Removal of Cadmium(II) from Crystallized Ferritin

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The cadmium content of crystallized horse spleen ferritin, usually about 2% by weight without special treatment, can be substantially decreased by prolonged dialysis against certain chelating agents, chaotropic ions, or weakly reducing anions. For example, neutral bisulphite buffer (2M) removed 95% of the bound cadmium of crystallization without affecting the iron content, and may thus be valuable for preparing 'metal-free' holoferritin for physical-chemical studies.

The iron-storage protein ferritin has been widely studied by biochemists and physiologists because of its central role in iron metabolism, the heterogeneity of its subunit structure (Ishitani et al., 1975), and the unusual sensitivity of its iron content to the iron requirement of the cell (Harrison et al., 1974; Sirivech et al., 1974). Many of these properties are now being exploited to diagnose haematological and iron-storage disorders (Drysdale et al., 1977). Since the time of Laufberger (1937) and Granick (1942), mammalian ferritins have been purified most commonly by heat treatment, salting out, and crystallization with Cd(II) ion. The exceptional avidity of the protein moiety of ferritin for Cd(II), Zn(II) and other bivalent cations has been well documented by Macara et al. (1973) and by Coleman & Matrone (1969). It has been assumed that ferritin could be rendered free of the cadmium of crystallization by repeated precipitation with (NH₄)₂SO₄ (Granick, 1942), but personal experience and the high cadmium content of many commercial horse spleen ferritins (J. Hegenauer & L. Hatlen, unpublished work) suggest that this claim is erroneous. The residual cadmium in so-called 'Cd-free' ferritin has not been properly appreciated and may seriously affect the interpretation of physical-chemical data for the holoprotein. The present paper surveys some methods for removal of bound cadmium to provide a 'metal-free' holoprotein for metal-binding studies. Our results suggest that prolonged dialysis against certain reducing or chelating agents can substantially decrease, but not totally eliminate, the residual cadmium content of crystallized horse spleen ferritin.

Materials and Methods

Ferritin

Horse spleen ferritin ('electron-microscopic grade') was purchased from Polysciences Inc., Warrington,

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Results and Discussion

Without special treatment the cadmium content of crystallized horse spleen ferritin is over 2% by weight (Table 1); values approaching 3% have been reported for dialysed ferritin (Granick, 1942). Table 1 ranks some representative dialysis regimens in order of their increasing effectiveness in removing bound cadmium of crystallization from horse spleen ferritin. Prolonged dialysis against dilute solutions of certain neutral salts (NaCl, Na2SO4) removed some cadmium. Since sodium acetate at the same concentration had little effect, the ability of certain anions to strip cadmium from the protein is evidently related to the Hofmeister (lyotropic) series (von Hippel et al., 1973) and not to the ionic strength of the medium. Concentrated thiocyanate solution removed over 80% of the total cadmium, but no iron, at neutral pH. The strongly chaotropic thiocyanate anion (Sawyer & Puckridge, 1973) has not been reported to cause permanent disruption of the subunit structure of apoferritin, but may have assisted the removal of cadmium by exposing metal-binding sites to ligand exchange with thiocyanate in the surrounding medium. In dilute solution, the weak chelator Bicine [NN-bis-(2-hydroxyethyl)glycine] (Chaberek & Martell, 1959) removed about 90% of the cadmium without affecting the iron content. It has been our experience that more concentrated solutions of Bicine may cause some loss of iron during prolonged dialysis and are also prohibitively expensive if used with the dialysis schedule described in the present paper. Although glycine is a good ligand for bivalent cations such as Cu(II) and Zn(II), it ranked poorly in removing cadmium. Concentrated solutions of the weakly reducing bisulphite anion removed about 95% of the cadmium; dialysis at neutral pH did not affect the iron content significantly, but appreciable iron was lost as iron(II) during dialysis at pH 6.4. Neutral bisulphite also displaced some phosphate from the surface of the ferric oxyhydroxide core (Treffry et al., 1977); more phosphate was lost during dialysis against acidic bisulphite, presumably because of loss of iron. Ferritin was salted out by the high ionic strength of the 2M-bisulphite buffers, but redissolved promptly on final dialysis against dilute NaCl.

The low cadmium:subunit ratios obtained after dialysis against bisulphite (Table 1) clearly indicate the loss of all loosely bound cadmium and most of the 'stoichiometric' cadmium held by specific cation-binding sites on the surface of the apoferritin moiety (Macara et al., 1973). Cd(II) ion may be removed effectively by chelation or, better, by ligand exchange with such compounds as thiocyanate or bisulphite, which may displace Cd(II) from thiols. A more extensive methodological survey was beyond the scope of the present investigation. On the basis of the limited data available, however, we recommend prolonged dialysis against neutral bisulphite to remove most of the residual cadmium from commercial ferritin that is used for critical physical-chemical studies.

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References

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Table 1. Cadmium content and elemental composition of horse spleen ferritin

<table>
<thead>
<tr>
<th>Dialysis treatment</th>
<th>Elemental composition (% Cd)</th>
<th>Cd/subunit* (molar)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solute</td>
<td>Conc. (m) pH</td>
<td>Cd</td>
</tr>
<tr>
<td>1. H2O</td>
<td>—</td>
<td>2.11</td>
</tr>
<tr>
<td>2. Sodium acetate</td>
<td>0.15 7.0</td>
<td>1.72</td>
</tr>
<tr>
<td>3. Glycine</td>
<td>2.0   7.0</td>
<td>1.04</td>
</tr>
<tr>
<td>4. NaCl</td>
<td>0.15 7.0</td>
<td>0.57</td>
</tr>
<tr>
<td>5. Na2SO4</td>
<td>0.15 7.0</td>
<td>0.47</td>
</tr>
<tr>
<td>6. NaSCN</td>
<td>4.0   7.0</td>
<td>0.35</td>
</tr>
<tr>
<td>7. Bicine</td>
<td>0.1   7.0</td>
<td>0.25</td>
</tr>
<tr>
<td>8. NaHSO3/Na2SO3</td>
<td>2.0   7.0</td>
<td>0.11</td>
</tr>
<tr>
<td>9. NaHSO3/Na2SO3</td>
<td>2.0   6.4</td>
<td>0.09</td>
</tr>
</tbody>
</table>

* The molecular weight of the apoferritin subunit is 18600 g/mol (Leach et al., 1976). The nitrogen content of apoferritin is 16.3% (J. Hegenauer & L. Hatlen, unpublished work), or 16.3 g of N/100 g of protein. Therefore, 18600 g of protein/mol of subunit x 16.3 g of N/100 g of protein = 3032 g of N/mol of subunit protein. The nitrogen content of each holoferritin sample can be attributed exclusively to the apoferritin moiety and is given in the Table. Sample No. 1 above, for example, contains 2.11% Cd and 11.06% N, or 2.11 g of Cd/11.06 g of N. Since the atomic weight of Cd is 112.40 and there are 3032 g of N/mol of subunit protein, we calculate 18772 μmol of Cd/3648 μmol of subunit protein, or 5.1 atoms of Cd/mol of subunit.

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