

Heterogeneity Between Two Mouse-Toxic Protein Polymers from *Pasteurella pestis* Indicated by Electrophoresis Patterns in a Phenol-Acetic Acid-Urea Gel System

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Highly purified plague murine toxins A or B dissociate to form single uncommon polypeptide bands and another polypeptide band common to both toxins.

Experiments have been reported demonstrating that the murine toxin A (240,000 molecular weight) or toxin B (120,000 molecular weight) dissociates into the same size, biologically active subunits proposed to be 12,000 molecular weight, based on one cysteine/polypeptide chain (3). Recent determinations with Sephadex indicate subunits of approximately 24,000 molecular weight are formed after treatment of either toxin with 1% SDS (sodium dodecyl sulfate) and assayed in columns not containing SDS (submitted for publication). Dissociation in organic acids yielded similar size subunits from both toxins (Montie et al., *Bacteriol. Proc.*, p. 96, 1968). These subunits were not toxic. The strongest evidence to date for possible heterogeneity between the two polymeric forms was indicated by the 30% lower tryptophan content in toxin B, compared to toxin A (2). Because toxin proteins dissociate in organic acids to rather unstable forms, we proposed to compare the toxin subunits on the basis of charge difference by dissociation in an acid-organic solvent electrophoretic system.

Rottem and Razin (4) employed a phenol-acetic acid-water solvent to separate and dissociate membrane components in an acetic acid-urea gel system. Initial attempts to examine the toxin components by acid gels or by acetic acid-urea gels without phenol were unsuccessful. The phenol addition to the sample along with acetic acid and urea in the gel gave an effective separation system.

Initial patterns using toxin A or B purified by Sephadex G-100 columns usually showed 10 to 15% impurities. These impurities gave somewhat confusing patterns, with one or two extra bands appearing in the stained acrylamide gels.

It appeared that the phenol-acetic acid-urea system was very sensitive for detecting impurities in the toxin samples. Partially purified toxin, consequently, was subjected to preparative gel electrophoresis on a Buchler apparatus. Representative samples from each protein peak were assayed on pH 9.0 gels (Fig. 1). This preparative method clearly resolved each protein comparable to separation on the small analytical gels. The stepwise protein elution pattern (Fig. 1) showed single banded toxins of close to 100% purity. Injection into 20-g mice indicated retention of good toxin activity ($LD_{50} = 1$ to $2 \mu\text{g}$).

The lyophilized samples from each toxin were dissolved in phenol-acetic acid-water (2:1:0.5, v/v/v) and subjected to electrophoresis for 2 hr at room temperature through 7.5% (w/v) acrylamide gels containing 35% (v/v) acetic acid and 5 M urea. The patterns obtained in acid are compared in Fig. 2. Toxin A gave two bands: the least electropositive band is designated no. 1 and the more electropositive band no. 2. Toxin B exhibits two bands also, a band corresponding to the no. 2 band of toxin A and an additional no. 3 band of greater electrophoretic mobility than no. 2 band. The no. 2 band (common to both toxins) always appears as the heaviest band of the three. Measurements by densitometry of the areas under the peak tracings indicate that toxin B is composed of 60% no. 2 band and 40% no. 3 band. Toxin A is composed of 55% no. 2 band and 45% no. 1 band.

We have tentatively concluded that toxins A and B contain a different polypeptide component (band no. 1, band no. 3). A third polypeptide is common to both toxins (band no. 2).

Takayama (6) utilized the phenol-acetic acid-water system to dissociate membrane proteins to

