Two Molecular Species of Mouse L Cell Interferon Differing in Lectin Binding

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SUMMARY

Binding of L cell interferon to lectins, Wistaria floribunda agglutinin (WFA) and concanavalin A (Con A) was studied by affinity chromatography. Of the two molecular species of L cell interferon, F (mol. wt. 24000) and S (mol. wt. 36000), only the latter was bound efficiently to WFA-Sepharose and eluted quantitatively with α-galactose followed by a pH 3 buffer, suggesting a substantial difference between the two interferon species in their carbohydrate structure. Both were partially bound to Con A-Sepharose and eluted with α-methyl-α-mannoside, indicating that at least some of the F species are also glycoprotein, and that both F and S interferons are heterogeneous as regards their affinity to this lectin.

INTRODUCTION

Mouse L cell interferon induced by Newcastle disease virus contains two molecular species differing in mol. wt., interferon F (24000) and S (36000) [Yamamoto & Kawade, 1976]. As will be reported elsewhere, they differ in antigenicity and in cross-species activity (Kawade et al. 1978; Y. Yamamoto et al. unpublished data). They were also found to be quantitatively different in cell growth inhibitory activity (Ohwaki & Kawade, 1972; Matsuzawa & Kawade, 1974). It was therefore of interest to clarify the structural differences between the molecules of the two interferon species.

In a previous report (Yamamoto & Kawade, 1976), it was suggested from electrophoretic analyses that the two interferons differ in their carbohydrate content and that each of them is somewhat heterogeneous in apparent molecular size and charge. In this report, binding of the interferon molecules to plant lectins was investigated by the technique of affinity chromatography. A galactose-specific lectin from Wistaria floribunda was found to bind the S species, but not the F species, of L cell interferon, suggesting a marked difference in their carbohydrate moiety. Both species bound to Con A at least partially. Heterogeneity in the carbohydrate moiety of each of the two interferon species was suggested by their behaviour.

METHODS

Lectins. Wistaria floribunda agglutinin (WFA) purified to homogeneity in SDS-polyacrylamide gel electrophoresis (Tsuda et al. 1975) was kindly donated by Dr M. Tsuda and Dr Y. Sugino of the Biological Research Laboratories, Takeda Chemical Industries, Ltd., Osaka, Japan. Partially purified WFA used in the early phase of this work and purified Ricinus communis agglutinin (RCA) were kindly donated by Dr T. Osawa of the University of Tokyo.

Lectin-Sepharose. WFA (4·2 A_{280} units) was coupled to CNBr-activated Sepharose
4B (0·5 g; Pharmacia Fine Chemicals) by the standard procedure in the presence of 0·2 M-D-galactose (Axen et al. 1967). From the u.v. absorbance of the bead washings, the bound WFA was estimated to be 1·7 $A_{280}$ units/ml of gel sediment. The beads, packed in a column, were extensively washed with 0·2 M-sodium acetate buffer at pH 4, 0·1 M-tris-HCl at pH 8, 0·1 M-D-galactose in 0·1 M-acetic acid, and finally with the loading buffer to be used for interferon binding. A second batch of WFA-Sepharose with 1 mg WFA/ml of gel sediment was generously supplied by Dr M. Tsuda. Identical experimental results were obtained with these two batches. RCA-Sepharose was prepared similarly and had 6·5 $A_{280}$ units of bound RCA/ml of gel sediment. Con A-Sepharose 4B (Pharmacia Fine Chemicals) had approx. 8 mg of bound Con A/ml of gel sediment.

**Affinity chromatography on lectin-Sepharose.** For WFA-Sepharose, the loading buffer was 0·01 M-sodium phosphate buffer at pH 7·4 containing 0·5 M-NaCl (buffer A). Elution was carried out in two steps, first with 0·1 M-D-galactose in buffer A and second with glycine buffer at pH 3·0 (Table I). In some experiments, Dulbecco’s phosphate buffered saline (PBS) without CaCl$_2$ and MgCl$_2$ was used as the loading buffer and elution was in three steps; first, with 0·1 M-D-galactose in PBS; second, with 0·1 M-acetic acid and third, with 2 M-NaCl (Table 2).

For Con A-Sepharose, two kinds of loading buffers were used and the elution buffer were as specified in Results. All buffers contained 0·02 to 0·05 % bovine plasma albumin, 250 units/ml of penicillin and 300 $\mu$g/ml of streptomycin. The albumin was added because it stabilized the interferon activity without changing the binding characteristics. Chromatography was done at 4 °C at a flow rate of 4 to 6 ml/h. The eluates were assayed for interferon after dialysis in a collodion bag (Sartorius Membrane Filter, Gottingen, no. SM13200 pore size 8 nm).

