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NOTES

Purification and Crystallization of Fimbrial Hemagglutinin from *Bordetella pertussis*

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A procedure to purify and crystallize fimbrial hemagglutinin from *Bordetella pertussis* is described. Redissolved crystals had the same specific activity as the original, purified solution of fimbrial hemagglutinin. About 97% of the weight of washed crystals was accounted for by amino acids.

Keogh et al. (3) discovered that cultures of *Bordetella pertussis* produced a substance capable of agglutinating erythrocytes from various animal species. This observation was soon confirmed by others (2, 4, 5, 8, 9). The substance was demonstrated in cells during early phases of growth and was released into the culture fluid as the incubation period was prolonged (5). The role of this hemagglutinin (HA) on immunity to whooping cough has not been conclusively defined. Although some have thought it played an important role (2, 4), other workers have not found HA to protect mice from intracerebral infection with *B. pertussis* (5, 6, 8). Since the mouse protection test is accepted as an indicator of effectiveness of pertussis vaccines, it was assumed that HA did not play a role in the prophylaxis of whooping cough. Recently, this problem has been reopened by the observations of Arai and Sato (1), who found that *B. pertussis* produced two hemagglutinins. One was associated with the fimbriae, had a filamentous structure (2 by 40 nm), was nontoxic, and, in their hands, was mouse protective. The other hemagglutinin was associated with spherical structures (6 nm in diameter), was toxic, produced leukocytosis, induced histamine hypersensitivity, and was referred to as leukocytosis-promoting factor-HA. This substance is similar to what one of us has called pertussigen (J. J. Munoz, Fed. Proc. **35**:813, 1976). Arai and Sato (1) found that leukocytosis-promoting factor HA did not protect mice at the doses they tested. The role of these two hemagglutinins on protection against experimental intracerebral infection with *B. pertussis* will be a topic of another communication. In this note we describe the purification and crystallization of the fimbrial HA.

The smooth agglutinogen type 1,3 strain $3779B1_2S_4$, originally obtained from Eli Lilly & Co., was used in this work. This strain corresponds to culture no. 114 of the Bureau of Biologics. It was grown in stationary cultures in the medium used by Sato et al. (7). Two-liter Erlenmeyer flasks containing 500 ml of medium were inoculated heavily with a 24-h-old Bordet-Gengou agar culture and incubated for 5 days at 35°C. At the end of this incubation period the cultures were treated with 0.01% thimerosal and centrifuged in the cold, and the supernatant was collected. The remaining steps of purification were carried out at 2 to 5°C. The pH of the supernatant (6 liters) was adjusted to 6.5 with glacial acetic acid and then passed through a CM-Sepharose CL-6B column (4.7 by 8 cm) equilibrated in 20 mM phosphate buffer, pH 6.5. The fimbrial HA was absorbed onto the CM-Sepharose. After washing the column with 1 liter of 20 mM phosphate buffer, HA was eluted with 0.5 M NaCl in 20 mM phosphate buffer, pH 6.5, containing 5% ethanol. The active fractions were pooled and concentrated 22-fold by vacuum dialysis. The concentrated pool was passed through a Sepharose 6B column (1.5 by 88 cm) equilibrated in 50 mM tris(hydroxymethyl)-aminomethane-hydrochloride buffer containing 1 M NaCl and 5% ethanol. HA, as measured by hemagglutination of chicken erythrocytes, was obtained in the second protein peak. The active fractions were pooled and concentrated 20-fold at 2 to 5°C by vacuum dialysis. This produced a crystalline precipitate of HA. These crystals had a typical refractive appearance when struck by direct light. Some crystals were a few millimeters in length but broke into small fragments when shaken or when compressed under a glass

cover slip. The appearance of these crystals under the light microscope at 143 times magnification is shown in Fig. 1.

The crystals could be washed in distilled water and then dissolved in 50 mM tris(hydroxymethyl)aminomethane-hydrochloride buffer containing 1 M NaCl, pH 8. The specific activity (1.4×10^5 HA units/mg of protein) of the washed and redissolved crystals was as high as that of the purified solution of HA from which the crystals were obtained (1.2×10^5 HA units/mg of protein). In Table 1 the average recovery and specific activity of the various active fractions from two different experiments are given.

