AN INVESTIGATION OF THE CYANOGEN BROMIDE FRAGMENTS OF THE POLYHEDRAL PROTEIN OF Borrelinavirus bombycis

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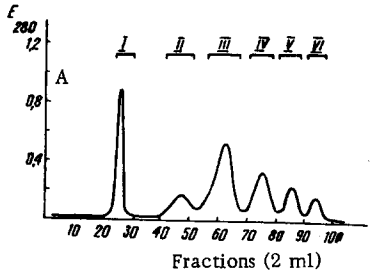
UDC 547,964+537,363

In the present paper we give the results of an analysis of the cyanogen bromide fragments of the polyhedral protein of Borrelinavirus bombycis by electrophoresis in polyacrylamide gel in the presence of sodium dodecyl sulfate (SDS).



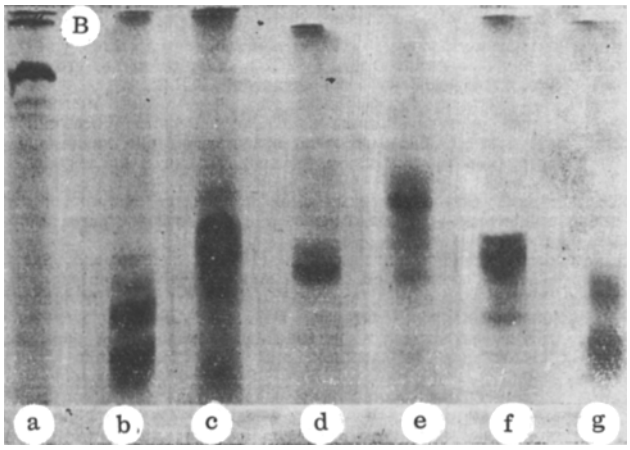


Fig. 1. Fractionation of the product of BrCN cleavage on a column (1.8 × 90 cm) of Sephadex G-75 (A); electrophoresis in polyacrylamide gel with sodium dodecyl sulfate of the carboxymethylated protein (a), of insulin (b), of the BrCN mixture (c), and of fractions I-IV (d, e, f, g) (B).

The protein isolated by dissolving the polyhedra in acetic acid [1] was reduced and carboxymethylated [2]. The protein derivative (S-RCM) was cleaved with cyanogen bromide in the modification of Steers et al. [3]. The mixture of fragments was fractionated on Sephadex G-25* in 50% formic acid (Fig. 1A).

Fractions I-IV (see Fig. 1A), after rechromatography, were analyzed by electrophoresis in polyacrylamide gel in the presence of SDS under the conditions worked out by Swank and Munkres [4] for oligopeptides, using 15% of acrylamide (N,N'-methylenebisacrylamide: acrylamide 1:15). The acrylamide gel contained 0.1% of SDS (Ferak, Berlin), 0.07% of ammonium persulfate (Reanal, Hungary), 0.075% of N,N,N',N'-tetramethylethylenediamine (Reanal, Hungary) 0.1 M H_3PO_4, 8 M urea, and tris buffer, pH 6.8. Samples with a concentration of 1 mg/ml were treated with a solution containing 1% of SDS, 8 M urea, and 1% of β-mercaptoethanol brought to pH 6.8 by the addition of tris buffer. The mixture was heated at 60°C for 15 min and was left at room temperature for 15 h before electrophoresis was performed. A buffer solution consisting of 0.1% of SDS, and 0.1 M H_3PO_4 brought to pH 6.8 by the addition of tris buffer was used. From 10 to 20 μl of the sample was deposited on each column of gel (diameter 5 mm, length 70 mm), the voltage gradient was 8 V/cm, and the current 2 mA/gel, 7 h). After 2 h, the gel was stained with a 0.25% solution of Coomassie Blue in a mix-

*Inconsistency in name of column material present in Russian original – Publisher.

Sector of Molecular Biology and Genetics, Academy of Sciences of the Ukrainian SSR. D. K. Zabolotnyi Institute of Microbiology and Virology, Academy of Sciences of the Ukrainian SSR. Translated from Khimiya Prirodnykh Soedinenii, No. 5, pp. 683-685, September-October, 1972. Original article submitted March 14, 1972.

ture of 90 ml of 50% methanol and 9 ml of glacial acetic acid. The gel was washed with several portions of a mixture of 37.5 ml of acetic acid, 125 ml of methanol, and 337 ml of water. The excess of the dyestuff was eliminated over 48 h. Figure 1B shows the results of the electrophoresis in polyacrylamide gel with SDS of fractions I-IV, the carboxymethylated protein, the mixture of cyanogen bromide fragments, and insulin. Fractions V and VI (see Fig. 1A) were not revealed by this method, since they were apparently eluted from the gel in the staining process. We have found that these fractions contain peptides with molecular weights less than 800 daltons.

Thus, the method considered is an extremely valuable analytical procedure which permits the analysis of cyanogen bromide fragments of the polyhedral protein consisting of comparatively large oligopeptides with a considerable tendency to aggregation and interpeptide interaction.

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