Tryptic Hydrolysis of Human Serum Albumin

THE PATTERN OF INITIAL FRAGMENTATION*

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GABOR MARKUS, DAVID K. MCCLINTOCK,‡ AND BARBARA A. CASTELLANI

From the Department of Experimental Biology, Roswell Park Memorial Institute, New York State Department of Health, Buffalo, New York 14203

SUMMARY

The fragmentation of human serum albumin by trypsin was studied by electrophoresis and by gel filtration. Brief hydrolysis at pH 8.5 yields a characteristic pattern of fragmentation, the most conspicuous feature of which is the appearance of a 51,000 mol wt fragment of distinct electrophoretic mobility. Concomitant with the formation of this fragment, an 18,500 mol wt fragment is released. In addition to this predominant pattern, a second breakdown mechanism seems to operate which yields 13,500 mol wt fragments and still smaller peptides. The yield of the 51,000 fragment per bond cleaved is pH-dependent and is maximal at pH 10. The pH dependence of this yield appears to be different from that of the “alkaline transition” as revealed by optical rotatory measurements. The “subunit” hypothesis of albumin is discussed in the light of these findings.

Recent investigations of the proteolytic fragmentation of serum albumin have lent tentative support to the proposals of Harrington, Johnson, and Ottewill (1) and of Foster (2), that this single chain molecule may consist of a small number of compact regions connected by short and flexible segments. Evidence for such a subunit structure can be convincing if it can be shown that several proteolytic enzymes with different bond specificities produce fragments of similar size and composition. In the well known case of γ-globulin, at least six proteolytic enzymes have been shown to yield univalent antibody fragments of about 50,000 mol wt (3). For serum albumin such evidence is more difficult to obtain, in large part because of the lack of “markers” that would readily identify specific regions of the molecule. However, indications of a subunit structure can be found in the recent work of Adkins and Foster (6) on the subtilisin cleavage of this molecule. It seemed of interest, therefore, to examine the breakdown products of albumin obtained by digestion with a third protease, trypsin, and to attempt to correlate the results with those obtained earlier. A study of this kind was also necessary as a basis for the investigation of ligand-stabilized conformational changes (reported in the subsequent paper (7)), in which a comparison of trypsic digestion patterns is utilized as a sensitive indicator of conformational changes. Although the present study was carried out on human, rather than on bovine, serum albumin, a comparison of the fragments obtained by trypsin digestion of human and bovine albumin detected no significant differences in the net charge or the size distribution of the trypsic fragments in the two species.

In the present study, the digested human serum albumin was analyzed by gel filtration chromatography and by electrophoresis on paper or cellulose acetate. The largest fragment was isolated and studied in some detail. An analysis of the digestion patterns as a function of time served to establish a tentative sequence of initial events in the tryptic breakdown of HSA1. The results of digestion at different pH values were correlated with the pH-dependent conformational transition known to exist in this molecule.

MATERIALS AND METHODS

Human serum albumin (Pentex, crystallized, Lot 9) was used for most of the experiments reported here. For some experiments Cutter's 25% albumin solution was used. HSA was deionized by passage through an Amberlite MB-3 mixed bed resin column, exhaustively dialyzed against water, and stored frozen as a 5% solution. Neither preparation was defatted. Radioiodination of HSA was carried out according to the method of Grossberg, Radzimski, and Pressman (8) with 125I to yield a total of 2 iodine atoms per mole of HSA and about 50,000 cpm per mg of HSA. "Lyophilized trypsin" (twice crystallized, Lot TRL 6EA) and soy bean trypsin inhibitor (puro, noncrystalline) were products of Worthington.

1 The abbreviations used are: HSA, human serum albumin; BSA, bovine serum albumin; STI, soybean trypsin inhibitor; Fragment L, the 51,000 molecular weight fragment of human serum albumin, obtained by tryptic hydrolysis.
Measurement of Digestion Rates—Digestion rates were measured by continuous titration with 0.05 N NaOH from a syringe microburette to maintain the desired pH. A stream of N$_2$ was blown on the surface of the solution titrated. The small pH drop caused by the addition of trypsin (blank value) was determined on an identical sample lacking substrate protein. When the titrations were carried out above pH 8.5, the moles of base consumed were taken to be equivalent to the moles of peptide bonds hydrolyzed. Since between pH 7 and 9 a pH-dependent fraction of the hydrogen ions liberated is absorbed by the α-amino groups formed, factors were needed to convert moles of base to moles of bonds hydrolyzed. These were determined as follows. HSA was digested by trypsin at the “low” pH (less than pH 9) for the desired period; the pH was maintained constant by titration, and the moles of base used were recorded (A). The digestion was then arrested by the addition of STI, and the amount of base necessary to titrate this solution to pH 9 was noted (B). From the sum of these two titrations was subtracted the amount of base required to titrate an undigested sample (containing STI) from the “low” pH to pH 9 (C). The resulting moles of base (A + B - C = D) is equivalent to the moles of bonds hydrolyzed. The ratio D/A is constant for a given pH and can be used as a factor to correct titration values obtained at “low” pH values to yield the full equivalence of hydrogen ions liberated, without repeating the above procedure. All digests were carried out at 25°C.