**Interferon.** L cell interferon was prepared and partially purified as described before (Yamamoto & Kawade, 1976). F and S interferons were separated from each other by polyacrylamide gel electrophoresis at pH 4·3, or by gel filtration, using a preparation with a specific activity of $2 \times 10^7$ to $3 \times 10^7$ units/$A_{280}$ (step 6 in Table 1 of Yamamoto & Kawade, 1976). In Expt. 2 in Table 2, each interferon had been further purified on affinity columns containing the respective antibodies. There was little cross reactivity between interferon S and anti-F serum and between interferon F and anti-S serum, as will be described elsewhere (Y. Yamamoto et al. unpublished data).

**Assay of interferon.** The method was as previously reported (Yamamoto & Kawade, 1976). It involves the inhibition by interferon treatment of $^3$H-uridine incorporation into vesicular stomatitis virus RNA in L cells under single cycle conditions (Kawade et al. 1976). The titles in this report are expressed in terms of the National Institutes of Health mouse interferon reference preparation, Catalogue no. G-002-904-511.

**RESULTS**

**Chromatography of unfractionated L cell interferon on galactose-specific lectin-Sepharose**

In view of the results of Dorner et al. (1973) and Davey et al. (1976), galactose-specific lectins were first examined. WFA, whose specificity was examined in detail by Irimura et al. (1975), was mainly used. When this was added at 0·1 or 0·01 $A_{280}$ units/ml of culture medium with or without interferon, it was not cytotoxic, and it did not affect uridine incorporation into vesicular stomatitis virus RNA or the interferon titre.

When whole L cell interferon was chromatographed on a WFA-Sepharose column, part
Interferon-lectin binding

Table 1. Chromatography of L cell interferon on WFA-Sepharose.

<table>
<thead>
<tr>
<th>Interferon (units x 10^-8)</th>
<th>Unfractionated†</th>
<th>Unbound</th>
<th>Bound</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loaded</td>
<td>300</td>
<td>24</td>
<td>35</td>
</tr>
<tr>
<td>Total recovery</td>
<td>345 (100)‡</td>
<td>24 (100)</td>
<td>33 (100)</td>
</tr>
<tr>
<td>Unbound</td>
<td>88 (26)</td>
<td>21 (87)</td>
<td>3 (9)</td>
</tr>
<tr>
<td>Bound</td>
<td>257 (74)</td>
<td>3 (13)</td>
<td>30 (91)</td>
</tr>
</tbody>
</table>

* Column size: 8 x 11 mm.
† Crude interferon concentrated by zinc acetate (1 x 10^8 units/A280).
‡ Total recovered from column taken as 100%.

of the activity was not adsorbed, as shown in Table 1. The adsorbed fraction (74%) was recovered quantitatively when eluted first, with D-galactose (0.1 M) and then with acid (glycine buffer at pH 3). Upon re-chromatography, the bound and unbound fractions behaved essentially as in the first chromatography (Table 1), indicating that L cell interferon consists of two species differing in their binding to the lectin.

Elution was possible also with D-galactosamine (0.1 M) but the efficiency was less than with D-galactose.

RCA, another galactose-specific lectin (Irimura et al., 1975), coupled to Sepharose, also bound L cell interferon to a similar degree, but it was difficult to measure its elution: small amounts of the lectin released from the column often caused severe damage to the L cells used for interferon assays. Nevertheless, there were indications that elution was incomplete with the system which was used for WFA.

Comparison of the binding of S and F interferons to WFA-Sepharose.

In a previous report (Yamamoto & Kawade, 1976), the apparent mol. wt. of interferon S (but not that of interferon F), as determined by SDS-polyacrylamide gel electrophoresis, altered with changes in the gel concentration, suggesting that S may contain more carbohydrate than F. This and the results described in the preceding section suggested that the fraction of L cell interferon bound to galactose-specific lectins might represent S interferon, and that which was not bound, F interferon. Experiments using isolated S and F interferons substantiated this.

