JOURNAL OF BACTERIOLOGY, Mar. 1972, p. 1322-1324
Vol. 109, No. 3

Characterization of Colicin Ia and Colicin Ib: Antigenic Homology

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Received for publication 26 October 1971

Immunological techniques have been employed to determine antigenic homology between colicin Ia and colicin Ib. Results of these experiments demonstrate that the two colicins are antigenically similar.

Physical homologies between colicin Ia and colicin Ib have been demonstrated (4). Both colicins are of similar molecular weight (~80,000) and have an axial ratio of approximately 15:1. Chemical homology between these colicins is suggested by their similar amino acid composition (J. Konisky et al., Bacteriol. Proc., p. 62, 1970). The biological modes of action of colicin Ia and Ib appear to be identical in that both colicins cause cessation of all macromolecular synthesis and inhibition of adenosine triphosphate production (5). Furthermore, both colicins appeared to adsorb to identical receptors on the *Escherichia coli* cell surface. However, the two colicins exhibit different immunity specificity in that cells which produce one of the two colicins are immune to the action of the colicin produced but sensitive to the heterologous colicin (e.g., cells producing Ia are immune to Ia, but sensitive to Ib; reference 7). Thus, some structural diversity between the two molecules is indeed necessary.

In this communication, antigenic homology between colicin Ia and Ib is demonstrated. Antiserum was prepared against purified colicin Ib-P9 (4) by immunization of an adult albino virgin female rabbit. The purified colicin emulsified in Freund's complete adjuvant (0.147 mg of colicin/ml) was used for the primary immunization and was injected as follows: 0.1 ml divided among four foot pads and 0.6 ml subcutaneously. Four weeks later, a secondary booster of 0.257 mg of colicin in Freund's complete adjuvant was injected subcutaneously. One week after the secondary immunization, the rabbit was bled from the lateral ear vein. Collected blood was allowed to clot in the cold for 24 hr, and the supernatant fluid was decanted. The crude serum was partially purified by 14% sodium sulfate precipitation (3) and applied to a diethylaminoethyl cellulose column equilibrated with 0.02 M potassium phosphate, *p*H 7.0 (6). The column was developed with the same buffer, and the eluate (1.63 mg of protein/ml) was collected and stored at −20 C.

Purified colicins E2, Ia, and Ib (197 μg of protein/ml in 0.05 M potassium phosphate buffer, *p*H 7.0) were placed in separate peripheral wells of an Ouchterlony plate (1) containing partially purified gamma globulin in the center well. The fourth well contained phosphate buffer (PB). As seen in Fig. 1, two distinct lines of identity are observed with colicin Ia and colicin Ib. Spurring or partial identity is not observed. The lack of a precipitin line with E2 or no inhibition by E2 of the anti-Ib-Ib reaction indicates the absence of antigenic homology between colicin E2 and colicin Ib.

Quantitative neutralization of colicin activity was measured to confirm cross-reaction between the two colicins. To 0.1-ml portions of diluted colicin (4.4 μg/ml) was added 10 μliter of buffer solution containing various amounts of gamma globulin. After thorough mixing and incubation at 37 C for 10 min, the reaction mixtures were serially diluted twofold in tris-(hydroxymethyl)aminomethane salts (5), spotted onto a freshly seeded lawn of sensitive strain *E. coli* K-12 W 3110 Str-r, and incubated for 4 hr at 37 C. The units of colicin activity were defined as the reciprocal of the highest dilution yielding a complete zone of inhibited cell growth. This assay is semiquantitative, yielding deviations of 20 to 30%. It is clear (Fig. 2) that the Ib antiserum is capable of inactivating both I colicins to almost the same extent. As a control, it is seen that colicin E2 activity is unaffected by the antiserum.

Herschman and Helinski (2) studied structural homology between colicin E2 and colicin E3 by similar immunological techniques. In double diffusion tests, they found common

