The Interaction of Immobilized Transition-Metal Ions with some Gastrointestinal Polypeptides

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Amino acid residues in proteins that possess an ionizable proton can display ligand properties. Nitrogen donors include the imidazole group of histidine, the ε-amino group of lysine and the guanadinium group of arginine. The carboxyl groups of aspartate and glutamate and the hydroxyl group of tyrosine can function as oxygen donors, and the thiol group of cysteine can act as a sulphur donor. The nitrogen atom of the peptide linkage and the N-terminal amino group can also co-ordinate to metal ions (Vallee & Wacker, 1970). The affinity of proteins for heavy metal ions has been used by Porath et al. (1975) in the development of a chromatographic technique in which columns of immobilized zinc and copper ions were used to fractionate serum proteins. In the present study, the interaction of immobilized transition-metal ions with the 125I-labelled polypeptide hormones, gastrin and glucagon and with glucagon-like-immunoreactivity polypeptide material, which competes with glucagon, for binding sites on antibodies raised against glucagon, was investigated. The glucagon-like-immunoreactivity material (mol.wt. approx. 12000) was purified from extracts of pig colon by immunoaffinity chromatography (Murphy et al., 1973).

The metal-binding conjugate (I) was synthesized by the method of Porath et al. (1975) by coupling iminodiacetic acid to Sepharose-4B activated with 1,4-bis-(2,3-epoxypropoxy)butane.

\[
\text{Agarose} - \text{O} - \text{CH} = \text{CH(OH)} - \text{CH}_2 - \text{O} - \text{(CH}_2\text{)}_4 - \text{O} - \text{CH}_2 - \\
\text{CH(OH)} - \text{CH}_2 - \text{N(CH}_2\text{CO}_2\text{H}) - \text{CH}_2\text{CO}_2\text{H} \\
(1)
\]

Transition-metal ions (Table 1) were immobilized on columns (3 cm x 1 cm) of this gel by irrigation at 20°C with aqueous solutions of the metal sulphates (2 mg/ml). The columns were washed with water and equilibrated with 0.1 M-NaH$_2$PO$_4$/Na$_2$HPO$_4$ buffer, pH7.4 (solvent I). Solutions of 125I-labelled polypeptide hormones (20 ng) or of glucagon-like-immunoreactivity material (immunoreactivity equivalent to 20 ng of glucagon) were applied at a flow rate of 0.5 ml/min. Columns were irrigated with solvent (I) (10 ml) and with 20 ml of each of the following solvents: 0.1 M-NaH$_2$PO$_4$/Na$_2$HPO$_4$, pH7.4, containing 1 M-NaCl (II); 0.1 M-sodium acetate adjusted to pH4.0 with acetic acid (III); 0.05 M-EDTA containing 0.5 M-NaCl adjusted to pH7.0 with 5 M-NaOH (IV); 0.05 M-NH$_4$HCO$_3$ adjusted to pH10.5 with aq. NH$_3$ (sp.gr. 0.88) (V).

The chromatographic behaviour of the polypeptides is summarized in Table 1. 125I-labelled glucagon did not bind to immobilized Mn$^{2+}$ and Fe$^{3+}$ ions. Binding was observed with

<table>
<thead>
<tr>
<th>Polypeptide</th>
<th>Metal ion immobilized</th>
<th>Solvent required for elution</th>
</tr>
</thead>
<tbody>
<tr>
<td>125I-labelled glucagon</td>
<td>Mn$^{2+}$, Fe$^{2+}$, Fe$^{3+}$</td>
<td>(I)</td>
</tr>
<tr>
<td>125I-labelled gastrin</td>
<td>Zn$^{2+}$, Cd$^{2+}$, Ni$^{2+}$</td>
<td>(III)</td>
</tr>
<tr>
<td>Glucagon-like-immunoreactivity material</td>
<td>Co$^{2+}$, Cu$^{2+}$</td>
<td>(IV)</td>
</tr>
<tr>
<td></td>
<td>Ca$^{2+}$, Cd$^{2+}$, Cu$^{2+}$</td>
<td>(I)</td>
</tr>
<tr>
<td></td>
<td>Zn$^{2+}$</td>
<td>(IV) or (V)</td>
</tr>
<tr>
<td></td>
<td>Cu$^{2+}$, Co$^{2+}$</td>
<td>(III)</td>
</tr>
</tbody>
</table>
the Ni$^{2+}$, Zn$^{2+}$ and Cd$^{2+}$ conjugates and the hormone could be eluted in a sharp band with solvent (III). Stronger binding occurred with immobilized Co$^{2+}$ and Cu$^{2+}$ ions, as evidenced by the need for solvents (IV) or (V) for elution of the hormone. Elution of the labelled glucagon from the immobilized metals could also be accomplished by irrigation with concentrated (>10mg/ml) aqueous solutions of the respective metal sulphate. The $^{125}$I-labelled glucagon could not be eluted from the Co$^{2+}$ and Cu$^{2+}$ conjugates by solutions of high salt concentration (solvent II) or by 8M-urea, suggesting that binding to the columns was through metal–ligand interactions and not through strong electrostatic or hydrophobic interactions with conjugate (I). Further, the hormone could be eluted from the metal-free conjugate with solvent (I).

The strong binding of the $^{125}$I-labelled glucagon to the immobilized Co$^{2+}$ and Cu$^{2+}$ ions may be due to chelation by the N-terminal histidine residue and the aspartate residue at position 9, metal binding being enhanced by hydrogen bonding of the histidine with the acidic residue (Liljas & Rossmann, 1974). In a study of the sites of binding of copper ions by the N-terminal (residues 1–24) peptide of bovine serum albumin, Bradshaw et al. (1968) implicated the N-1 atom of the imidazole group of histidine, the amide nitrogen atoms of histidine and threonine, and the terminal amino group of aspartic acid in chelate formation. Similarly Brill & Venable (1967) have confirmed that in zinc–insulin complexes the imidazole residues are the most important binding sites. Gastrin does not contain a histidine residue and $^{125}$I-labelled gastrin did not bind to immobilized Cu$^{2+}$ ions. This hormone contains a polyglutamate sequence at residues 6–9, but unexpectedly did not bind to immobilized Ca$^{2+}$ or Cd$^{2+}$ ions, which can form stable complexes with oxygen donors (Irving & Williams, 1953). The electrostatic repulsion between vicinal negative charges may force the molecule to adopt a conformation in which the glutamate residues are not available for chelate formation.

In contrast with $^{125}$I-labelled glucagon, glucagon-like-immunoreactivity material bound to columns of immobilized Zn$^{2+}$ ions could not be eluted by using solvent (III), but was readily eluted by solvent (V). This difference in chromatographic properties indicates that glucagon-like-immunoreactivity material binds to metal ions through a region of the molecule that is not homologous with glucagon.

Chelate-forming adsorbents are both easy to prepare and to manipulate and the present study confirms the conclusion of Porath et al. (1975) that immobilized metal ions may have an important application in the purification of polypeptides and proteins. In addition, the chromatographic behaviour of proteins on those conjugates may be used to complement the technique of equilibrium dialysis in the study of protein–metal interactions.

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