Ion Exchange Chromatography of Glucagon in Urea-containing Buffers*

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Although the existence of the pancreatic hyperglycemic factor was postulated by Kimball and Murlin (1) in 1923, it was only recently that glucagon was isolated in crystalline form by Staub et al. (2). Glucagon was shown to be a basic polypeptide at that time. Such a peptide would be expected to yield successfully to chromatography on the carboxylic ion exchange resin Amberlite IRC-50, except for the fact that the hormone is insoluble in aqueous buffers in the region of pH 6. Glucagon is soluble, however, in this region of pH if urea is present, and may thus be used as an example of a special application of chromatography in urea-containing buffers (3) to peptides and proteins with similar solubility characteristics.

Furthermore, it was of interest to apply chromatography to glucagon with the use of a buffer system similar to that used for insulin (3) to determine whether or not glucagon could be detected in the chromatograms of insulin.

EXPERIMENTAL PROCEDURE

The samples of glucagon used were from Lots 258-234B-54-2 and 258-234B-167-1, generously given by Dr. Edward Grinnan and Dr. William W. Bromer of Eli Lilly and Company, Indianapolis, Indiana. Dr. Grinnan also supplied the crystalline bovine insulin (Lot 693502).

The resin used was Amberlite IRC-50 (XE-64) screened (wet) through 200-mesh wire gauze, or Amberlite IRC-50 (XE-97). The resin was prepared and the columns operated as described by Hirs et al. (4) except that the columns were run at 3-5°C with buffers of 0.13 M phosphate-0 M to 8 M urea prepared by the method described elsewhere (3). Samples of 1 to 50 mg of glucagon were applied to columns 0.64 x 30 cm, and the buffer was allowed to flow through the column by gravity at a rate of 3 to 5 ml per hour.

Analysis of effluent fractions was carried out with the technique published by Lowry et al. (5). For assay, the glucagon was recovered from the eluent buffer by diluting with 10 volumes of water, and collecting the precipitate by centrifugation. The precipitate was then washed with water and dried with ethanol and ether (yield, 75 to 80%).

RESULTS

Since insulin had already been chromatographed on Amberlite IRC-50 with 0.13 M phosphate-7 M urea buffer at pH 6.0, these were the first conditions tried for glucagon in the hope of determining the position of the latter hormone on the chromatograms of insulin. These conditions were tried in spite of the fact that glucagon has a more basic isoelectric point than insulin and would therefore be expected, on the basis of simple ion exchange, to be excessively retained under conditions appropriate for insulin. Chromatography in 0.13 M phosphate-7 M urea gave the results shown in Fig. 1, from which it may be observed that the main component of glucagon is eluted at 21 ml. This elution volume is slightly less, rather than greater, than the elution volume (30 ml) of insulin on the same column. The greater retention of insulin might be due to hydrogen bonding (in addition to the retention due to ion exchange), an effect which would be expected to be less in the case of the smaller glucagon molecule. The data given in Table I, however, show that retention was increased by lowering the urea concentration and so suggest that hydrogen bonding plays a significant role in the case of glucagon as well as in the case of insulin. Clearly, the order of elution depends not only on net charge, but also on such undetermined characteristics as the distribution of charges and the molecular shape, and may not be accurately predicted for untried proteins.

The main component shown in Fig. 1 was shown to be active, and the recovery of peptide protein material (based on the yield of color) was quantitative (97%). That the minor peaks were not artifacts derived from the main component, either reversibly or irreversibly during chromatography, was demonstrated by reapplying to the column eluate taken from the main band of a previous chromatogram. The results of such an experiment are given in Fig. 2 and show a single peak at the appropriate elution volume.

The small amount (15 to 20%) of material in addition to the main component may well represent products of transformation similar to those noted by Staub et al. (2). These workers subjected crystalline hormone to zone electrophoresis on starch and found 10 to 15% of the material in minor peaks. When, however, peptide from the main electrophoretic peak was isolated by crystallization and resubmitted to zone electrophoresis, the minor peaks were again present. Staub et al. concluded that these minor components were artifacts of the isolation procedure. In the rechromatography (Fig. 2) of material from the main band of the chromatogram, there was a slight suggestion

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FIG. 1. The chromatography of glucagon on Amberlite IRC-50 in 0.13 M phosphate-7 M urea at pH 6.0. The column was 0.64 X 30 cm.

TABLE I

Retention volume of glucagon and insulin at different concentrations of urea

<table>
<thead>
<tr>
<th>Urea concentration</th>
<th>Glucagon ml</th>
<th>Insulin ml</th>
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<tbody>
<tr>
<td>8</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>7</td>
<td>13</td>
<td>22</td>
</tr>
<tr>
<td>6</td>
<td>22</td>
<td>32</td>
</tr>
</tbody>
</table>

* The retention volume used here represents the total elution volume minus the holdup volume (8 ml) of the column. Chromatography was performed using 0.64 X 30 cm columns of Amberlite IRC-50 with urea-containing buffers of 0.13 M phosphate at pH 6.0.

FIG. 2. The rechromatography of material from the main peak of a previous chromatogram. A 0.64 X 30 cm column of Amberlite IRC-50 was eluted with 0.13 M phosphate-7 M urea, pH 6.0.

Fig. 3. The chromatography of a mixture of glucagon and insulin on Amberlite IRC-50, eluting with 0.13 M phosphate-6 M urea, pH 6.0.

that some transformation had occurred. Since it was possible that such a transformation was largely avoided by working at low temperatures, but might occur at higher temperatures, a sample of glucagon was submitted to chromatography after prior incubation in the phosphate-urea buffer at room temperature for 8 hours. The results obtained (15 to 20% minor components) showed no perceptible increase in the faster moving components. These minor components may be artifacts of previous isolation steps but are not produced readily by treatment with phosphate-urea buffers.

Finally it was of interest to see if this chromatographic system could resolve a mixture of glucagon and insulin. A mixture of the two hormones yielded the chromatogram shown in Fig. 3. Although the separation of the main components of these two hormones is not complete, the pattern indicates that such a chromatographic system might be adapted to the estimation of these hormones in biological tissues, and might find further application to their isolation from limited amounts of tissue, such as those which might be obtained from laboratory animals. Work along these lines is now in progress.

SUMMARY

Glucagon has been submitted to chromatography on the ion exchange resin Amberlite IRC-50, eluting with a 0.13 M phosphate-urea buffer of pH 6.0. The concentration of urea was varied from 6 M to 8 M in different experiments, and the lower concentration resulted in increased retention of the glucagon.

As in the work of Staub et al. (2), crystalline glucagon was found to be relatively homogeneous, with only 15 to 20% in the form of minor components. Even on the short columns used it was possible to partially resolve insulin and glucagon.

REFERENCES

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