Gel Filtration—For this procedure, Sephadex G-100 (Pharmacia) was used. The material was washed with distilled water and equilibrated with a buffer containing 0.05 M borate-0.1 M NaCl, pH 8.5. Before filling the columns, the suspension was heated in a boiling water bath for 15 min. Two columns were used: Column I was 2.5 X 70 cm and Column II (part of the LKB Recychrom apparatus) was 4 X 80 cm. The columns were filled by gravity and equilibrated for 48 hours with borate-NaCl buffer before use. The flow was regulated by peristaltic pumps to give elution rates of 7 ml per hour (Column I) and 15 ml per hour (Column II). Fractions of 2 and 4 ml, respectively, were collected. The elution of protein was monitored by ultraviolet absorption measurements at 280 μμ, or by radioactivity in a Packard Autogamma spectrometer. Since albumin was trace-labeled with lz61, the labeling was nonuniform, and thus the areas under the peaks in the plots of radioactivity with respect to elution volume were not proportional to the amount of protein eluted. In order to arrive at accurate values, the specific activities characteristic of each kind of breakdown product had to be determined in a preliminary experiment. To do this, the contents of tubes corresponding to each peak were pooled, their volume was reduced by pervaporation, and an aliquot thereof was hydrolyzed in 6 N HCl for 24 hours. The amino acid content of the hydrolysate was then determined by ninhydrin analysis (9). The protein content thus derived was evaluated quantitatively. The amino acid content of the hydrolysate was then determined by ninhydrin analysis (9). The protein content thus derived was correlated with the radioactivity to give specific activities for each kind of peak. It was found that these activities were characteristic for each kind of fragment, but varied little in different batches of iodinated HSA. Sephadex columns were calibrated by the use of the following test proteins: soybean trypsin inhibitor (21,500), myoglobin (16,700), and ribonuclease (13,680). These substances encompassed the size range of most of the albumin breakdown products. Gel filtration was carried out at room temperature.

Zone electrophoresis was carried out in a Beckman/Spinco apparatus with either paper strips or cellulose acetate (Sephrachrome III, Gelman). For paper electrophoresis, 15 μl of a 1% tryptic digest were applied to each strip; separation was carried out in Veronal buffer, pH 8.6, ionic strength 0.075, at 2.5 ma for 16 hours. Quantitative determination of the bromophenol blue-stained strips was done with the Beckman/Spinco Analytrol. Electrophoresis on cellulose acetate, with 10-μl samples, was done at 1.25 ma per strip for 2 hours in a Veronal buffer of ionic strength 0.06, pH 8.6. The strips were stained with Ponceau 3 (Allied Chemical). For quantitative evaluation, the stained bands were cut out, the dye was eluted in 1 ml of 0.1 N NaOH, and the purple color was determined spectrophotometrically at 550 μμ. Correlation of electrophoresis bands and Sephadex elution peaks was achieved by electrophoresis of material eluted from the column, after dialysis and freeze-drying. Only HSA, Fragment L, and Fragment A were found to have well-defined electrophoretic mobilities; dimer migrated as the monomer of HSA, and Fragments B and C appeared as diffuse staining between the origin and Fragment L.

Amino Acid Analyses—Amino acids were analyzed with a Technicon automatic analyzer; elution from a single column of Dowex 50 was achieved by a continuous salt and pH gradient.

Equilibrium Centrifugation—Equilibrium centrifugation for the molecular weight determinations of HSA and Fragment L was done by the method of Yphantis (10).

Optical Rotation—Optical rotation was measured at 546 μμ in a Bendix-Ericsson polarimeter at 25°C.