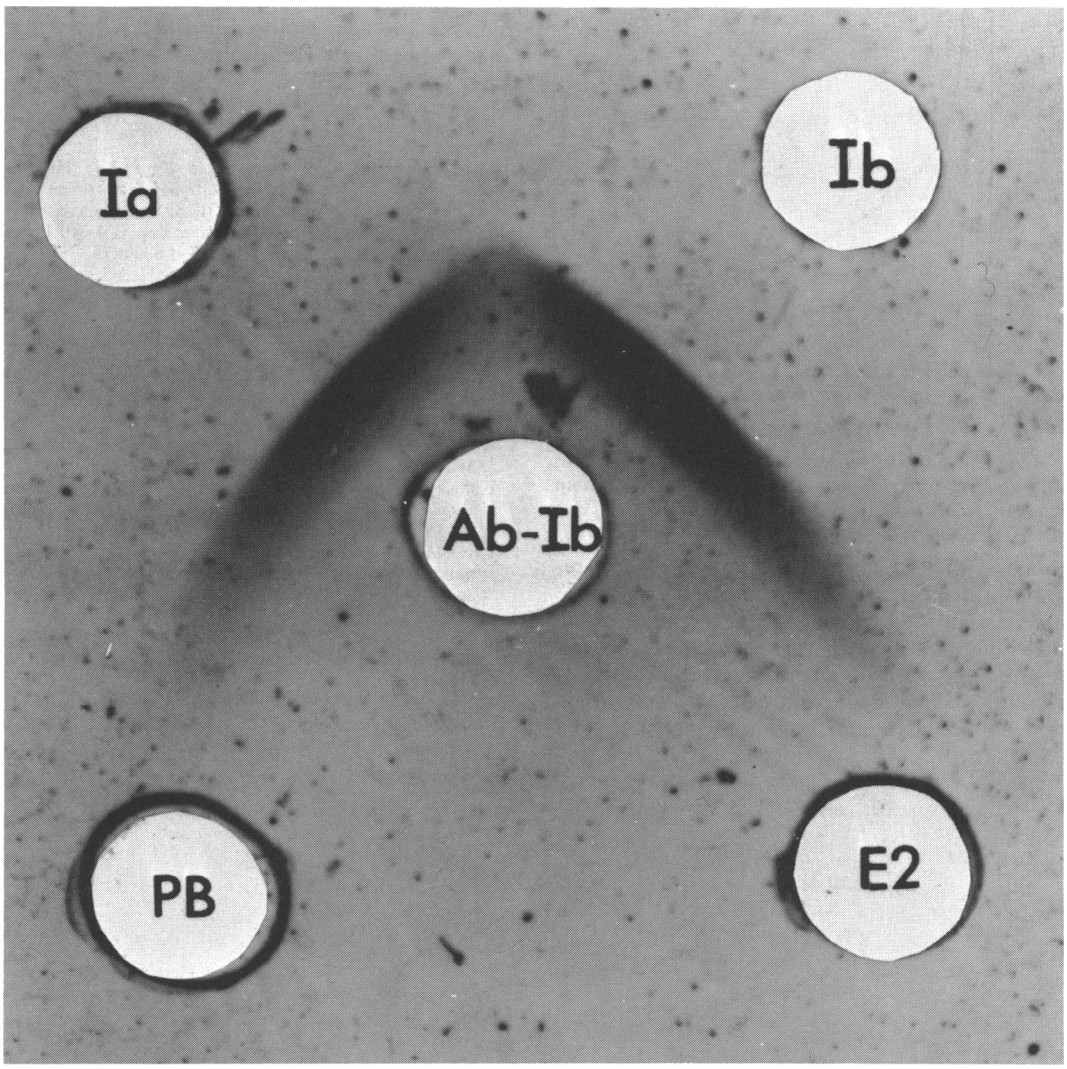

FIG. 1. *Immunodiffusion of colicins Ia, Ib, and E2. Diffusion proceeded for 24 hr at room temperature in a humidity chamber. Reactions were terminated by soaking for 24 hr in 1% NaCl and then staining with 1% Buffalo Black in 7.5% acetic acid.*

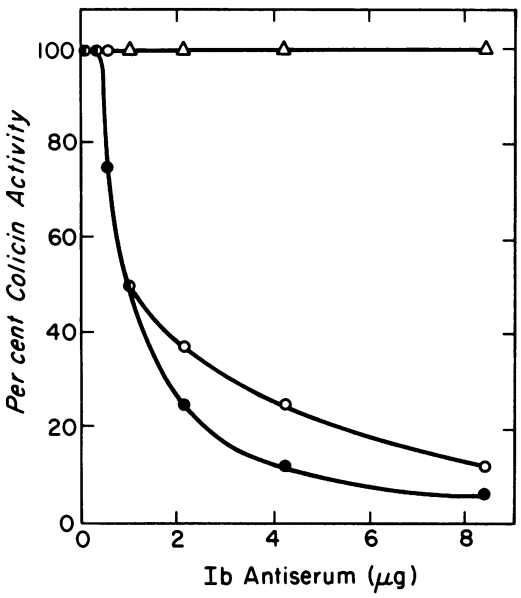

FIG. 2. *Quantitative neutralization of colicin Ia and colicin Ib by Ib antiserum. Symbols:* (○) *colicin Ia,* (●) *colicin Ib,* (△) *colicin E2.*

lines of identity as well as spurring. Their results indicated that colicin E2 and colicin E3 are partially homologous by antigenic criteria. The double diffusion data shown in Fig. 1 show no spurring with a smooth connection of identity lines. This would indicate that colicins Ia and Ib are structurally more closely related than are colicins E2 and E3. This close relationship is also demonstrated in the experiment described in Fig. 2. Results obtained here confirm our previous conclusion, obtained from both chemical and physical studies (4; J. Konisky et al., Bacteriol. Proc., p. 62, 1970), that the biological similarities of colicins Ia and Ib are reflected in common structural features.

Colicin E2 was a gift from Lowell P. Hager. We thank E. W. Voss, Jr., for suggestions.

This investigation was supported by Public Health Service grant AI-10106 from the National Institute of Allergy and Infectious Diseases. Richard E. Isaacson was supported by Public Health Service traineeship GM-510 from the National Institute of General Medical Sciences.

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