of 60 μA, an ionizing voltage of 50 V, and a temperature of the ionization chamber of 150°C. The accuracy of the mass measurement was $5 \cdot 10^{-6}$. The conditions for chromato-mass spectrometric analysis have been published previously [1].

SUMMARY

The structures of the two main products of the biotransformation of deoxypeganine and deoxyvasicinone have been established by the PMR method as 11α-hydroxydeoxyvasicinone and 2-(β-ethoxycarbonyl)ethyl-4-quinazolone. The fragmentation of the compounds has been elucidated on the basis of the structure and measurements of the elementary compositions of the ions.

A minor component of the total metabolites has been isolated by chromato-mass spectrometry, and for this the structure of 6-methoxydidehydrodeoxyvasicinone is suggested.

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DNA-CELLULOSE — A NEW AFFINITY SORBENT FOR HUMAN LEUCOCYTIC INTERFERON

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The possibility has been studied of using affinity chromatography on DNA-cellulose for the purification of interferon. It has been shown that interferon at a low ionic strength binds to DNA-cellulose, and on this basis a new method is proposed for purifying interferon which permits its 200-fold purification with small losses.

The interferons are proteins of vertebrate cells (from fish to Man) which form wide controlling and regulating functions directed to preserving cell homeostasis. The most important of these functions are their antiviral, anticellular, immunomodeling, and radioprotective functions [1, 2]. In spite of the voluminous material on the study of interferon over more than 20 years, its use in medical practice has not previously been broad enough. This can be explained by the fact that there is no sufficiently effective and economic method of purifying interferon, since such demands are made on the preparation as the complete absence of toxicity and antigenicity in homologous organisms. The known methods are associated with great losses or envisage the use of difficultly accessible expensive materials [4-7]. In the present paper we give the results of a study of the possibility of using affinity chromatography on DNA-cellulose, preceded by ion-exchange chromatography, for purifying human leucocytic interferon.

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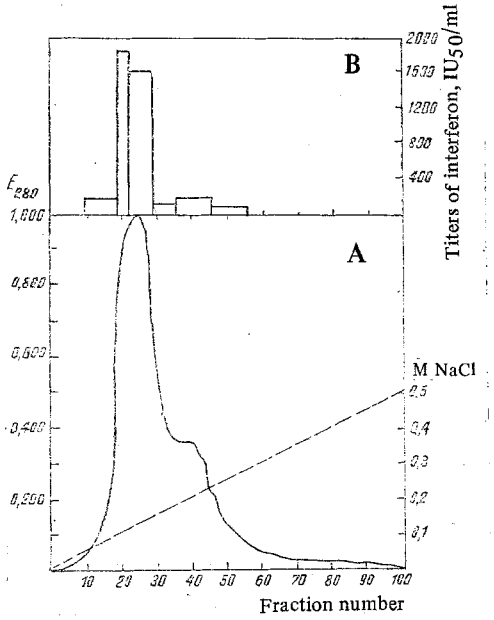

Fig. 1. Elution of interferon from QAE-Sephadex A-50 by a linear NaCl gradient: A) elution profile of the protein (full line — UV absorption; dashed line — concentration of salt); B) titer of the antiviral activity in the fractions (columns).

The crude interferon was concentrated by precipitation with KSCN and, after dialysis, it was subjected to ion-exchange chromatography on QAE-Sephadex. Figure 1 shows the elution of interferon from the ion-exchange material with a linear NaCl gradient. Antiviral activity was detected in the fractions containing concentrations of the salt of from 0.05 to 0.30 M, and therefore elution was performed subsequently with 0.30 M NaCl. Our results on ion-exchange chromatography agree with those given in the literature: the interferons are acidic proteins [3].

Below we give the results of the affinity chromatography of interferon on DNA-cellulose:

Fraction	Amount of protein, mg	Interferon titer, IU_{50} (thousands)	Activity per 1 mg of protein	Activity yield, %
1. Deposited on the DNA-cellulose	170.0	32.0	190	100
2. Breakthrough	159.6	8.4	52	26
3. Elution with 0.65 M NaCl	1.4	20.0	14285	62
4. Elution with 0.50 M NaCl	1.6	8.0	5000	25
5. Elution with 2.0 M NaCl	0.92	-	-	-

As can be seen from these figures, on subsequent deposition on DNA-cellulose the bulk of the interferon is bound to the column, and 62% of the bound interferon is eluted at an NaCl concentration of 0.05 M, the remaining 25% being bound more strongly and appearing in the fractions eluted by 0.5 M NaCl: these fractions were therefore combined and the subsequent elution of interferon was carried out with a 0.5 M concentration of the salt.

As a result of the successive ion-exchange and DNA-cellulose chromatography, it was possible to purify the interferon approximately 200-fold. Known affinity sorbents, such as poly-(V)-Sepharose, (dextran blue)-Sepharose, and copper and zinc chelates give a purification of several tens-fold only after preliminary 100-fold partial purification (by fractionation with ethanol). The possibility of using the method for the purification of crude interferon and the accessibility of the materials make the method of affinity chromatography on DNA-cellulose a valuable one.

The affinity of human interferon for DNA, which has not previously been described in the literature, and the nature of this interaction is still a subject of study; nevertheless,

affinity chromatography on immobilized natural DNA may prove to be effective in the purification of interferon as an independent method or in combination with other methods. We may note that the QAE-Sephadex can be replaced by another anion-exchange material, for example, DEAE-cellulose, but the ion-exchange chromatography should not be cut, since it is at this stage that the main proteins that may also bind to DNA, and also possible contamination with DNase, are eliminated.

EXPERIMENTAL

We used interferon obtained by the induction with the virus of Newcastle disease of a steady-state culture of human leucocytes isolated from peripheral blood. After the inactivation of the viral inductor, the antiserum was precipitated with KSCN at a concentration of 0.5 M, the pH was brought to 3.5, and the precipitate was collected by centrifugation (2000g, 15 min) and was dissolved in 0.1 M potassium phosphate buffer, pH 8.0.

Ion-exchange chromatography on QAE-Sephadex A-50, and also subsequent affinity chromatography on DNA-cellulose, were carried out by the method of Parsons et al. [8], developed for the DNA-bound proteins of serum in 10 mM potassium phosphate buffer with 1 mM EDTA, pH 6.8. The DNA-cellulose was obtained by Litman's method [9]. The column used in the experiment (1.2 × 5 cm) contained 50 mg of calf thymus DNA. Quantitative protein determination was carried out by Lowry's method [10]. The elution profile of the protein was measured on a SF-26 spectrophotometer. The NaCl concentration gradient was created with the aid of an Ultrograd.

All stages of purification were monitored by determining the interferon activity by means of the test system generally adopted (50% protection of a monolayer of primary-trypsinized human embryo fibroblasts from cytopathic action of the vesicular stomatitis virus, IU_{50}) [3], using as standard the interferon produced by the Gamalei Institute of Epidemiology and Microbiology, Series No. 219-1, which exhibited an activity of 160 IU_{50} in 1 ml.

SUMMARY

It has been shown that human leucocytic interferon binds to DNA-cellulose. The optimum concentration of salt for eluting interferon from QAE-Sephadex and from DNA-cellulose have been determined.

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