ADSORPTION OF RIBONUCLEASE ON GLASS

Raymond Shapira

Department of Biochemistry
Emory University
Atlanta 22, Georgia

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When 10 microliter amounts of ribonuclease solution (1 mg in 10 ml distilled water) were assayed in duplicate, the second aliquot always indicated a larger amount of enzyme. Subsequent assays using the same micropipette showed similar increases until about six aliquots had been tested. From then on, the assay remained constant at a level about double the initial assay.

The pipette at this point was washed exhaustively with distilled water. Repeating the test with this pipette gave a maximum initial and duplicate assay. If the pipette was washed with acid, base, or salt solutions followed by rinsing with distilled water, the phenomenon of increasing activities was again observed.

When the pipette, which had been cleaned with concentrated sulfuric acid, was first equilibrated with enzyme solution for several minutes, the assays were always maximal and similar. This pipette was washed with distilled water and an aliquot of pH 5, 4 M sodium acetate buffer was assayed. All of the enzyme activity lost in the initial assays was recovered from the pipette.

Apparently a 10 microliter pipette containing 1 microgram of ribonuclease in water removed about one half of the enzyme from solution. This glass-bound enzyme could not be removed with distilled water. When acid washed 'pyrex' cuvettes were used in the assay, no hydrolysis of RNA was observed in the absence of added enzyme. If the cuvettes were allowed to stand with enzyme solution (1 mg in 10 ml distilled water) for several minutes, and then rinsed thoroughly with distilled water, subsequent determin-

ations of ribonuclease activity in these cuvettes without added enzyme corresponded to 0.5 micrograms of enzyme in the first assay, 0.2 micrograms in the second assay, and 0.1 micrograms in the third assay.

In order to obtain more quantitative data, adsorption of ribonuclease on a 15 ml 'pyrex' fine porosity fritted disk Buchner funnel and on finely powdered 'pyrex' glass was investigated. The Buchner funnel was found to have a capacity of 0.16 mg of enzyme. After washing with water and increasing molarities of pH 5.0 sodium acetate buffer, the glass-bound enzyme was found to be unaffected by distilled water, but was slowly released by dilute 0.01 M - 0.05 M buffer. Higher molarities of 0.5 M - 5.0 M caused rapid and complete removal of the activity from the funnel.

In 'pyrex' test tubes, 5 ml of ribonuclease solution containing 0.5 mg of enzyme was thoroughly mixed with 0 - 400 mg quantities of finely powdered 'pyrex' glass which had been passed through a 0.044 mm sieve. After shaking for several minutes, the mixtures were centrifuged and the supernatant analyzed for enzymatic activity. One-half of the enzyme (0.25 mg) was removed by 90 mg of glass. When the glass-bound enzyme was washed with distilled water, no activity was recovered from the glass. Washing with 0.5 - 5.0 M acetate buffer or KCl released all of the enzyme from the glass.

Some of the analytical and kinetic problems which have been encountered with ribonuclease may be resolved when the adsorption of this enzyme on glass and similar materials is investigated.

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