Physicochemical Properties and Amino Acid Composition of Chymotrypsinogen B*

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SUMMARY

The physicochemical properties and amino acid composition of chymotrypsinogen B have been examined. The partial specific volume has been determined as 0.733 ml per g. From sedimentation velocity measurements, $s_{20,w}$ has been found to be 2.58 S at pH 3.0. At pH values above 4, the zymogen dimerizes as indicated by both sedimentation velocity and approach to sedimentation equilibrium measurements. From light scattering and approach to sedimentation equilibrium measurements, an average molecular weight of 24,850 was calculated. Amino acid analyses performed in this laboratory are consistent with this value. The weight intrinsic viscosities of chymotrypsinogens A and B were found to be 2.18 and 2.26 ml per g, respectively. Comparison of the effective hydrodynamic volume calculated from the weight intrinsic viscosity and the viscosity increment with the value calculated from the partial specific volume of the anhydrous protein indicates that the molecule is relatively compact and sparingly hydrated in solution. Optical rotatory dispersion measurements of chymotrypsinogens A and B show that both have similar low helical contents and similar tertiary structures. An anomalous Cotton minimum at 221 m$_\nu$ in chymotrypsinogen A is absent in chymotrypsinogen B and must represent some structural differences between the two proteins.

Amino acid analyses have shown significant differences in the levels of several of the amino acids, including lysine, arginine, aspartic acid, threonine, serine, glutamic acid, proline, methionine, and tyrosine. The half-cystine content has been determined as 10 and is consistent with the isolation of five unique disulfide sequences from this protein (3).

A comparison of the enzymatic, chemical, and physical properties of closely related enzymes may shed light on the relationships between structure and function of these protein molecules. The chymotrypsinogens are a convenient subject for such comparisons since they are readily available in high purity and yield and since much information on the structure and activity of chymotrypsin A has accumulated. In this laboratory we have undertaken a study of the structure and properties of chymotrypsinogen B (1-3), first isolated and characterized by Laskowski (4). Recently, we developed a chromatographic procedure for the purification of this zymogen in a relatively pure form (1) and showed that both chymotrypsin A and B have similar enzymic activities (2).

In this paper we present some of the physicochemical parameters and amino acid analyses of the chymotrypsinogen B isolated by this procedure. Molecular weight measurements by the methods of approach to sedimentation equilibrium and light scattering have yielded an average molecular weight of 24,850. Amino acid analyses are consistent with this value. Partial specific volume, sedimentation velocity, and viscosity measurements indicate that both chymotrypsinogens are compact and sparingly hydrated in solution. A comparison of the optical rotatory dispersion properties of the two zymogens has shown that both have a low helical content. Although they are apparently very similar in tertiary structure, an anomalous Cotton minimum at 221 m$_\nu$ in chymotrypsinogen A indicates that they are not identical.

EXPERIMENTAL PROCEDURE

Chymotrypsinogen B—This protein was prepared as previously described (1) with the following modifications. The protein, precipitated between 0.2 and 0.4 saturated (NH$_4$)$_2$SO$_4$, was dissolved in 0.005 M HCl and centrifuged at 9000 rpm for 60 min. The supernatant was dialyzed overnight against 0.001 M HCl, centrifuged at 9000 rpm, and filtered through cheesecloth. After the protein concentration was adjusted to 1.75%, saturated (NH$_4$)$_2$SO$_4$ (adjusted to pH 3.0 with 1 M HCl) was added to a final concentration of 0.28% saturation. The resulting precipitate was collected by centrifugation and discarded. The supernatant was brought to 0.4 saturation with the saturated ammonium sulfate solution (pH 3.0). The resulting precipitate (0.28 to 0.40 ammonium sulfate fraction) was collected, dissolved in a minimal volume of 0.005 M HCl, dialyzed for 24 hours against 0.001 M HCl, and freeze-dried. This material had a potential $K'$ against N-acetyl-L-tyrosine ethyl ester of 1.7 to 1.9 and a low free chymotrypsin activity (0.1 to 0.2%). This material has been used for chromatography on carboxymethyl cellulose as previously described (1).

