Adsorption of Testicular Hyaluronidase upon Glass

POUL STRØIER RASMUSSEN

Biochemical Institute, University of Copenhagen, Denmark

It has been observed quite accidentally by the author that bovine testicular hyaluronidase (h.dase) is adsorbed upon filterpaper, glass and quartz sand. Adsorption also takes place on cotton wadding and permutit. Up to now attempts to elute from filterpaper and wadding have been unsuccessful.

EXPERIMENTAL

The h.dase preparation used in the investigations described below was the commercial "Penetrase Leo", but different preparations have given essentially the same results.

The activities were measured in proportion to "Penetrase" as a standard, using the turbidimetric method of Dorfman and Ott¹ with slight modifications.

The adsorption upon glass and quartz sand is influenced by pH, ionic strength and the presence of other substances. Fig. 1 shows the effect of pH. Two ml portions of "penetrase", 50 mg/100 ml, in phosphate buffers of different pH were put in centrifuge tubes with 200 mg of pulverized quartz sand (particle size unknown). In one investigation (curve B), the buffers were 0.1 *M* and therefore the ionic strength increased with pH, in another (curve A) the ionic strength was kept constant (0.1) by appropriate dilution of the buffers. Adsorption is accomplished almost at once. After stirring 2 or 3 times with a glass spatula, the tubes were centrifuged and the supernatants analysed for activity.

The influence of the ionic strength is seen from Fig. 2. Two ml "penetrase", 50 mg/100 ml, in pH 7.2 phosphate buffers of different ionic strength was adsorbed on 50 mg of pulverized quartz sand.

Many attempts to elute the h.dase from quartz and glass have been made. Several monohexoses, fructose, glucose, galactose and glucosamine and also glycerol, all in concentrations of about 1—10 %, were able to hinder, or almost so, the adsorption of h.dase on quartz sand; moreover at an acid pH (3.8) these substances were able to elute some of the adsorbed h.dase. Urine has been found capable of eluting 70 % of the adsorbed h.dase. The best elution has been obtained with 1 *M* phosphate buffer, pH 5.6 (pH of minor importance).

Fig. 1. Effect of pH on adsorption of testicular hyaluronidase upon pulverized quartz sand. In curve A a constant ionic strength of 0.1 is used. In curve B 0.1 M phosphate buffer is used.

Fig. 2. Effect of ionic strength on adsorption of hyaluronidase upon pulverized quartz sand. Phosphate buffers, pH 7.2.

H.dase may be purified by adsorption upon a column of glass beads followed by elution with 1 M phosphate, pH 5.6 (see Fig. 3).

A 33×1 cm column of small glass beads ("Ballotini", diameter about 0.1 mm) was washed with phosphate buffer, pH 7.7, ionic strength 0.1, until the pH of the effluent was 7.7. The flow rate was 0.5 ml per minute. "Penetrase", 50 mg/100 ml, in this buffer was run through the column until considerable activity was obtained in the effluent, the column was thus "saturated" (76 ml, Fig. 3). After washing with nearly 20 ml solvent, 1 M phosphate, pH 5.6 was put on the column to elute the enzyme (beginning at 95 ml on the abscissa of Fig. 3). The effluent was collected as before in *ca.* 3 ml fractions. As a measure of protein concentration the absorption of the clear effluent fractions at 280 $m\mu$ is given in per cent of that of the original 50 mg/100 ml "penetrase" (for this solution the extinction was 0.465 in a 1 cm cell).

From Fig. 3 it is seen that a purification of h.dase is obtained in the third and fourth elution fraction (about 10 and 4.5 times respectively).

With a taller column containing twice the amount of glass beads, one of the elution fractions had a h.dase activity 15 times greater than that of the solution put on the column. Nevertheless, the purification was only of the same order of magnitude (10 times) as in the first experiment because the absorption at 280 $m\mu$ was also elevated (about 1.5 times).

The ability of glass to adsorb h.dase is especially important to keep in mind when working with purified h.dase. It has been found that h.dase is more easily adsorbed the purer it is. Consequently, it is also then more difficult to elute. A diluted (2 mg/100 ml) solution of Wyeth h.dase (declaration:

Fig. 3. Purification of hyaluronidase by adsorption upon a 1×33 cm column of glass beads followed by elution with 1 M phosphate, pH 5.6. Diameter of beads about 0.1 mm. 76 ml h.dase was run through the column, followed by washing with buffer. After collecting 95 ml effluent, elution with 1 M phosphate, pH 5.6 was begun.

1 400 T.R.U. per mg) in borate buffer, pH 7.3, 0.1 M NaCl, will lose more than 50 % activity if kept in a test tube half an hour, 3°. With this preparation (about 3 times more pure than "penetrase") it could be proved that much of the loss was due to adsorption, *i. e.* much of the lost activity could be eluted. Solutions of h.dase at least 50 times more pure than "penetrase", about the same purity as the best hitherto reported, very easily lose activity from contact with glass and other surfaces. Elution, however, has not been effected. Such purified h.dase preparations are said to be very unstable². The author thinks that some of the "instability" of purified h.dase is due to adsorption upon glass, but investigations not yet completed seems to show that this "instability" is more complex.

These observations concerning the adsorption of h.dase upon negatively charged substances suggested that h.dase was possibly a basic protein. H.dase was therefore tried on a column of Amberlite IRC 50 in the same way as Moore, Stein and co-workers have done with ACTH³, ribonuclease⁴, and lysozyme⁵. Indeed the Wyeth preparation has now been purified 30-fold by this method. Details will be published later.

SUMMARY

Testis-hyaluronidase can be adsorbed upon glass, quartz sand, permutit, filterpaper and cotton wadding. Attempts to elute from filterpaper and wadding have not been successful.

Adsorption upon pulverized quartz sand is influenced by pH and ionic strength.

Purification of testicular hyaluronidase was obtained by adsorption upon a column of fine glass beads, followed by elution with 1 *M* phosphate, pH 5.6.

The author is indebted to Løvens Kemiske Fabrik, Copenhagen, for the "Penetrase Leo" used in this investigation.

REFERENCES

1. Dorfman, A. and Ott, M. L. *J. Biol. Chem.* **172** (1948) 367.
2. Meyer, K. and Rapport, M. M. *Advances in Enzymol.* **13** (1952) 199.
3. Dixon, H. B. F., Moore, S., Stack-Dunne, M. P. and Young, F. G. *Nature* **168** (1951) 1044.
4. Hirs, C. H. W., Moore, S. and Stein, W. H. *J. Biol. Chem.* **200** (1953) 493.
5. Tallan, H. H. and Stein, W. H. *J. Biol. Chem.* **200** (1953) 507.

Received January 23, 1954.