AN ISOELECTRIC FOCUSING STUDY OF CHICK AND MOUSE INTERFERONS

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Chick and mouse interferons were studied by isoelectric focusing in sucrose and in polyacrylamide gel. The advantages of the focusing in gel method compared with focusing in sucrose for interferon analysis are demonstrated. KEY WORDS: isoelectric focusing; interferon.

Interferon is currently one of the most promising antiviral agents and, for that reason, the problem of obtaining it free from contamination, in a biologically active form, is very urgent. It is closely connected with the problem of the study of the physicochemical parameters of interferon. In turn, this last problem necessitates a search for the most adequate methods for studying the various characteristics of interferons.

With the above considerations in mind the isoelectric points of chick and mouse interferons were investigated by the electric focusing method in sucrose and polyacrylamide gel.

EXPERIMENTAL METHOD

Chick interferon was obtained from culture fluid collected from a culture of chick fibroblasts infected with Venezuelan equine encephalomyelitis virus [14]. Mouse interferon was obtained from culture fluid collected from a continuous culture of mouse cells of strain 929, superinduced by the polynucleotide poly(I:C) by the method described previously [13].

Interferon activity was studied either by determination of the protection of cells against the cytopathic action of an indicator virus or by the method of inhibition of proliferation of indicators virus [12]. Electric focusing was carried out by the method of Davis et al. [3] in an electric focusing column from LKB (Sweden), using sucrose as the fractionation medium, or in an apparatus for electrophoresis in polyacrylamide gel from Reanal (Hungary). In the latter case electric focusing was carried out in 7% gel. The pH range used in the two types of experiment was 3-10. The experimental conditions are given in captions to the figures.

EXPERIMENTAL RESULTS

In many publications different, and sometimes contradictory, values of isoelectric points (pI) are shown for different interferons [1, 2, 4, 5, 8-11]. This may be due to true variations in the values of this parameter, due to the use of different inducers or interferons obtained from different types of cells of an animal of the same species, or perhaps the result of differences in the sensitivity of the methods used. To test this last hypothesis experiments were carried out to study electric focusing of chick interferon in sucrose and in gel. In both cases interferon from culture fluid was subjected to preliminary purification, including incubation of the material at pH 2 and precipitation with zinc acetate. The concentrated and partially purified material was dialyzed against deionized water and used for isoelectric focusing. In both types of isoelectric focusing the interferon-containing material was distributed initially either throughout the volume of the column or along the whole length of the slab of gel. The results of determination of the isoelectric point of chick interferon by these two methods are given in Figs. 1 and 2.

As Fig. 1 shows, chick interferon fractionated in sucrose occupied the zone corresponding to pH 7.87-8.70, and a volume of 8 ml. During isoelectric focusing in gel the same interferon preparation was distributed within a narrower zone with a value of pI = 8.05.

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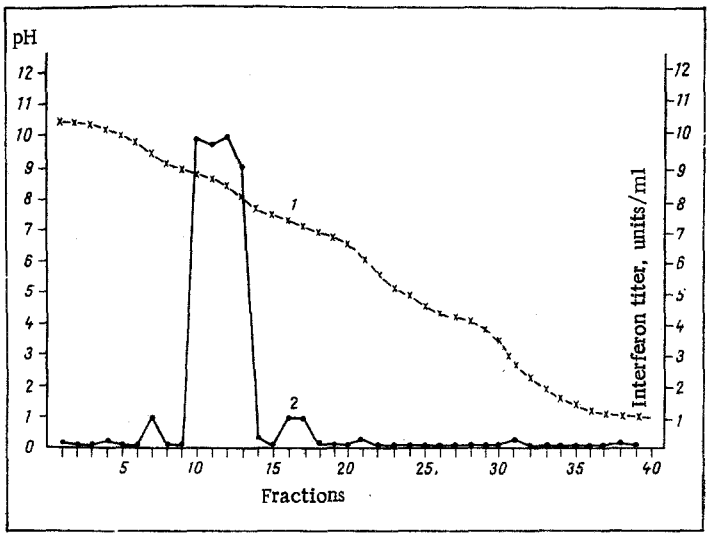

Fig. 1. Isoelectric focusing of chick interferon in sucrose. Focusing time 72 h, voltage 300 V, current 6.5 mA; volume of fractions 2.5 ml. Here and in Figs. 2 and 3: 1) change in pH; 2) interferon activity.