HA was a protein; at least 97% of its weight was accounted for by amino acids as determined in an amino acid analyzer. By electron microscopy, the washed and redissolved crystals were

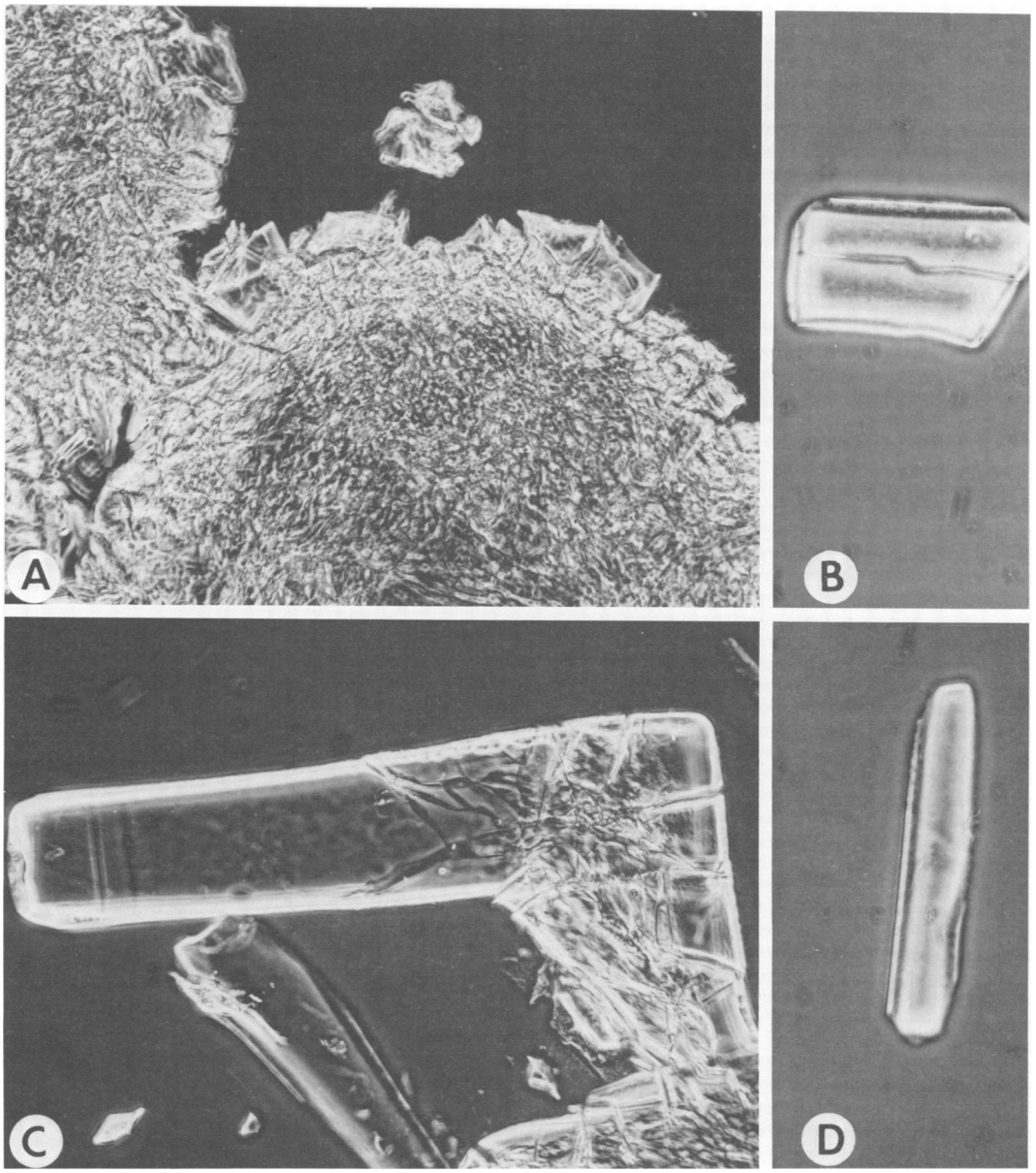

FIG. 1. *Different crystals from fimbrial HA. (A) and (C) Two different clusters of crystals. (B) and (D) Isolated single crystals.*

TABLE 1. *Specific activity and recovery of fimbrial HA from supernatant of B. pertussis cultures*

Sample	Protein (mg)[a]	Sp act (U/mg of protein)	% Recovery of HA activity
Culture supernatant	466.0[b]	1.9×10^4	100
CM-Sepharose CL-6B	31.7	6×10^4	21
CM-Sepharose CL-6B concentrated	25.2	7.2×10^4	20
Sepharose 6B	10.2	1×10^5	12
Sepharose 6B concentrated	7.7	1.2×10^5	10

[a] Protein was determined by Folin Ciocalteau reagent employing bovine serum albumin as a standard.

[b] The total protein in culture supernatant was determined on a dialyzed sample.

shown to be made up of filamentous structures similar to those previously reported for HA (1). This is, to our knowledge, the first time that an HA from bacterial origin has been crystallized. The method employed was simple and consisted of using only two types of column chromatography. The biological properties of this material are now under further study.

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