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SHORT COMMUNICATION

AN IMPROVED METHOD FOR PURIFICATION OF MEGACIN A INHIBITOR

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In the previous report (Ochi et al., 1970) on megacin A inhibitor produced by producing strains megacin A, a method for partial purification of the inhibitor was described. However the yield was poor and the only information obtained on the chemical nature of the inhibitor was that it was inactivated by heating 70 C for 5 min.

This report describes an improved method for purification of the inhibitor and evidence is given suggesting that the inhibitor is a protein.

The bacterial strains, medium and assay method used were as described in the previous report (Ochi et al., 1970).

Strain 265, a mutant of *Bacillus megaterium* strain 216 which is less sensitive to megacin A than the parent, was grown in P-Y meduin. The culture supanatant was obtained in an early part of the stationary phase when megacin activity was undetectable using excess inhibitor. The NaCl concentration was adjusted to 1.0 M, and the fluid was concentrated about 50-fold by ultrafiltration using a Visking cellophane tube. Concentration without addition of NaCl resulted in considerable loss of the inhibitor. After concentration, the fluid was adjusted to pH 4.0 by adding 1 N-HCl, kept at 4 C for 30 min and then centrifuged. By this treatment, most of the megacin A was precipitated. The supernatant was adjusted to 7.5 by adding 1 N NaOH and centrifuged. At this step, the apparent recovery of activity in the supernatant was almost complete, although the amount of inhibitor which had previously been inactive because it was combined with co-existing megacin A could not be estimated.

The supernatant contained RNA which was previously shown to have no role in the activity of megacin inhibitor (Ochi et al., 1970). Accordingly it was digested by bovine pancreatic RNase (50 μg per ml in final concentration) at 37 C for 60 min. The digest was diluted with 0.01 M Tris buffer (pH 7.5) to reduce the NaCl concentration to 0.4 M and applied to a DEAE cellulose column (3$\times$ 50 cm) equilibrated with 0.4 M NaCl in 0.01 M Tris buffer (pH 7.5). The column was washed with the same solution until no megacin activity could be detected in the eluate. Then the column was eluted with 2 liter of a linear concentration gradient (0.4–0.9 M) of NaCl containing 0.01 M Tris buffer, pH 7.5 at a flow rate of 100 ml per hr and 10 ml fractions were collected.

As ahown in Fig. 1, megacin inhibitor was eluted after the peak of RNA. The fractions containing more than 40 units of activity were pooled and concentrated about 100-fold by ultrafiltration and the NaCl concentration was adjusted to 1.0 M. The recovery of activity

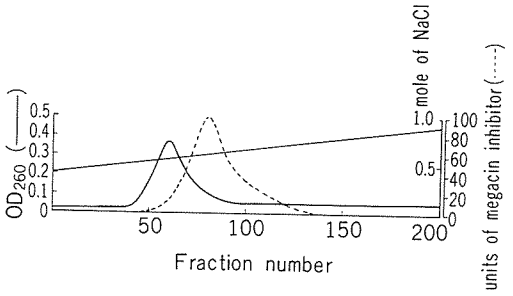

FIGURE 1. *DEAE cellulose chromatography of megacin inhibitor.*

was about 50%.

Ultracentrifugal studies were made in linear gradients of sucrose (25 ml, 5–20%) containing 1.0 M NaCl and 0.01 M Tris buffer (pH 7.5). Samples of 1 ml containing 3000 units and 5% sucrose in the buffered saline were applied and centrifuged at 24000 rev/min for 40 hr in a Spinco, model L, ultracentrifuge with a model SW 25 rotor. Fractions of approximately 1 ml were collected and their megacin inhibitor activity and OD_{235} and OD_{750} values in the Folin reaction were measured (Lowry et al., 1951).

As shown in Fig. 2, there were two peaks in the curve of OD_{235}. The larger peak was found to contain RNA. The smaller peak did not contain RNA and was superimposed on the single peak obtained in the Folin reaction and on the peak of megacin inhibitor activity. These results and the heat lability, described previously (Ochi et al., 1970), indicate that the megacin A inhibitor is a protein.

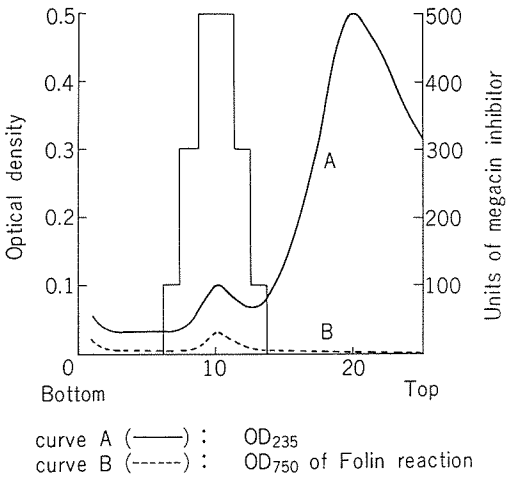

FIGURE 2. *Sucrose density gradient ultracetrifugation of megacin inhibitor.*

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