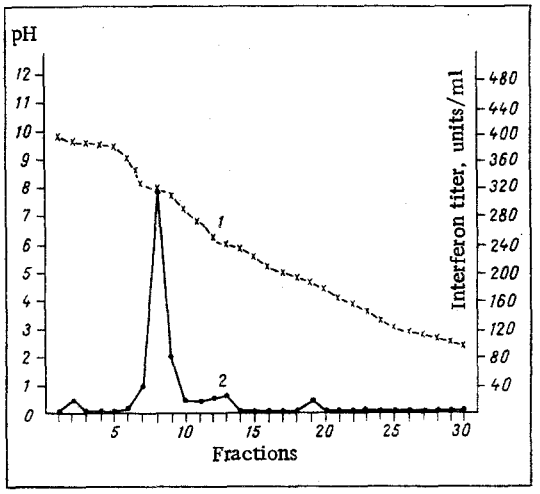

Fig. 2. Isoelectric focusing of chick interferon in polyacrylamide gel. Focusing time 2 h, voltage 200 V, current 2 mA.

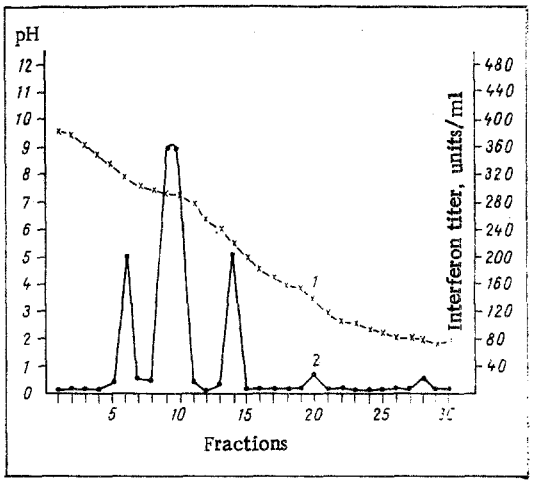

Fig. 3. Isoelectric focusing of mouse interferon in polyacrylamide gel. Focusing time 100 min, voltage 240 V, current 2.5 mA.

The quantitative yield of interferon in these two cases should be noted. Practically all the interferon activity applied to the column and to the gel was concentrated in the above-mentioned zones and could be collected from the colum or eluted from the gel.

The data given above thus indicate that during isoelectric focusing in gel the material is concentrated in narrower zones of gel than during focusing in a colum with a sucrose carrier and, consequently, the results of focusing in gel enable the value of pI of the material for analysis to be determined more accurately.

Having confirmed the advantages of focusing in gel, an attempt was made to determine pI of mouse interferon. The results of this determination are illustrated in Fig. 3. Clearly mouse interferon was distributed mainly in three zones with pH values of 7.9, 7.38, and 5.5 respectively.

Interferon is known to be a population of molecules heterogeneous with respect to various parameters; for that reason the question of the use of the most adequate methods for the determination of particular characteristics of interferon is particularly acute.

In the present investigation two methods of isoelectric focusing were studied for use with interferon. The conclusions drawn from the results is that focusing in gel is a more adequate method of obtaining information about isoelectric points of different interferons than the method of focusing in sucrose. Although the two methods give quantitative yields of material, the zone of distribution of chick interferon in sucrose is wider than in gel. Consequently, the values of pI for interferon determined by focusing in gel are more accurate than those obtained by focusing in sucrose. One evident reason for this phenomenon is the different degree of diffusion of the material in the liquid and solid phases and the possibility of contamination of the fractions during elution from the sucrose column. The lower degree of diffusion and the more reliable system of fractionation and elution give the method of focusing in gel definite advantages over focusing in sucrose.

The results of the present investigation demonstrate that the errors of the isoelectric focusing method in a liquid phase may play a definite role in the heterogeneous distribution of interferon during isoelectric focusing. Although these observations in no way dispose of the question of heterogeneity of interferon preparations, they are nevertheless evidence that only results obtained by the same method are suitable for comparison. On this basis it is difficult to interpret differences in the values of pI for chick interferon obtained by several workers [2, 6, 8] and the values obtained by ourselves. It may be that the differences observed are due to the use of different inducers and of different cell lines. However, the possibility of artifacts connected with details of the techniques used likewise cannot be ruled out.

Analysis of mouse interferon demonstrated unequivocally the heterogeneity of this substance with respect to electric charges. The nature of this heterogeneity was not specially studied, but it may be supposed that the observed heterogeneity is due to differences in the content of the carbohydrate component in the different interferon molecules. Such an interpretation would be in full agreement with data in the literature obtained by the study of rabbit interferon [4, 9] and also relating to the heterogeneity of mouse interferon [7].

Another factor which must be emphasized when the method of isoelectric focusing of interferon is analyzed is the resistance of interferon to the action of ampholytes. As the focusing experiments in sucrose and gel showed, practically no inactivation of interferon takes place during isoelectric focusing. This fact enables isoelectric focusing to be used in conjunction with other methods in order to obtain purified samples of interferon.

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