THE SEDIMENTATION BEHAVIOR AND MOLECULAR WEIGHT OF PANCREATIC CARBOXYPEPTIDASE*

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Previous studies of the physical properties of crystalline carboxypeptidase have included investigations of the electrophoretic behavior (1, 2) and measurements of viscosity and diffusion (2). It was reported that the several times recrystallized protein migrated essentially as a homogeneous substance in the electrophoresis apparatus (2).

We have investigated the sedimentation behavior of carboxypeptidase in the ultracentrifuge, since no previous studies of this type have been reported. It was found that the carefully recrystallized protein behaved as a homogeneous substance. From the sedimentation constant and the previously determined diffusion constant (2), the molecular weight was found to be 33,800. This value is in reasonable agreement with the molecular weight of 31,600 estimated from diffusion and viscosity measurements by Putnam and Neurath (2).

EXPERIMENTAL

The enzyme was prepared from frozen bovine pancreas as described by Anson (3) and recrystallized four to six times by the procedure of Neurath, Elkins, and Kaufman (4). The protein concentration of the carboxypeptidase solutions was evaluated from the protein N content, the conversion factor of 7.0 being used, since the protein has been reported to contain 14.4 per cent N (3).

All of the sedimentation studies were made in the Spinco¹ electrically driven ultracentrifuge. A brief description of this instrument, and the controls and procedures, has been given in a previous publication from this laboratory (5).

The results obtained with various preparations of carboxypeptidase are presented in Table I. Most of the runs showed that the protein sedimented essentially as a homogeneous substance with a sedimentation constant of 2.5.

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¹ Specialized Instruments Corporation, Belmont, California.
constant of about $3.1 \times 10^{-13}$. A representative experiment is shown in Fig. 1, A. However, in some experiments it was found that a frequent contaminant of carboxypeptidase is an accompanying protein with a sedimentation constant of about $9.0 \times 10^{-13}$; such a run is illustrated in Fig. 1, B. This heavier protein is apparently a globulin which is relatively in-

### Table I

**Sedimentation of Carboxypeptidase**

The temperature is the average for the duration of the run. The results incorporate the usual corrections for the temperature, and for the viscosity and density of the solvent (6). The values for the sedimentation constants ($S_1$, $S_2$, and $S_3$) are for $S_{20,w}$ in cm. per second. The parenthetical values give the amount of each component estimated from its percentage of the total sedimenting area.

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Solvent</th>
<th>pH</th>
<th>Temperature °C</th>
<th>Protein concentration (per cent)</th>
<th>$S_1 \times 10^{13}$</th>
<th>$S_2 \times 10^{13}$</th>
<th>$S_3 \times 10^{13}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.04 M phosphate + 0.4 M NaCl</td>
<td>7.1</td>
<td>25.0</td>
<td>0.42</td>
<td>3.25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.015 M veronal + 0.185 M NaCl</td>
<td>7.7</td>
<td>24.8</td>
<td>0.23</td>
<td>3.19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.015 M veronal + 0.185 M NaCl</td>
<td>7.7</td>
<td>27.1</td>
<td>0.12</td>
<td>3.29</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.015 M veronal + 0.185 M NaCl</td>
<td>7.7</td>
<td>22.2</td>
<td>0.06</td>
<td>3.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.015 M veronal + 0.185 M NaCl</td>
<td>7.7</td>
<td>25.3</td>
<td>0.23</td>
<td>3.45</td>
<td></td>
<td></td>
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<tr>
<td>6</td>
<td>0.25 M NaCl</td>
<td>5.9</td>
<td>24.4</td>
<td>0.12</td>
<td>3.12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>0.1 M veronal + 0.1 M NaCl</td>
<td>7.8</td>
<td>26.2</td>
<td>0.14</td>
<td>2.97</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>0.1 M veronal + 0.3 M NaCl</td>
<td>7.8</td>
<td>26.4</td>
<td>0.16</td>
<td>2.83</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9†</td>
<td>1.0 M NaCl</td>
<td>6.8</td>
<td>24.7</td>
<td>0.58</td>
<td>2.82</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>1.0 M NaCl</td>
<td>6.5</td>
<td>25.1</td>
<td>0.42</td>
<td>2.94</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11†</td>
<td>0.015 M veronal + 0.385 M NaCl</td>
<td>7.6</td>
<td>25.3</td>
<td>0.91</td>
<td>3.02 (86%)</td>
<td>9.0 (14%)</td>
<td></td>
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<tr>
<td>12</td>
<td>1.0 M NaCl</td>
<td>5.9</td>
<td>21.8</td>
<td>0.50</td>
<td>3.05 (50%)</td>
<td>8.2 (8%)</td>
<td>3.9 (42%)</td>
</tr>
<tr>
<td>13</td>
<td>0.5 M NaCl</td>
<td>5.9</td>
<td>23.9</td>
<td>0.25</td>
<td>3.32 (65%)</td>
<td>9.7 (8%)</td>
<td>5.8 (27%)</td>
</tr>
<tr>
<td>14</td>
<td>0.04 M glycine + 0.11 M NaCl</td>
<td>9.0</td>
<td>24.5</td>
<td>0.44</td>
<td>3.15 (87%)</td>
<td>9.4 (13%)</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>0.2 M phosphate</td>
<td>7.6</td>
<td>22.2</td>
<td>0.28</td>
<td>2.88 (90%)</td>
<td>8.6 (10%)</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>0.2 M NaCl</td>
<td>8.7</td>
<td>22.0</td>
<td>0.26</td>
<td>3.26 (87%)</td>
<td>9.1 (13%)</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>0.1 M glycine + 0.1 M NaCl</td>
<td>11.4</td>
<td>22.3</td>
<td>0.89</td>
<td>2.02</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* These values have been omitted from the average sedimentation constant.
† These are the experiments shown in Fig. 1, A (Sample 9) and Fig. 1, B (Sample 11).
soluble at low ionic strengths, since extracts of a stock preparation of the crystalline enzyme made near neutrality at 0.2 ionic strength do not show this substance. When extracts are made at higher salt concentrations (0.4 to 1.0 ionic strength), or at about pH 9, this substance is dissolved. In two instances, large amounts of additional contaminants ($S_3$) were observed, as in Samples 12 and 13. These proteins, $S_2$ and $S_3$, do not appear to possess any carboxypeptidase activity. Enzymatic tests with homogeneous samples yielded values for the proteolytic coefficient ($C$) of 12 to 13, with carbobenzoxyglycyl-L-phenylalanine as the substrate at 0.05 M concentration. Tests with Samples 12 and 13 gave somewhat lower activities ($C = 7$ to 9). Previous results with highly purified carboxypeptidase gave proteolytic coefficients in the neighborhood of 12 to 14 (4, 7, 8).

