelled mouse IFN showed no displacement. Attempts to reverse the displacement by incubating an unlabelled complex with labelled IFN, were not successful; nor was it possible to reconstitute the complex in vitro, using a digitonin extract of Daudi cells and labelled IFN. This suggests that dissociation, when it occurs, is irreversible and that the stability of the complex is maintained by IFN as well as by digitonin.

Most of the extractions were done at room temperature. Differences in stability at up to 37°C were not remarkable. This is in striking contrast to what happens to cell-bound IFN at different temperatures. At 4°C, cell-bound IFN has a 'half-life' of $\geqslant$10 h. At 37°C, turnover is very rapid: the 30 min peak values are displaced with a 'half-life' of 15 min [13]. We must assume therefore that the metabolic activity of the cell is responsible for the instability of the cellular IFN–receptor complexes.

We have reasoned elsewhere, that one of the functions of IFN receptors is to transfer IFN to an activation site [13]. Fig.4 shows the results of separating digitonin complexes extracted after 90 min incubation with labelled IFN. The '30 min' complex is seen in diminished quantity together with a larger complex appearing within the void volume of the column, and a quantity of dialyzable radiolabel which may represent degraded ^{125}I-IFNα_2.

The transfer of IFN from one complex to another with time supports the kinetics evidence for sequential binding of IFN to two cellular sites [13]. It is significant for it offers an explanation for the disparity of the kinetic and dose–response parameters seen for different effects of IFN. There exists a group of effects, often apparent around 10 h after the addition of IFN, easily saturable, with maxima 1–2 days afterwards. For Daudi cells this includes such effects as inhibition of viral multiplication and of cellular growth [1,2]. These effects correlate with the amount of IFN bound at 3 h [13]. There exist other effects of IFN that occur earlier, that become very rapid as the dose in-

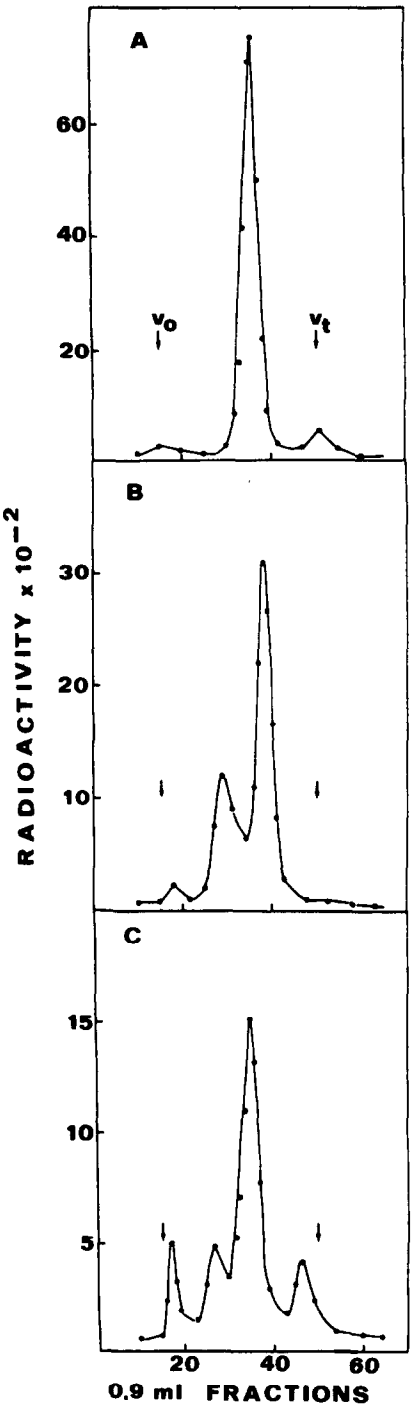

Fig.4. Comparison of separated complexes extracted from Daudi cells by digitonin after 30 min and 90 min incubations with ^{125}I-IFNα_2. All samples were passed on the same column of Sephacryl S-400 (1.6 × 22 cm; flow 13 ml/h) in 0.05% digitonin. (A) Free IFN; (B) 30 min extract; (C) 90 min extract; (⟶) void volume and bed volume of column.

creases; e.g., augmentation of histocompatibility antigens [3], and in Daudi cells, enhancement of cyclic GMP levels [4]. There are also effects that show a different response upon dose in Daudi cells; e.g., the enhancement of expression of Epstein-Barr virus early antigens is not saturated until the dose of IFN approaches 10000 units/ml [5], in contrast to the growth effects which are saturated at 100 units/ml.

Published kinetic and dose–response data are often incomplete. Yet it is, nevertheless, clear that some of the effect of IFN will correlate well with the amount bound to receptors; i.e., with the concentration of the 30 min digitonin complex described here; whereas other effects will correlate with the concentration of the second complex formed later [13]. We see then a divergence of pathways right from the first steps of IFN binding. The stability of the digitonin complexes thus offers the means of examining the composition and activity of the first components in these pathways.

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