In Expt. 2 of Table 2, F and S interferons of high purity isolated from each other by gel filtration (step 8 in Table 1 of Yamamoto & Kawade, 1976) were separately charged to a WFA-Sepharose column. With F, most of the activity (86% of total recovered) was not retained. This column could bind at least 5 x 10^6 units of unfractionated interferon, precluding flow-through due to overcharge. On the contrary, most of the S interferon was bound. The results of Expt. 2 in Table 2, obtained with F and S interferon preparations purified by affinity chromatography using respective antibodies, were essentially as in Expt. 1. The elution pattern of these interferons from the WFA-Sepharose column are shown in Fig. 1. Most of the F interferon (Fig. 1a) appeared in the flow-through fractions, only about 1% being eluted with D-galactose and acid. On the other hand, S interferon was almost totally bound (Fig. 1b). About 30% of the activity was eluted with D-galactose and the remainder with a pH 3 buffer. Upon re-chromatography of the galactose-eluted fraction, complete elution again required the pH 3 buffer, so that clear-cut fractionation was not obtained.
Table 2. Comparison of binding of F and S interferons to WFA-Sepharose

<table>
<thead>
<tr>
<th>Experiment no.</th>
<th>Interferon (units × 10⁻³)</th>
<th>1*</th>
<th>2†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interferon</td>
<td>F</td>
<td>S</td>
<td>F</td>
</tr>
<tr>
<td>Loaded</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total recovery</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unbound</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bound</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Interferons separated by gel filtration. Column size: 8 × 20 mm.
† Interferons separated by antibody affinity chromatography. Column size: 5 × 30 mm.
‡ Total recovered from column taken as 100%.

Chromatography on Con A-Sepharose

Con A, a binding agent with a specificity different from WFA and RCA was next used. It was particularly interesting to see whether interferon F, which did not appreciably bind to WFA, would show any binding to Con A.

In the first experiment, unfractionated L cell interferon was charged to a Con A-Sepharose column in 0.01 M-phosphate buffer, pH 7.4, containing 1 M-NaCl (buffer B), a system similar to that in which efficient binding of human fibroblast interferon was observed (Davey et al. 1974). When 5 × 10⁵ units of interferon were loaded, most of the activity came through unadsorbed and 8% was eluted with 0.1 M-α-methyl-D-glucoside in buffer B (total recovery 98%). After this, α-methyl-D-mannoside (0.2 M), which has a stronger affinity to Con A, eluted only 10% of the activity eluted with α-methyl-D-glucoside, indicating that the latter can elute the bound interferon essentially completely. Little or no activity was eluted with 50% (v/v) ethylene glycol in buffer B used after the sugar, in contrast to human fibroblast interferon (Davey et al. 1974). This column (about 1 ml of gel sediment) was found to bind 3 mg of ovalbumin (about 2% carbohydrate; Marshall & Neuberger, 1972). The column capacity will then be large enough, considering the estimated specific activity of interferon of 10⁶ units/mg protein or higher (Iwakura et al. 1978), even though the carbohydrate content of interferon molecules is not known.

Another buffer system used by Besançon & Bourgeade (1974), who reported binding of L cell interferon to Con A, was used. The loading buffer contained divalent cations involved in the binding structure of Con A (0.001 M each of CaCl₂ and MnSO₄) in 0.1 M-sodium acetate, pH 6.0. As seen in Table 3, Expt. 1, a large portion of the unfractionated interferon used in the previous experiment was bound in this case. A major portion of the bound interferon was eluted with 0.1 M-α-methyl-D-glucoside, and the remainder with 0.2 M-sodium tetraborate-0.8 M-potassium phosphate, pH 6, containing 1 M-NaCl (Svenson et al. 1970). Upon re-chromatography, the unbound fraction of Expt. 1 showed a decreased amount of binding, whereas most of the bound fraction was bound, indicating, though not very clearly, that there was some fractionation. In another experiment (not shown), the unfractionated interferon of an amount only 1/1000 that of Expt. 1 was found to give a similar result with perhaps a small shift towards more binding. Results with separated F and S interferons are shown in Table 3, Expt. 2. Again, both interferons were partially bound and eluted with the specific sugar followed by tetraborate. The extent of binding in Expt. 2 was less than in Expt. 1, the reason for which is not clear, but it might be that different batches of interferon differ quantitatively in their binding to Con A.
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Fig. 1. Chromatography of (a) interferon F and (b) interferon S on WFA-Sepharose (Expt. 2 of Table 2). The loading buffer was PBS. Elution buffers were: E-1, 0.1 M-o-galactose in PBS; E-2, 0.1 M-acetic acid, and E-3, 2 M-NaCl. The fraction volume was 4.5 ml in (a) and 3.5 ml in (b). The titre of interferon in the first fraction in (a), designated △, was 3.6 × 10⁴ units/ml.