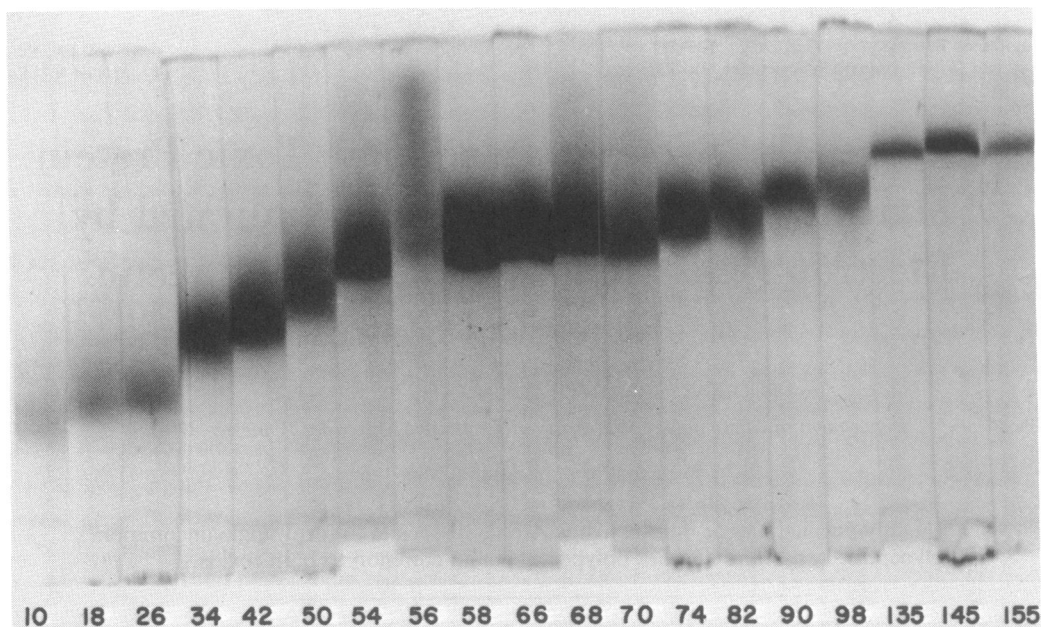


FIG. 1. Separation and isolation of toxins A and B by preparative acrylamide gel electrophoresis. Electrophoresis was performed generally as described by the Buchler Poly-Prep Instruction manual. An 80-mg amount of a crude toxin (precipitate fraction obtained between 35 and 70% $(\text{NH}_4)_2\text{SO}_4$ saturation, LD_{50} 4.0 μg of protein) was added to 3.5 ml of tris(hydroxymethyl)aminomethane-glycine upper buffer and 0.5 ml of 40% sucrose. This sample was clarified by centrifugation at 10,000 rev/min for 5 min. Run conditions were: gel length 6 cm, 6 C, run time 36 hr (pH 8.9), elution rate 20 ml/hr. Representative fractions (0.02 ml of a 2.0-ml fraction) from protein peaks are shown assayed by analytical acrylamide gel electrophoresis at pH 9.0 (proteins negatively charged). Toxin B fractions, 58 to 70; toxin A fractions, 135 to 155. Fractions 10 to 56 and 74 to 98 show nontoxin protein bands, and in subsequent acid electrophoresis assay they exhibited different bands (polypeptides) from the toxin fractions.

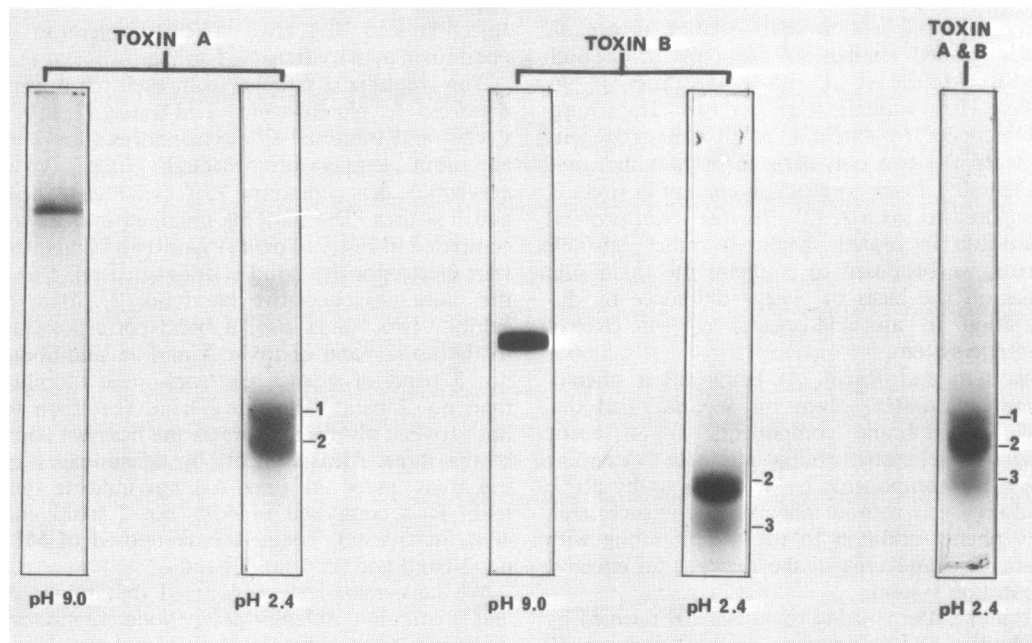


FIG. 2. Pure toxins A and B dissociated by electrophoresis in phenol and acetic acid-urea acrylamide gels. Representative samples (pH 9.0 gels) from preparative gel separation were electrophoresed in phenol-acetic acid-urea at pH 2.4, toward the negative pole. Bands were stained with amido black. Three different polypeptide bands are identified. Number 2 band is common to both toxins as confirmed by co-electrophoresis of toxins A + B.

their monomeric forms. Bagdasarian et al. (1) and Stanley et al. (5) presented evidence that indicates phenol-acetic acid-water solvent does not break peptide bonds. We suspect, therefore, that the subunits are heterogenous as judged by electrophoretic criteria, although they are homogenous in size. Possibly, this heterogeneity is also reflected in the difference in tryptophan content between the two toxins. This hypothesis is being investigated.

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