Chymotrypsinogen A—This protein was purchased from Worthington as the four times crystallized material. It was dissolved in 0.001 M HCl, dialyzed extensively against this solution, and

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freeze-dried. For measurements in solutions above pH 4, both the A and B zymogen preparations were dissolved directly in buffer, readjusted to the appropriate pH, and used without dialysis. For the optical rotatory dispersion measurements, crystalline chymotrypsinogen A (Lot P) was kindly supplied by Dr. P. E. Wilcox. This material is said to contain only traces of chymotrypsin-like material upon high resolution chromatography. Upon rapid tryptic activation it had a potential k', against N-acetyl-t-tyrosine ethyl ester of 50.1 (1).

Protein Concentration—This was calculated from the absorbance at 280 μm of appropriately diluted solutions in 0.001 M HCl, measured in a Beckman DU spectrophotometer. The extinction coefficient, E280 = 18.7, was determined from measurements of dry weight, percentage nitrogen (16.06 ± 0.08%), and light absorbance on simultaneous samples of the lyophilized protein, previously equilibrated with the laboratory air at room temperature for 24 hours.

Partial Specific Volume—This was determined at 20°C ± 0.05°C from density measurements in a 10-ml pycnometer equipped with a thermometer and overflow tube. Solutions of chymotrypsinogen B were clarified by filtration through Millipore filters ( pore size, 0.45 or 0.8 μm). The partial specific volume was calculated according to Schachman (5).

Sedimentation Velocity—The sedimentation velocity measurements were carried out in a model E Spinco ultracentrifuge at 59,780 rpm. The calculations and corrections were made as previously described (6).

Viscosity—Ostwald-Fenske viscometers requiring 5.0 ml and with a water flow time of 540 sec at 20°C ± 0.01°C were used to measure the viscosities of chymotrypsinogen A and B solutions. Solutions were routinely passed through a Millipore filter ( pore size, 0.80 μm) immediately before the measurements. Viscosities were calculated, relative to the solvent, neglecting the kinematic correction. The method of least mean squares was used to calculate the weight intrinsic viscosity and slope term.

Light Scattering—All measurements were performed at a wave length of 436 μm and at room temperature (23°C ± 2°C) with the Brice-Phoenix light scattering photometer as previously described (6). Solutions were passed through a Millipore filter ( pore size, 0.80 μm) immediately before the measurements. A refractive index increment of 0.1940 at 436 μm, the value determined for chymotrypsinogen A (7), was assumed for the B zymogen. From this, the Debye factor, H, was calculated to be 1.025 x 10^-5.

Approach to Sedimentation Equilibrium—Molecular weights were measured at both cell top and bottom positions as previously described (6) except that a rotor speed of 12,590 rpm was found most satisfactory. Pictures were taken throughout the run at phase plate angles of 60° and 75°. For measurements, the 75° angle position gave the sharpest outline.

Optical Rotatory Dispersion—Measurements were carried out with a Cary model 21 recording spectropolarimeter at 27°C. For the measurements in the range of 300 to 600 μm, 0.2 to 0.3% protein solutions were used with a cell of 1-cm path length. The data were treated by the method of Yang and Doty (8), which involves plotting -N[α] with respect to -[α] to determine the dispersion constant, λe. The Moffitt-Yang equation (9) was also applied, wherein [m]' = [(α² - λ²e²)/α²] was plotted with respect to λe²(α² - λ²e²). A value of 212 μm was assigned to λe in order to linearize the plots, and an average amino acid residue weight of 105, based on current estimates of the amino acid composition and molecular weight, was taken for both proteins in the calculation of the mean residue rotation, [m]'e. The dispersion parameters, α and β, of the Moffitt-Yang equation were obtained from the ordinate intercept and slope of the plots, respectively (10). In calculating helical contents from λe, the assumption was made that this parameter is of the order of -640° for a completely helical molecule of right-handed screw sense. Optical rotation data in the wave length region of 200 to 250 μm were obtained with approximately 0.03% protein solutions in a 1-mm cell. The data were plotted as the mean residue rotation, [m]'e, with respect to λ. Both zymogens showed characteristic Cotton troughs at 231.5 μm, from which helix contents were calculated with the use of limits of -2,000° and -16,600° for the completely random and 100% helical conformations, respectively (11). It should be noted that Yang and McCabe (11) caution against the unreserved use of polyglutamic acid as a model polymer, upon which the above values were based. The values quoted in this study are therefore to be regarded as operational values which may have to be revised.

