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Purification of the Protein Crystal from *Bacillus thuringiensis* by Zonal Gradient Centrifugation

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A method is described for the large-scale purification of the *Bacillus thuringiensis* protein crystal by zonal gradient centrifugation. NaBr gradients are employed in a Beckman J21-B centrifuge equipped with a JCF-Z rotor.

*Bacillus thuringiensis* is an economically important microbial insecticide which differs from most other spore formers by synthesizing a discrete parasporal protein crystal in addition to the endospore. This protein crystal appears to be the insecticidal agent because crystal preparations which do not contain any viable cells are still highly toxic when ingested by lepidopteran insects (2). However, efficient separation of spores from parasporal crystals is a technical prerequisite of studying the chemistry of the crystal itself. The earliest attempts to obtain purified crystals relied on spontaneous germination and autolysis of the spores, followed by repeated differential centrifugation of the crystals (4, 8). These techniques were followed by several diphasic systems in which organic solvent emulsions were formed; the crystals remained in the aqueous phase. Among the solvents employed were trifluorotrichloroethane (3), tetrabromoethane (8), carbon tetrachloride (10), and chloroform (9). Unfortunately, the extractions had to be repeated several times to achieve acceptable purity, yields were low, and the danger of crystal modification by the organic solvents was ever present. This last objection was overcome by the use of diphasic systems containing dextran sulfate 500 and polyethylene glycol 6000; the spores preferentially entered the phase richer in polyethylene glycol (5, 7).

Fast (6) later described a method of crystal purification employing isopycnic density gradient centrifugation in CsCl, whereas Sharpe et al. (11) recommended the use of Renografin gradients. Both of these methods are convenient and give crystal preparations of high purity. However, the biochemical characterization of the *B. thuringiensis* crystal requires larger quantities of purified crystal than are conveniently available from density gradient centrifugation in a swinging bucket rotor.

One solution to this requirement for greater capacity is zonal gradient centrifugation (1). Zonal centrifugation employs a hollow bowl instead of tubes. Thus, the entire 360° rotor radius is available for the density gradient, and the consequent larger gradient volume allows a larger sample capacity. We report here an isopycnic zonal centrifugation method for the large-scale purification of the insecticidal *B. thuringiensis* protein crystals employing NaBr gradients in a Beckman J-21B centrifuge equipped with a JCF-Z 40-1900 titanium rotor.

Figure 1 shows the results of a typical separation. Satisfactory resolution of the spores and crystals has been achieved with both *B. thuringiensis* var. *thuringiensis* (NRRL B-4039) and *B. thuringiensis* var. HD-1. A sample size of 100 ml was employed routinely, containing 3.0 to 7.0 g (dry weight) of spores and crystals. Purified crystal free of NaBr could subsequently be prepared either by dialysis or by dilution followed by centrifugation. The final crystal yields ranged between 15 and 20%. No systematic attempts were made to modify gradient shape or to maximize sample loading capacity.

The buoyant densities we have observed for the crystals and spores in NaBr (1.32 and 1.38 g/cm³, respectively) are, of course, different from those previously reported in CsCl (6) and Renografin (11–13). These differences are to be expected because buoyant densities are not strictly comparable between gradients of different types due to changes in the extent of hydration and possible specific ion binding. Significantly, the difference between our spore and crystal buoyant densities (0.06 g/cm³) in NaBr is of approximately the same magnitude as those reported by other workers (6, 11–13). The two lighter debris peaks (Fig. 1) are similar to those observed previously in tube gradients (6, 11).

Selection of an appropriate gradient type is especially important because of the large gradient volumes employed in zonal centrifugation. The rotor volume is 1,900 ml, and an additional 1,900 ml of heavy-density material (1.42 g/cm³)
of NaBr) must be pumped in through the edge in order to unload the rotor from the center. Both Renografin and CsCl are too expensive to be used in such large amounts. Thus, the ideal gradient must be of sufficient density (up to 1.42 g/cm³), cheap, and of low viscosity because low viscosity allows the use of less expensive gravity-flow gradient makers. NaBr was considered the best candidate. It can be purchased in bulk (350 lb. [ca. 159-kg] drums) from Great Lakes Chemical Corp., West Lafayette, Ind.

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LITERATURE CITED


