Differences in Sialic Acid Content of Human Interferons

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SUMMARY

Human leucocyte, lymphoblastoid and fibroblast interferons were separately treated with sialidase and the effect of this on their isoelectric focusing was examined using a system in which full dissociation of complexes occurred. Both leucocyte and lymphoblastoid interferons showed a single form with an isoelectric point which was unaltered by treatment with sialidase. In contrast, fibroblast interferon showed three forms which were reduced to one by treatment with the enzyme.

Several different methods have been used to establish that interferons are glycoproteins (Spiro, 1969; Fantes, 1970a; Schonne et al. 1970; Dorner et al. 1973). Investigations of the structural composition of the carbohydrate moiety have been made with indirect probes such as binding to lectins (Davey et al. 1976; Jankowski et al. 1975), or treatment with sialidase or other glycosidases (Schonne et al. 1970; Dorner et al. 1973; Bose et al. 1976). The results obtained have been equivocal either because non-specific binding reactions can occur with lectins (Huang et al. 1975) or because the enzymes used could contain other species such as proteases. In addition, the separation methods used to analyse the results of the enzyme treatment would not necessarily have led to the total dissociation of protein complexes. In this paper we report an investigation of the properties of human interferon after treatment with pure sialidase using an analytical method in which full dissociation of protein complexes is known to occur (O’Farrell, 1975).

Lymphoblastoid and fibroblast interferons were prepared from Namalva and HEF-9 cells by published techniques (Atherton & Burke, 1975; Strander et al. 1975). Partially purified human leucocyte interferon was a kind gift from Professor K. Cantell. All samples were assayed by the inhibition of virus nucleic acid synthesis in HEF-9 cells (Atherton & Burke, 1975). In this assay 1 unit of interferon activity is equal to 1 reference research unit (BS 69/19). The specific activities were: leucocyte interferon 4.5 x 10^6 units/mg; lymphoblastoid interferon 3 x 10^6 units/mg; fibroblast interferon 1.1 x 10^5 units/mg. Protease-free sialidase (EC 3.2.1.18) was prepared from Streptomyces griseus (Kabayo & Hutchinson, 1977). The incubation mixtures contained 2500 units of leucocyte or lymphoblastoid interferon, or 1000 units of fibroblast interferon, in 250 µl of 0.05 M-sodium acetate buffer pH 5.3. Samples were incubated for 3 h in the presence and absence of sialidase (50 µg). They were then prepared for analysis by a modification of the isoelectric focusing technique described by O’Farrell (1975), in which 2-mercaptoethanol was omitted from all buffers and 0.25% sodium dodecyl sulphate was added to the sample before it was loaded on the gel. After electrophoresis, the gels were sliced into 0.25 cm segments, placed in phosphate buffered saline (1 ml) and dialysed exhaustively against phosphate buffered saline before their interferon content was determined.

We demonstrated that, under these reaction conditions, all the sialic acid residues are removed from fetuin, used as a model substrate. Fetuin was prepared from foetal calf serum (Spiro, 1960). Incubation mixtures contained fetuin (300 µg) and sialidase (50 µg),
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Fig. 1. Release of sialic acid from fetuin when treated with sialidase for the times shown.

0.05 M-sodium acetate buffer (250 μl), pH 5.3. The sialic acid released after given time intervals was determined by the addition of phosphotungstic acid (0.1 ml, 20%, w/v, in 12.5% H₂SO₄). The samples were centrifuged, and the sialic acid present in the supernatants was estimated (Warren, 1959). The results are shown in Fig. 1 and it may be seen that after 60 min of incubation with sialidase there was no further release of sialic acid. The amount released corresponded to that expected from the acid hydrolysis of fetuin (Svennerholm, 1958), and therefore we believe this treatment removes all of the sialic acid from fetuin. Since the concentration of fetuin was much higher than that of the interferon used in the experiments described above, it is very probable that the treatment employed would remove all of the sialic acid residues from interferon.

The pH gradient in the isoelectric focusing gels was determined and the results are shown in Fig. 2(b). The isoelectric points of human fibroblast, leucocyte and lymphoblastoid interferons were then determined.

Both leucocyte and lymphoblastoid interferon gave a single broad peak of interferon activity with an isoelectric point of 5.4 (Fig. 2a and c); the recovery of interferon activity was, respectively, 68% and 75%. In the case of fibroblast interferon, three peaks of interferon activity could be detected with isoelectric points of 5.2, 5.8, and 6.3, and the overall recovery was 30% (Fig. 2e). Treatment of the three sorts of human interferon with sialidase did not lead to any loss of antiviral activity. When the treated samples were analysed, there was no change in the isoelectric point of either leucocyte or lymphoblastoid interferon (Fig. 2b and d) and their recovery was unaltered. However there was a dramatic change in the case of fibroblast interferon. Instead of the three peaks found in the untreated sample, only one peak of interferon activity could now be detected, with an isoelectric point of 6.3. In addition, the recovery was increased to 70% (Fig. 2f). The results shown in Fig. 2 were obtained from a single set of experiments in which the samples were analysed concurrently. We have obtained similar results when different samples of both interferon and sialidase were used.

These results show that human fibroblast interferon consists of three forms, separable on the basis of their intrinsic charge. This contrasts with the human leucocyte and lymphoblastoid interferons, both of which apparently contain only one form. Recent reports have shown that leucocyte interferon consists of two forms with isoelectric points of
5·5 and 5·7 (Törmä & Paucker, 1976). The particular technique of slicing gels and eluting the interferon that we used may not be able to separate molecules that have such close intrinsic charges. In one experiment two peaks of activity were detected in the leucocyte and lymphoblastoid interferons with very similar isoelectric points (5·3 and 5·5). Other reports (Fantes, 1970b; Havell et al. 1977) indicate that leukocyte interferon is heterogeneous and contains several species with isoelectric points that are different and higher than those reported here. In our experiments the high concentrations of urea used could have caused changes in the apparent isoelectric points (O’Farrell, 1975). However, our method does fully dissociate aggregates, so that any separations that are detected are real and not due to contaminants in the preparation interacting with the interferon. We have been unable to detect any interferon present in the preparations of leucocyte or lymphoblastoid interferons that has the characteristic isoelectric points of fibroblast interferon; in this respect our results differ from those of Havell et al. (1978).

When the leucocyte and lymphoblastoid interferons were treated with sialidase, no change in their isoelectric point could be detected. Therefore we conclude that these interferons lack sialic acid. On the other hand treatment of the fibroblast interferon with sialidase converted the interferon into a single form, suggesting that the other two forms, observed in untreated samples, were distinguished because they contained differing amounts of sialic acid. The form of fibroblast interferon with an isoelectric point of pH 6·3 is probably the form lacking sialic acid, because our treatment did not increase its isoelectric point. Hence this study has confirmed that leucocyte and lymphoblastoid interferons are similar in their lack of sialic acid but different from fibroblast interferon.
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Note added in proof: L. E. Mogenson, L. Pyhala, E. Torma & K. Cantell (1974, Acta Pathologica et Microbiologica Scandinavica, Section B 82, 305-331) have also observed that the isoelectric focussing pattern of human leucocyte interferon does not change appreciably after treatment of the interferon with sialidase.

REFERENCES


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