Short Communications and Preliminary Notes

PURIFICATION OF TESTICULAR HYALURONIDASE BY CHROMATOGRAPHY

by

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In a previous paper[1] it was mentioned, that a column of Amberlite IRC-50 may be used for purification of testicular hyaluronidase (h.dase).

The chromatographic procedure for the present purpose was, in general, the same as that described by MOORE, STEIN and co-workers for ribonuclease[2] and lysozyme[3]. The chromatography was carried out at room temperature *i.e.* ca. 20° C. Flow rate was about 2 ml per hour. Wyeth Hyaluronidase (declaration: 1400 T.R.U. per mg) was used in the described experiments. H.dase activity was measured turbidimetrically[4] with the Wyeth as a standard. A measure of protein concentration in the fractions was obtained with Folin phenol reagent[5]. In the figures the protein (Folin color) and h.dase content of the single fractions are converted in per cent of total used in the experiment.

The results of an experiment are shown in Fig. 1. It is seen that most of the protein passes directly through the column. After about 20 ml effluent the fractions contain very little protein. Most of the h.dase, however, is found in the fractions after 20 ml. At about 25 ml, the fractions, 1.4 ml each, contain ca. 20 γ protein or 0.2% of the total 10 mg applied. Here the fractions have a h.dase activity corresponding to 500 γ Wyeth or 5% of 10 mg Wyeth used. At about 20 ml in Fig. 1 the activity per unit of protein is increased ca. 8 fold; at 25 ml and above it is further increased to ca. 25 times, but the protein content is here too low for exact measurement. Eighty three per cent of the h.dase used was eluted during the first 66 ml, in the next 110 ml, collected during the night, the remaining activity was found. Only 87% of the total protein could be accounted for in the effluent, as measured by the Folin reaction. The h.dase peak is broad; no better results in this respect have been obtained at other pH values or concentrations of the buffer.

The chromatographic behaviour of h.dase on IRC-50 is very dependent on pH and concentration of the buffer. The effects of pH, ionic strength and sodium ion concentration have not been investigated systematically, but very often lowering the pH about 0.1 unit has resulted in no h.dase being eluted at all, while a rise of about 0.1 pH unit caused poor separation of h.dase from the protein peak. Good results, however, are easily obtained with 0.2 *M* sodium phosphate buffer at pH 6.8. Because IRC-50 has a high buffering capacity, a thorough treatment of the resin with the buffer is recommended before preparing the column.

In experiments of the type illustrated in Fig. 1 the capacity for h.dase of a 0.9 × 37 cm column is only about 10 mg Wyeth. As the purified h.dase fractions are very dilute it is better for preparative work to adsorb at one pH and salt concentration and elute under other conditions. With sodium phosphate buffers of 0.1 *M*, maximum adsorption was found at pH 6.

Fig. 2 shows an experiment in which adsorption of 100 mg Wyeth was carried out under these circumstances; elution was effected by 0.3 *M* sodium phosphate buffer at pH 7.7 beginning at 20 ml effluent. Flow rate was about 7 ml per hour.

Most of the protein passed directly through the column. The pH of the effluent did not rise above 6 until after 175 ml had passed through. At the same time the h.dase was eluted in about 5 ml together with a few mg of protein. The midmost of the three h.dase rich fractions contain 64% of all the h.dase and 2.3% (2.3 mg) of the protein. This would mean a purification of 28 times if the Folin reaction gives a correct measure of protein. Thus far, the purified h.dase on a weight basis (freeze dried) only gives about 70% of the colour with Folin reagent as that given by the Wyeth h.dase, therefore, a 20 fold purification is more likely. Ninety two per cent of the h.dase could be accounted for, 90% appearing in the three most purified fractions. Only 85% of the protein was obtained based upon the reaction with Folin reagent. One hundred mg Wyeth h.dase was used in this

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experiment, but as the h.dase is eluted in a sharp peak, one would presume the column to work equally well with much greater amounts.

Until recently only small amounts of the purified material have been available; physico-chemical data, therefore, concerning the relative purity of the preparation are not yet ready for presentation.

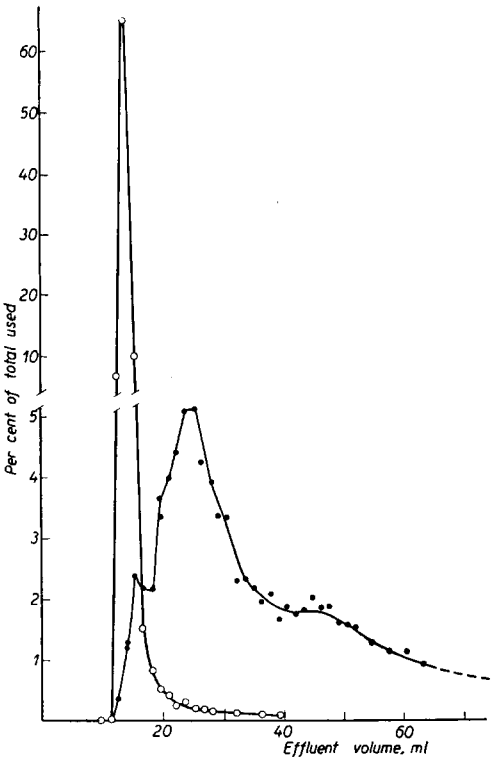

Fig. 1. Chromatography of 10 mg h.dase (Wyeth, 1400 T.R.U. per mg) on a 0.9 × 37 cm column of IRC-50. Eluant: 0.12 *M* sodium phosphate buffer, pH 7.4. Volume of fractions 1.4 ml. ○ protein content of fractions in per cent of total used. ● h.dase content of fractions in per cent of total activity.

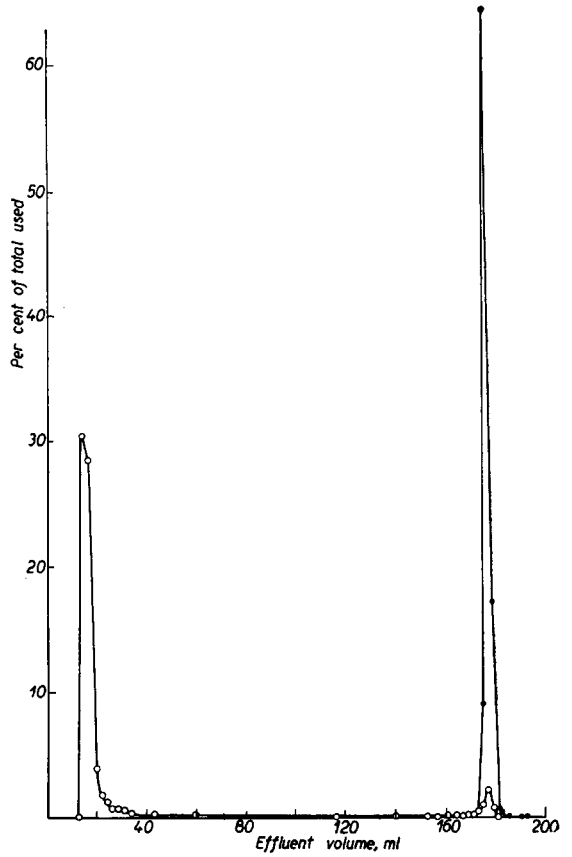

Fig. 2. Purification of h.dase (100 mg Wyeth) by adsorption upon a 0.9 × 40 cm column of IRC-50 with 0.1 *M* sodium phosphate buffer, pH 6, and elution with 0.3 *M* sodium phosphate buffer, pH 7.7. ○ protein determined by Folin reaction; ● h.dase.

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