Amino Acid Analysis—After equilibration with the laboratory air, 3-μg samples were accurately weighed into Pyrex test tubes, 18 x 250 mm. A simultaneous sample (15 μg) was taken for estimation of dry weight. After the addition of 1 ml of 6 M HCl (a 1:1 dilution of reagent concentrated HCl), the tubes were evacuated and sealed. Hydrolysis was carried out at 110°C ± 2°C in an oven for 7, 22, 30, and 70 hours.

At the end of the hydrolysis period, the tubes were opened and taken to dryness in a Buchler rotary Evapo-Mix. The residue was dissolved in 0.5 ml of water, and the solution was brought to about pH 6.5 by the addition of 0.5 ml of 0.2 M sodium phosphate buffer, pH 6.5. After standing for 4 hours, the solution was adjusted to about pH 2 by the addition of 0.06 ml of N HCl and then quantitatively transferred to a 5-ml volumetric flask with 0.2 ml sodium citrate buffer at pH 2.2. The flask was brought to volume with the buffer used to rinse the hydrolysate tube. Aliquots were analyzed by ion exchange chromatography (12) in a Spinco model 120B automatic amino acid analyzer. An independent determination of cystine as cysteic acid was made by the method of Moore (13). The values for tryptophan and amide NH values have been taken from Kassell and Laskowski (14).

RESULTS

Partial Specific Volume—The values of Vapp calculated for five different concentrations (0.64 to 1.44%) of chymotrypsinogen B in 0.1 M NaCl-0.001 M HCl, pH 3.0, were independent of concentration, and the average value, 0.733, was taken as the partial specific volume, Φ.

Sedimentation Velocity—In Fig. 1 are presented the plots of corrected sedimentation constants as a function of protein concentration at pH 3.0, 4.0, and 5.0. The sedimentation patterns from which these constants were calculated showed no unusual features. It is apparent that chymotrypsinogen B shows normal concentration dependence at and below pH 4.0. The Φapp values of the protein in 0.1 M NaCl-0.001 M HCl at pH 3.0, over the concentration range 0.1 to 2.0%, fall closely on a line given by least squares as Φapp = 2.56 ± 0.02 (±0.01), c, where c is expressed in grams per 100 ml. The best straight lines drawn through the points at the other pH values and extrapolation to zero protein concentration yield Φapp values close to 2.60. These values are in good agreement with those of Smith, Brown, and Laskowski (15), who reported an Φapp value of 2.49 S at pH 3.88.
The latter figure, when corrected by 2.5% for adiabatic expansion and contraction (16), is raised to 2.55 S. Keller, Cohen, and Neurath (17) reported an \( \eta_{\text{in}}/c \) value of 2.53 S for anionic Component 2 (chymotrypsinogen B) of bovine pancreatic juice.

**Intrinsic Viscosity**—The least squares straight line of the data for chymotrypsinogen B, over the concentration range 0.6 to 2%, is given by \( \eta_{\text{in}}/c = 2.26 (\pm 0.04) + 0.62 (\pm 0.04)c \), where \( c \) is in grams per 100 ml. For chymotrypsinogen A, over the same concentration range, the \( \eta_{\text{in}}/c = 2.18 (\pm 0.02) + 0.31 (\pm 0.06)c \). These are among the lowest values ever reported for proteins and indicate highly symmetrical and sparingly hydrated molecules. Only lysozyme has been reported to have an intrinsic viscosity as low as this (18).