Table 3. Chromatography of L cell interferon on Con A-Sepharose

<table>
<thead>
<tr>
<th>Experiment no.</th>
<th>Interferon (units × 10⁻²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interferon</td>
<td>Unfractionated †</td>
</tr>
<tr>
<td>Loaded</td>
<td>1700 (100)</td>
</tr>
<tr>
<td>Total recovery</td>
<td>1190 (100)§</td>
</tr>
<tr>
<td>Bound (1)</td>
<td>630 (53)</td>
</tr>
<tr>
<td>Bound (2)</td>
<td>90 (8)</td>
</tr>
<tr>
<td>F ‡</td>
<td>20.4 (100)</td>
</tr>
<tr>
<td>S ‡</td>
<td>12.0 (100)</td>
</tr>
</tbody>
</table>

* Unbound was the flow-through fraction in 0.1 M-sodium acetate at pH 6.0 containing 1 M-NaCl and 0.001 M each of CaCl₂, MgCl₂ and MnSO₄. Elution was done in two steps, Bound (1) with 0.1 M 2-methyl-D-glucoside in the loading buffer and Bound (2) with 0.2 M-sodium tetraborate-0.8 M-potassium phosphate, pH 6.0, containing 1 M-NaCl. Column size: 6 × 20 mm.
† Crude interferon concentrated by zinc acetate (1 × 10⁶ units/Asₙₙ).  
‡ F and S interferons were derived from a partially purified preparation (2 × 10⁷ units/Asₙₙ) different from the batch used for Expt. 1.
§ Total recovered from column taken as 100%.
The results thus indicate that both F and S interferons are at least partially bound to Con A in a sugar-specific manner, with no appreciable difference in their binding characteristics.

**DISCUSSION**

The glycoprotein nature of interferon was first suggested for rabbit interferon by Schonne et al. (1970) and then by Dorner et al. (1973), who inferred that it had a terminal carbohydrate sequence of sialic acid → galactose. Mouse L cell interferon (or part of it) was found to be bound to Con A (Besançon & Bourgeade, 1974) and Bandeaerea simplicifolia lectin (Davey et al. 1976) in a manner specific to mannose and galactose, respectively.

In this study, L cell interferon was found to be bound to lectins, WFA, RCA and Con A to certain extents and was eluted partially by monosaccharides, indicating the glycoprotein nature of at least some of the molecules.

The interferon bound to WFA was not completely eluted with galactose, but required an acidic buffer. It is not clear whether this means that some complex, rather than simple, sugars are necessary for complete elution, or that the binding involves bonds other than sugar-specific ones. Davey et al. (1974) inferred that hydrophobic interaction was important for human diploid interferon–Con A binding, since ethylene glycol was required for elution. In this study, however, L cell interferon which had bound to Con A under the same conditions as described by Davey et al. (1974) was completely eluted by α-methyl-D-glucoside and no further elution was observed when ethylene glycol was used after the sugar. When more extensive binding was effected by using a different loading buffer, the same sugar followed by borate (which may form a complex with sugar) gave complete elution. Thus L cell interferon appears to have a weaker affinity to Con A than human diploid interferon.

Of the two main species of L cell interferon, S, but not F, was bound to WFA. Davey et al. (1976) also fractionated L cell interferon by galactose-specific Bandeaerea lectin into bound and unbound species, which may well correspond to S and F interferons, respectively. This does not necessarily mean that F is free of carbohydrate, since it was partially bound to Con A in a sugar-specific way, and studies currently in progress in this laboratory indicate that glucosamine is incorporated into F as well as S interferon (J. Fujisawa et al. unpublished data). The structural basis of this clear-cut difference between F and S interferons must await further studies, but it seems reasonable to assume a major difference in their carbohydrate moieties. An obvious possibility is that both S and F molecules have mannose residues available for interaction with Con A, but in addition S molecules have galactose residues that bind to WFA. A lower carbohydrate content in F than in S was suggested previously (Yamamoto & Kawade, 1976). Another mouse fibroblast interferon from C-243 cells with properties very similar to L cell interferon (Stewart et al. 1977) was recently purified and the electrophoretic band of the 35 000 mol. wt. species could be stained for sugar but not that of the 22 000 mol. wt. species (De Maeyer-Guignard et al. 1978).

Both F and S interferons were bound to Con A only partially under the conditions examined, indicating heterogeneity in affinity to the lectin. This suggests that the carbohydrate moiety of both interferon species is heterogeneous in structure, although other interpretations should not be excluded.

The role of carbohydrates in interferon molecules is yet to be elucidated, although they appear not to be essential for the antiviral activity (Bose et al., 1976). Molecules which presumably had incomplete or aberrant carbohydrate chains were found to be less stable than normal molecules (Havell et al. 1975). This may explain the difference in stability of the activity of F and S (Matsuzawa & Kawade, 1974; Yamamoto & Kawade, 1976),
since they are presumably different in sugar moiety. Also, the sugar chain structure may vary in different batches of interferon from a single source and cause variations in stability.

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REFERENCES


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