The Existence of Hyaluronidase Substrate Factors in Microbiological Culture Media

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In the course of studies dealing with the production of hyaluronidase by bacteria, it was noted that the repeated subculture of hyaluronidase producing strains of group A *Streptococcus hemolyticus* in infusion broth (Baltimore Biological Laboratory) resulted in good enzyme titers which were maintained through successive transfers. Furthermore, a marked enhancement of hyaluronidase activity was evident when the recommended concentration of the dehydrated culture medium was doubled. In view of the reported increase of streptococcal hyaluronidase titers by the inclusion in culture media of hyaluronic acid (*1–3*), the possibility existed that mucopolysaccharide factors were retained in small quantities in the final dehydrated product, and that by increasing the concentration of the broth sufficient polysaccharide levels might be obtained which could be utilized as a substrate by the streptococci. It was of interest, therefore, to determine whether mucopolysaccharides could be isolated from microbiological culture media and, if present, to study their depolymerization by hyaluronidase. It must remain for further experiments to determine the extent and nature of the increase in hyaluronidase titers as correlated with bacterial growth or a specific enhancement factor.

Fifty-gram samples of infusion broth (B.B.L.), nutrient broth (Difco), and bacto-beef (Difco) were dissolved in 200 ml of distilled water. The concentrated broths were treated with 10% sodium acetate and 2 volumes of 95% ethyl alcohol. A precipitate formed which was recovered by centrifugation. The precipitate was dissolved in as little water as possible, the insoluble particles were centrifuged off, and the supernatant was dialyzed against running tap water for 24–48 hr and freeze-dried. The yields of the isolates in mg were as follows: infusion broth, 500; nutrient broth, 200; and bacto-beef, 90.

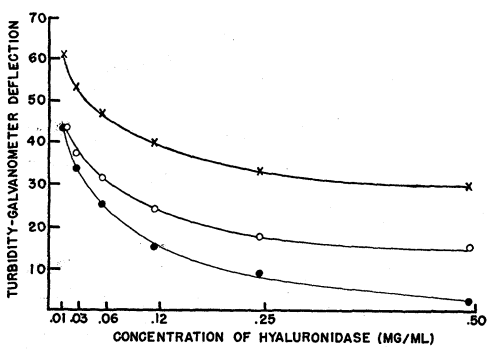

FIG. 1. The depolymerization of polysaccharide substrates by bovine testicular hyaluronidase. •——• = bacto-beef; ○——○ = infusion broth; ×——× = nutrient broth. Each system consisted of 0.5 ml of 3.0 mg/ml substrate and 0.5 ml of various dilutions of hyaluronidase. Incubated 30 min at 37° C. Reaction stopped and turbidity developed by addition of 3 ml of 0.5 *M* acetate buffer, pH 4.2, and 1 ml of 1:10 acidified horse serum, pH 4.2. Read in a Klett-Summerson colorimeter; red filter No. 66.

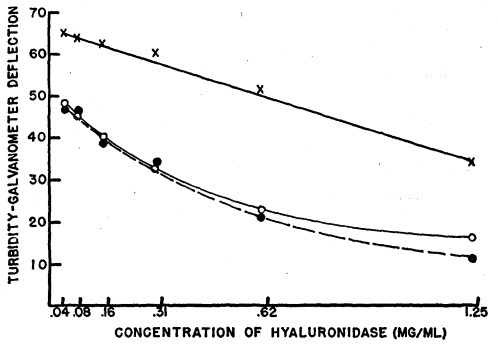

FIG. 2. The depolymerization of polysaccharide substrates by *S. hemolyticus* hyaluronidase. •——• = bacto-beef; ○——○ = nutrient broth; ×——× = infusion broth (see Fig. 1).

The depolymerization of the culture medium isolates by bovine testicular and *S. hemolyticus* hyaluronidase was measured turbidimetrically by a method described in a previous communication (*4*). The testicular hyaluronidase was prepared by essentially the method of Madinaveitia (*5*) and contained 1,400 turbidity reducing units per mg nitrogen. A crude group A *S. hemolyticus* enzyme preparation was employed and assayed 350 turbidity reducing units per mg nitrogen.

The isolated materials gave strong Molisch tests in dilutions ranging from 1:5000 to 1:20,000. Reducing substances were not present, as shown by negative Fehling's and Benedict's tests. Total sulfur as determined by the Carius method was as follows: infusion broth, 0.86%; nutrient broth, 1.77%; bacto-beef, 4.89%.[1]

The effect of testicular hyaluronidase on the depolymerization of the polysaccharide substrates is illustrated in Fig. 1. Although a difference in residual turbidities of the substrate occurred with the same enzyme dilutions, the curves produced were all on the same order. Heat-inactivated enzyme (60° C for 1 hr) did not reduce the initial turbidity of the substrates.

The depolymerization of the three substrate factors by streptococcus hyaluronidase is seen in Fig. 2. Since the bacterial hyaluronidase preparation did not possess the same degree of purification as the testicular enzyme, higher concentrations were necessary to obtain comparable reduction of the substrates. As previously reported for testicular hyaluronidase, heat-inactivated streptococcus hyaluronidase did not depolymerize any one of the three polysaccharides tested in the present study.

References

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[1] I am indebted to Thomas R. McCrea for these data.