**Approach to Sedimentation Equilibrium**—Table I lists the molecular weights obtained at the meniscus and cell bottom in runs performed with three different preparations of the B zymogen. From the standard errors, it is evident that calculations from data at the cell bottom are less accurate than those at the meniscus. This stems from the fact that the extrapolation procedure at the solution-silicone interface was rendered difficult by the thickening of the boundary. However, combination of both sets of results yields a mean molecular weight of 24,700 ± 900.

To ascertain whether chymotrypsinogen B undergoes association above pH 4.0, approach to equilibrium experiments were conducted at pH 5.0 in 0.1 M acetate buffer and at pH 7.0 in 0.1 M sodium phosphate buffer. Average molecular weights calculated from the meniscus and cell bottom were 36,220 ± 2,000 at pH 5.0 and 44,350 ± 1,000 at pH 7.0. It is clear from these data and the sedimentation velocity data that dimerization of chymotrypsinogen B occurs above pH 4.0.

**Light Scattering**—The values of \( Hc/r \) as obtained for chymotrypsinogen B are plotted as a function of protein concentration in Fig. 2. The best straight line drawn by the method of least squares intercepts the ordinate at 4.0 \( \times 10^{-5} \). The molecular weight was calculated from the reciprocal of the ordinate intercept as 25,000 ± 400, and the very small interaction constant, \( B \), was evaluated from the slope of the plot as 5.1 \( \times 10^{-3} \) mole per ml per g².

**Table I**

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Time of picture (min)</th>
<th>Mol wt at meniscus</th>
<th>Mol wt at cell bottom</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>16</td>
<td>25,780</td>
<td>23,170</td>
</tr>
<tr>
<td></td>
<td>32</td>
<td>25,000</td>
<td>23,580</td>
</tr>
<tr>
<td>II</td>
<td>16</td>
<td>25,430</td>
<td>23,150</td>
</tr>
<tr>
<td></td>
<td>32</td>
<td>25,860</td>
<td>25,120</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>25,740</td>
<td>25,120</td>
</tr>
<tr>
<td>III</td>
<td>16</td>
<td>24,000</td>
<td>23,420</td>
</tr>
<tr>
<td></td>
<td>32</td>
<td>25,550</td>
<td>24,660</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td>25,420 ± 300</td>
<td>23,900 ± 710</td>
</tr>
</tbody>
</table>

**Fig. 2.** \( Hc/r \) data plotted against concentration for chymotrypsinogen B in 0.1 M NaCl-0.001 M HCl, pH 3.0.

**Table II**

<table>
<thead>
<tr>
<th>Chymotrypsinogen</th>
<th>Yang-Doty plot</th>
<th>Moffitt-Yang plot</th>
<th>Cotton effect</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \lambda_s )</td>
<td>( m'_s )</td>
<td>( a_2 )</td>
</tr>
<tr>
<td>A</td>
<td>231.1</td>
<td>13.6</td>
<td>-436°</td>
</tr>
<tr>
<td></td>
<td>± 3.1</td>
<td>± 1%</td>
<td>± 18°</td>
</tr>
<tr>
<td>B</td>
<td>231.8</td>
<td>14.1</td>
<td>-465°</td>
</tr>
<tr>
<td></td>
<td>± 1.4</td>
<td>± 1%</td>
<td>± 32°</td>
</tr>
</tbody>
</table>

**Optical Rotatory Dispersion**—The optical rotatory dispersion parameters for both chymotrypsinogens A and B are given in Table II. The helix contents, calculated from \( \lambda_s, b_s, \) and [\( m'_s \)]₂₃₁.₅₀, agree within a few per cent, and on this basis alone no significant difference is evident between the two proteins. However, when a comparison is made of the optical rotatory dispersion of the two proteins between 250 and 207 mµ (Fig. 3), a distinct difference is evident. For chymotrypsinogen A, two
Amino acid recoveries from chymotrypsinogen B

Recoveries of amino acids after hydrolysis in 6 N HCl for the indicated times of hydrolysis are shown. Each value for the indicated time of hydrolysis represents the average of three to five separate analyses.

