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Properties of the Gentamicin Acetyltransferase Enzyme and Application to the Assay of Aminoglycoside Antibiotics

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The acetyltransferase technique of serum gentamicin assay was demonstrated to be specific for aminoglycoside antibiotics. The enzyme involved was isolated and stabilized by lyophilization.

There have been several publications describing the adenylyl transferase technique for the measurement of serum gentamicin concentrations (3, 4, 6-8), but relatively little attention has been drawn to the alternative acetyltransferase system (2), although it has recently been demonstrated that this technique is preferable in terms of speed and accuracy for a comparable cost of materials (1). We now wish to extend these observations to the general application of the specific assay of deoxystreptamine aminoglycoside antibiotics.

The acetyltransferase enzyme was prepared by sonication of washed *Escherichia coli* W677/R5 cells, as previously described (1). The gentamicin-Sepharose 4B column was prepared from cyanogen bromide-activated Sepharose 4B (Pharmacia Ltd.) and gentamicin sulfate powder (10:1, wt/wt), as described in the literature from Pharmacia Ltd. The ^{14}C-labeled acetyl coenzyme A was at a radioactivity concentration of 30 μCi/μmol. Each assay vial contained 1.2 nmol of acetyl coenzyme A, and not 2.75 nmol as used before (1, 2); otherwise the assays were performed as described previously (1). The assay procedure was unaffected by the presence of the following antibiotics in the serum (concentration tested in micrograms per milliliter): streptomycin (37.5), penicillin (7.5), ampicillin (7.5), cloxacillin (10.0), clindamycin (22.5), erythromycin (7.5), lincomycin (22.5), rifampin (7.5), fucidin (5.0), novobiocin (75.0), trimethoprim (6.0), nalidixic acid (10.0), cephacetrile (3.75), cephalothin (3.75), and cephazolin (15.0). Figure 1 illustrates the general applicability for assaying a variety of other deoxystreptamine antibiotics.

The only nonspecific interference found for the assay was the spuriously high results obtained for gentamicin serum samples from patients who were also on intravenous lipid solution. It is probable that the excess lipid present in the serum coated the phosphocellulose P-81 paper and thus prevented adequate washing of the unreacted ^{14}C-labeled acetyl coenzyme A from the paper, resulting in an artificially high value.

Figure 2 illustrates the preparation of purified enzyme by affinity chromatography. After this preparation the enzyme was lyophilized as described in the legend to Fig. 2.

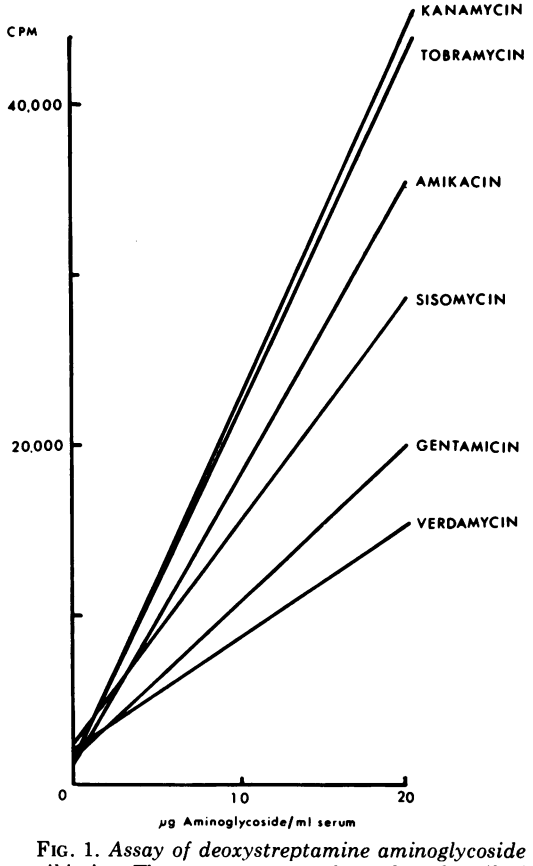

FIG. 1. *Assay of deoxystreptamine aminoglycoside antibiotics. The assays were performed as described in the text, using a crude E. coli extract.*

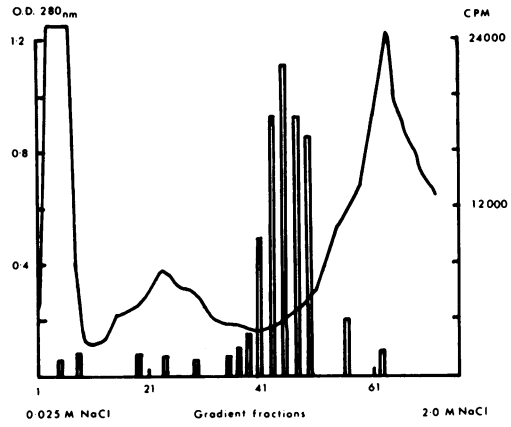

FIG. 2. *Elution profile from a gentamicin-Sepharose 4B column. The crude E. coli extract was applied to the gentamicin-Sepharose 4B column equilibrated with 0.025 M NaCl. A nonlinear gradient of 0.025 to 2.0 M NaCl was applied with the aid of a nine-compartment gradient former. After elution the most enzymatically active fractions were pooled and dialyzed against 4 liters of distilled water (two charges) for 12 h at 4 C. The enzyme dialysate was then divided into 1.0-ml aliquots and freeze-dried, followed by storage at −20 C.*

For reconstitution of the freeze-dried enzyme, 0.25 μl of 10 mM tris (hydroxymethyl)aminomethane-hydrochloride, pH 7.5, was added to each vial of lyophilized enzyme. The results obtained with this solution were identical with those obtained by using a crude enzyme sonic fluid. The lyophilized enzyme preparation is stable for at least 48 to 72 h at room temperature. At −20 C it is stable for several months or longer.

The results presented here are further indication of the viability of this assay for the general assay of commonly used aminoglycoside antibiotics. The specificity of the assay is especially useful in the light of a recent report that nearly 20% of clinical serum samples sent to one laboratory for gentamicin assay had other undisclosed antibiotics present (5). These may seriously affect conventional microbiological assay tecnhiques. By a reduction of 60% in the quantity of ^{14}C-labeled acetyl coenzyme A present in the assay, it is possible to considerably lower the cost of this technique. The scintillation fluid (1) may be reused three to four times by removing the P-81 papers after counting, since little radioactivity is lost from the papers. To assist other laboratories in trying this method, we are prepared to supply, on a limited basis for experimental use, the freeze-dried acetyltransferase enzyme.

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