We have taken advantage of the insolubility of the contaminating protein in 0.2 ionic strength sodium chloride in order to prepare routinely carboxypeptidase which behaves as a homogeneous substance in the ultracentrifuge, and which possesses a $C$ value of about 12 to 13. Stock preparations of 4 times crystallized carboxypeptidase are extracted with 0.2 M sodium chloride and centrifuged. The extracts are then dialyzed in the usual manner against gradually decreasing concentrations of salt solutions and finally against distilled water. Preparations of carboxypeptidase twice recrystallized in this way are homogeneous even when extracted into 1.0 M sodium chloride (Samples 9 and 10).

* The proteolytic coefficient is defined as the first order velocity constant expressed in decimal logarithms for a solution containing 1 mg. of protein N per cc.
A single determination (Sample 17) performed at pH 11.4 gave a preparation which showed only a single boundary, but which had a sedimentation constant of about $2.0 \times 10^{-13}$. This low value suggests that carboxypeptidase is split into half particles at this pH. It was found that this preparation was completely inactive enzymatically when tested at pH 7.4 in veronal buffer with carbobenzoxyglycyl-L-phenylalanine as the substrate.

The data in Table I indicate no significant variation of the sedimentation constant of carboxypeptidase ($S_1$) over the pH range of 5.9 to 9.0, over the tested range of protein concentrations from 0.06 to 0.91 per cent, or at different ionic strengths. Therefore, all of the values for $S_1$ were averaged with the exception of Sample 17, measured at pH 11.4, and Sample 5, which gave an unaccountably high result. The average value for $S_{20,w}$ is $3.07 \times 10^{-13}$ with an average deviation of $\pm 0.14$ and a standard deviation of $\pm 0.15$.

The diffusion constant of carboxypeptidase has been reported by Putnam and Neurath (2) to be $9.94 \times 10^{-7}$ sq. cm. per second at 25°. When this value is corrected for the difference in the viscosity of water between 25° and 20°, $D_{20,w}$ is $8.82 \times 10^{-7}$. Assuming that $V$ is 0.75, and incorporating these values in the usual formula (6), $M = RTS/D(1 - Vp)$, we obtain 33,800 for the molecular weight.

**SUMMARY**

Crystalline bovine pancreatic carboxypeptidase sediments as a homogeneous substance in the ultracentrifuge. The average value for the sedimentation constant, $S_{20,w}$, is $3.07 \pm 0.15 \times 10^{-13}$. From this and diffusion data, the molecular weight is 33,800.

The crystalline enzyme frequently contains a contaminating globulin with $S_{20,w} = 9.0 \times 10^{-13}$. This inactive substance is insoluble at low ionic strengths near neutrality and can be separated from carboxypeptidase.

**BIBLIOGRAPHY**

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