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Time of Hydrolysis</th>
<th>Average or Extrapolated Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7 hrs</td>
<td>22 hrs</td>
</tr>
<tr>
<td>Lysine</td>
<td>5.82</td>
<td>5.95</td>
</tr>
<tr>
<td>Histidine</td>
<td>1.22</td>
<td>1.24</td>
</tr>
<tr>
<td>Arginine</td>
<td>3.28</td>
<td>3.37</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>10.51</td>
<td>10.24</td>
</tr>
<tr>
<td>Threonine</td>
<td>9.75</td>
<td>9.63</td>
</tr>
<tr>
<td>Serine</td>
<td>8.43</td>
<td>7.70</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>10.77</td>
<td>10.85</td>
</tr>
<tr>
<td>Proline</td>
<td>5.68</td>
<td>5.88</td>
</tr>
<tr>
<td>Glycine</td>
<td>6.64</td>
<td>6.74</td>
</tr>
<tr>
<td>Alanine</td>
<td>7.90</td>
<td>7.78</td>
</tr>
<tr>
<td>Valine</td>
<td>7.31</td>
<td>10.16</td>
</tr>
<tr>
<td>Methionine</td>
<td>2.32</td>
<td>2.26</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>2.95</td>
<td>4.06</td>
</tr>
<tr>
<td>Leucine</td>
<td>9.07</td>
<td>9.56</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>2.21</td>
<td>2.25</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>4.04</td>
<td>4.42</td>
</tr>
</tbody>
</table>

* Average of 7-, 22-, 30-, and 70-hour hydrolysates.
* Average of 30- and 70-hour hydrolysates.
* Extrapolated to zero time.
* Average of 70-hour hydrolysates.
* Average of 22-, 30-, and 70-hour hydrolysates.
molecular weight calculated for each amino acid and the assumed number of residues (closest integral value to that calculated on the basis of a molecular weight of 24,850), a molecular weight for the protein was calculated. The average, 25,020, lends credibility to the value of 24,850 estimated from approach to sedimentation equilibrium and light scattering measurements.

When these data are compared with those of Kassell and Laskowski (14), the agreement is reasonably good, except that the total yields on a weight basis and on a percentage nitrogen basis are closer to theoretical in the present work. In addition, the levels of serine and glutamic acid are appreciably higher in our analyses (7.8% and 13.0% higher, respectively). A possible explanation for this discrepancy is the observation by Ikawa and Snell (25) that during evaporation of a hydrolysate in a vacuum desiccator, a compound of glutamic acid and serine may be formed. Our hydrolysates, which were taken rapidly to dryness on a rotary Evapo-Mix at 60°, would not be subject to this error. A further discrepancy in the two sets of data is in the levels of serine and glutamic acid; for which we obtain a value of 10.6 and 18.5%, respectively.

A comparison of the chymotrypsinogen B data from two laboratories with the amino acid analyses of chymotrypsinogen A is given in Table V. As previously described (14), there are significant differences in the levels of several amino acids, including lysine, arginine, aspartic acid, threonine, serine, glutamic acid, proline, methionine, and tyrosine. The very appreciable difference in amide content is consistent with the difference in isolectric points of the two proteins (4).

