Purification and properties of a protein having nemin activity

It was demonstrated recently[1] that morphogenesis in predacious fungi is induced by a metabolic product of nematodes, which was designated nemin. A biological assay was developed, evaluated, and used to determine the nemin activity of various animal sera and worm extracts[2]. Of these, ascarids collected from the swine intestine at the time of slaughter were the most suitable starting material for chemical fractionation. The present communication describes procedures for the isolation from ascarids of a purified protein having nemin activity.

A 100-g quantity of thoroughly washed ascarids was homogenized in 500 ml of cold 50% aq. ethanol, using a Waring Blendor. The homogenate was centrifuged at 3000 rev./min and the sediment extracted with an additional 300 ml of 50% ethanol. The combined extracts were concentrated *in vacuo* to a final volume of 30 ml. Insoluble material was removed by centrifugation and discarded, since the bioassay showed activity to be localized in the soluble portion of the concentrate. Several attempts to separate the active principle by partition of the crude extract with immiscible solvents were unsuccessful. Moreover, nemin activity did not move from the origin when the crude extract was chromatographed on paper using a variety of solvents.

Since the material of interest was not readily dissolved in organic solvents, the crude extract was diluted to 30% by volume in cold acetone, and the precipitate formed was harvested, washed, and dried. Bioassays showed that nemin activity was concentrated in the acetone-dried powder (Table I) and gel filtration of a water solution of this powder (1.2 g) was performed on a column (2.7 cm diameter) containing Sephadex G-50 (15 g). A flow rate of 40 ml/h was employed and the effluent was collected as separate 2.7-ml fractions. The absorbancy at 280 mμ, intensity of the ninhydrin reaction, and biological activity of each fraction were determined, and the results summarized in Fig. 1.

Nemin activity was concentrated primarily in tubes 19–23 and associated with maximum absorbancy at 280 mμ, which is characteristic of proteins. When the contents of tubes 19–23 were pooled and subjected to electrophoresis on cellulose acetate film using veronal buffer at pH 8.6, 5 components stained with Ponceau S, but bioassays indicated that nemin activity was associated only with the fastest moving acidic fraction. Isolation of this material in quantity was accomplished by preparative zone electrophoresis, using a trough 60 × 10 × 0.65 cm. Sephadex G-75

TABLE I

PURIFICATION OF A PROTEIN HAVING NEMIN ACTIVITY

Preparation		*Yield** (g)	*Bioassay*
Crude extract		4.11	+++
Acetone-dried powder		2.27	+++
Acetone-soluble material		1.84	±
Sephadex fractions 19–23		0.61	+++
Electrophoretic component**	I	0.122	+++
	II + III		—
	IV		—
	V		—

* From 100 g swine ascarids.

** Using Sephadex as supporting medium, numbered in order from the anode.

was employed as the supporting medium and the buffer was 0.1 *M* triethylamine-carbonate at pH 8.3. Bands were located by placing cellulose acetate film in contact with the gel surface and subsequent drying and staining with Ponceau S. The area of the gel that contained the active fraction was collected with a spatula, eluted with water, and the eluate was lyophilized. The yield was approximately 122 mg of biologically active material from 100 g of ascarids (Table I).

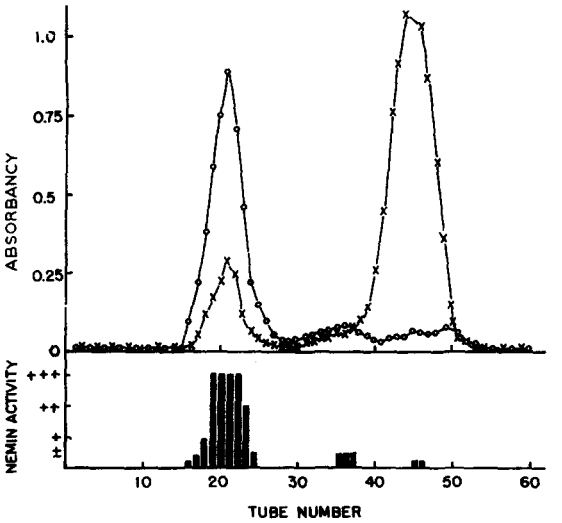

Fig. 1. Gel filtration on Sephadex G-50 of acetone-dried powder from swine ascarids. O—O, absorbancy at 280 mμ; ×—×, absorbancy at 570 mμ (ninhydrin reaction).

The preparation showed maximum absorption in the ultraviolet region at 278 mμ ($E_{1\%} = 10.2$ at pH 7.3) and the ratio $A_{278\,m\mu}/A_{260\,m\mu}$ was 1.45. It appeared homogeneous when examined by moving-boundary (NaCl–phosphate buffer at pH 7.3, $I = 0.1$), cellulose acetate film (pH 4.0, 5.4, 7.3, 8.6), disc[3], and polyacrylamide gel[4] electrophoresis. The sedimentation coefficient ($s_{20,w}$) for a 1.0% solution in 0.1 *M* phosphate buffer (pH 7.3) was 1.92. Although the preparation sedimented as a single boundary, the ultracentrifugation pattern was polydisperse. Experiments are in progress to further purify, crystallize, and elucidate the mechanism of action of the morphogenetic material.

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Department of Agricultural Microbiology, SHIMPEI KUYAMA
Rutgers–The State University, New Brunswick, N.J. (U.S.A.) DAVID PRAMER

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