### Table V

Comparison of amino acid analyses of chymotrypsinogen B and chymotrypsinogen A

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Chymotrypsinogen B</th>
<th>Chymotrypsinogen A</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>residues</td>
<td>residues</td>
</tr>
<tr>
<td>Lysine</td>
<td>10.0</td>
<td>10.3</td>
</tr>
<tr>
<td>Histidine</td>
<td>2.0</td>
<td>1.9</td>
</tr>
<tr>
<td>Amide</td>
<td>15.1</td>
<td>24.1</td>
</tr>
<tr>
<td>Arginine</td>
<td>5.1</td>
<td>4.0</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>19.5</td>
<td>21.8</td>
</tr>
<tr>
<td>Threonine</td>
<td>20.2</td>
<td>23.0</td>
</tr>
<tr>
<td>Serine</td>
<td>20.6</td>
<td>30.1</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>18.5</td>
<td>14.2</td>
</tr>
<tr>
<td>Proline</td>
<td>12.7</td>
<td>8.7</td>
</tr>
<tr>
<td>Glycine</td>
<td>22.4</td>
<td>23.3</td>
</tr>
<tr>
<td>Alanine</td>
<td>22.0</td>
<td>21.7</td>
</tr>
<tr>
<td>Half-cystine</td>
<td>9.5</td>
<td>10.0</td>
</tr>
<tr>
<td>Valine</td>
<td>23.5</td>
<td>22.4</td>
</tr>
<tr>
<td>Methionine</td>
<td>3.8</td>
<td>3.1</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>3.6</td>
<td>2.0</td>
</tr>
<tr>
<td>Leucine</td>
<td>18.6</td>
<td>18.9</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>6.8</td>
<td>6.5</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>6.4</td>
<td>6.2</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>6.2</td>
<td>7.0</td>
</tr>
</tbody>
</table>

### Discussion

The molecular weights of chymotrypsinogen B as deduced from approach to sedimentation equilibrium, light scattering, and amino acid analyses are given in Table VI for purposes of comparison. The good agreement obtained for the different methods employed lends credibility to the estimated average value of 24,850. Thus the molecular weights of chymotrypsinogens A and B are the same within experimental error.

From the parameters of viscosity, sedimentation, partial specific volume, and molecular weight, it is possible to calculate a β-function value from the equation of Scheraga and Mandelkern (31),

\[
\beta = \frac{N g_0 v}{k T M^2 (1 - \rho_0)}
\]

where \(N\) is Avogadro's number; \(g_0\), the viscosity of water at 20°; and \(\rho_0\), the density of water at 20°. On the basis of a molecular weight of 24,850, \(\beta\) was found to be 1.93 \(\times 10^6\) for chymotrypsinogen B. This is a value appreciably below the minimum possible value of 2.13 \(\times 10^6\) for a rigid ellipsoid of revolution of any shape. Similar low values of \(\beta\) have been observed for bovine serum albumin (32, 33). In this case, it was suggested that the discrepancy could be attributable either to a departure of the protein molecule from the ellipsoidal form or to an inaccuracy in the use of the Simha viscosity equation and/or the Perrin expression (on which the \(\beta\) derivation is based) for nearly spherical molecules. Similar arguments to explain the failure of the \(\beta\) derivation are clearly applicable to chymotrypsinogen B.

The effective hydrodynamic volume \(V_e\) of the chymotrypsinogen B molecule may be calculated from the expression

\[
V_e = \frac{100\sigma M}{N_\rho} = 0.368 \times 10^{-19} \text{ ml}
\]

where \(\nu\) is the viscosity increment and has been taken as 2.50 (value for a rigid ellipsoid of revolution of axial ratio equal to 0.4).

### Table VI

Hydrodynamic properties, molecular weights, and optical rotatory dispersion parameters of chymotrypsinogens A and B

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Chymotrypsinogen B</th>
<th>Chymotrypsinogen A</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\sigma) (ml/g)</td>
<td>0.733</td>
<td>0.721 (38)</td>
</tr>
<tr>
<td>(\delta_{20, w})</td>
<td>2.58 S</td>
<td>2.49 S (29, 60)</td>
</tr>
<tr>
<td>pH 3.0</td>
<td>2.00 S</td>
<td>2.58 S (29, 60)</td>
</tr>
<tr>
<td>pH 7.5</td>
<td>2.26</td>
<td>2.18</td>
</tr>
<tr>
<td>Molecular weight</td>
<td>24,700</td>
<td></td>
</tr>
<tr>
<td>Approach to sedimentation</td>
<td>25,000</td>
<td></td>
</tr>
<tr>
<td>Light scattering</td>
<td>24,850</td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>24,940</td>
<td>25,635*</td>
</tr>
<tr>
<td>Amino acid analyses</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Optical rotatory dispersion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\lambda), (m(\mu))</td>
<td>231.1</td>
<td>231.1</td>
</tr>
<tr>
<td>(a_0)</td>
<td>-465°</td>
<td>-436°</td>
</tr>
<tr>
<td>(b_0)</td>
<td>-97°</td>
<td>-86°</td>
</tr>
<tr>
<td>([m]_0)</td>
<td>-3,600°</td>
<td>-3,300°</td>
</tr>
<tr>
<td>Average % helix</td>
<td>13.5</td>
<td>12.0</td>
</tr>
</tbody>
</table>

* Calculated from the amino acid sequence (24, 27).
the partial specific volume of the anhydrous protein by means of
the expression

\[ \frac{M \bar{v}}{N} = 0.208 \times 10^{-3} \text{ ml} \]

is further strong evidence for the relative compactness and low
hydration of these molecules in solution.

The agreement in the estimates of helical content of the two
proteins by the several approaches employed is satisfactory and
is consistent with similar data obtained by Raual and Schellman
(19) for chymotrypsinogen A under different buffer and pH con-
ditions. Although the interpretation of optical rotatory dis-
proteins by the several approaches employed is satisfactory and
is further strong evidence for the relative compactness and low
helical content, the indicated low percentage helix in chymotrypsinogens
B and A is consistent with what is presently known of the three-
dimensional structure of chymotrypsinogen A from x-ray diffraction
studies. Kraut, High, and Sieker (35) have reported that
no recognizable helical sequences are visible from construction
of electron density maps of the structure at a resolution of 4 A.
With this protein, no rodlike structures straight or long enough
for to be more than one turn of helix have been observed. Clearly,
the \( \alpha \) helix plays only a minor role in the structures of these pro-
tiens.

The good agreement in all of the optical rotatory dispersion
parameters (\( \lambda_0, \alpha_0, \) and \( \beta_0 \)) between the A and B zymogens
indicates that these two proteins have similar secondary and tertiary
structures. In particular, the similar values for the \( \alpha_0 \) parameter,
which is dependent on the interaction of residues in the three-
dimensional structure, as well as upon residue-solvent interaction,
may be taken as an indication that these two proteins have simi-
lar tertiary structures as well as similar helical content. How-
ever, it seems unlikely that these 2 molecules have identical
three-dimensional structures in view of the anomalous Cotton
minimum at 221 nm that is present in chymotrypsinogen A but
absent in chymotrypsinogen B and other proteins studied to date.
Although the origin of this anomalous Cotton effect in chymo-
trypsinogen A is obscure at this time, it must represent some aspect
of structural difference between the A and B zymogens.

Whether this difference may be related to the different aromatic
amino acid contents of the two proteins remains to be elucidated.

The close similarity in the secondary and tertiary structures
of chymotrypsinogen A and B as indicated by the optical rota-
tory dispersion studies is consistent with what is currently known
of the primary structures of the two proteins. Since the three-
dimensional structure of the two proteins must be determined in
part by the number and location of disulfide linkages in the
primary structure, it is not surprising to find that there are five
such linkages in both proteins and that the sequence of amino
acid residues about these linkages is similar (3).

Although the gross hydrodynamic properties, molecular
weights, and internal three-dimensional structures of the two
chymotrypsinogens appear to be very similar, the amino acid
analyses reported in this paper and those previously documented
by Kassell and Laekowski (14) show very considerable differences
between the two proteins. As indicated above, these differences
are not appreciably reflected in the sequences about the disulfide
bonds of the two proteins. Since these sequences contain those
portions of the molecules believed to be involved in their catalytic
activity, including the “active” serine and the 2 histidine residues,

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