Results and Discussion

Formation of Large Fragment—The most conspicuous feature of the digestion of HSA by trypsin at pH 8.5 is the appearance of an electrophoretically distinct breakdown product, of lower apparent net negative charge than that of the parent HSA molecule. This component was first observed by Lapresle et al. (11) on starch gel electrophoresis and was found to contain most of the antigenic determinants of intact HSA. The initial rate of formation of this fragment and the number of bonds per mole of original HSA broken (Fig. 1) to yield the fragment were determined. Since the formation of fragment was too fast for a direct titration of the H+ ions released, the number of bonds cleaved was calculated from the measured change in pH by correlation with a titration curve for this pH interval, determined on an identical, undigested sample, after correction for the H+ ions brought in by trypsin. The small change in pH, from 9 to 8.4, during the experiment was of little consequence, since the rate and extent of Fragment L formation are very nearly constant in this pH interval (see below). The aliquots removed during digestion were analyzed by paper electrophoresis, and the conversion of HSA to Fragment L was evaluated quantitatively. Fig. 1c shows a plot of percentage of protein migrating as Fragment L against moles of bonds cleaved per mole of original HSA. The conversion to Fragment L is proportional to the number of bonds broken up to about three bonds; then the yield of Fragment L per bond cleaved declines sharply. An extrapolation of the linear portion of the curve to 100% conversion indicates that five bonds have to be cleaved per mole of HSA before 1 mole of Fragment L is released. If the error in the evaluation of the electrophoretic patterns and in the determination of the number of bonds is considered, the error in the extrapolation amounts to ± one bond. The five bonds should be regarded as a maximum estimate, since...
the cleavage of some of these bonds may be irrelevant to the formation of Fragment L. A conspicuous feature of this plot is the abrupt decline in the yield of intermediate after about 50% of HSA has been converted to Fragment L. This will be considered later. The kinetics of digestion is shown in Fig. 2. That the digestion is complex is evident, in that at least three different rates can be distinguished in the first 10 min of digestion. Extrapolation of the linear portions of the plot to the ordinate yields the extent of the process, or processes, that precede the linear segment. The first extrapolation yields an intercept of about three bonds and probably corresponds to the change in the pattern of digestion discussed above.

The degree to which HSA can be made to yield Fragment L depends on several factors. The effect of pH and ionic strength will be discussed below; the influence of ligands will be treated in the subsequent paper (7). In general, the process is seldom complete; even mild iodination tends to decrease the yield of this fragment. In most cases, after the first 10 bonds have been cleaved, the amount of Fragment L stays nearly constant while HSA is still disappearing at an appreciable rate (see below).

**Analysis of Large Fragment**—Slow passage through a Sephadex G-100 column has resulted in sufficient separation of Fragment L from intact HSA to permit determination of the molecular weight by equilibrium sedimentation on samples (0.01%) taken directly from the effluent, without the necessity of concentrating them. Concentration of dilute samples of Fragment L by pervaporation or by freeze-drying tended to induce the formation of a heterogeneous mixture of aggregates. Equilibrium sedimentation yielded a weight average molecular weight of 51,000 for the Fragment L. A control experiment with HSA done at the same time gave 68,000 for this parameter. The value of 51,000 is in good agreement with the estimate from

![Fig. 1. Initial events in the tryptic digestion of HSA. a, number of bonds cleaved as a function of time; b, paper electrophoresis of samples taken at times indicated in a; 1% HSA in 0.1% NaCl at 25°; 0.0337% trypsin. Digestion arrested by addition of soybean trypsin inhibitor (for details, see the text); c, formation of Fragment L ("Interm.") as a function of the number of bonds cleaved. Intercept of the dashed line with the abscissa indicates the number of bonds that have to be cleaved for complete conversion.](http://www.jbc.org/)

![Fig. 2. Time course of digestion of HSA by trypsin. Measurements were made by continuous titration with NaOH at pH 8.5, at 25°; 1% albumin in 0.1 M NaCl; 0.033% trypsin. Intercepts of the dashed (extrapolated) lines with the ordinate indicate the extent of the processes that precede the linear segments.](http://www.jbc.org/)
TABLE I
Amino acid composition of HSA, Fragment L and Fragment A

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>HSA</th>
<th>Fragment L</th>
<th>Expected composition of A (HSA - Fragment L)</th>
<th>Fragment A²</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>moles amino acid/mol wt 69,000</td>
<td>moles amino acid/mol wt 51,000</td>
<td>moles amino acid/mol wt 11,000</td>
<td>moles amino acid/mol wt 18,500</td>
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<tr>
<td>Aspartic acid</td>
<td>56.2</td>
<td>36.8</td>
<td>19.4</td>
<td>19.2</td>
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<tr>
<td>Threonine</td>
<td>28.4</td>
<td>18.5</td>
<td>9.9</td>
<td>8.9</td>
</tr>
<tr>
<td>Serine</td>
<td>22.9</td>
<td>20.0</td>
<td>2.9</td>
<td>4.4</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>85.8</td>
<td>54.9</td>
<td>30.9</td>
<td>23.4</td>
</tr>
<tr>
<td>Proline</td>
<td>24.9</td>
<td>16.9</td>
<td>8.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Glycine</td>
<td>12.7</td>
<td>11.5</td>
<td>1.2</td>
<td>4.4</td>
</tr>
<tr>
<td>Alanine</td>
<td>63.3</td>
<td>38.5</td>
<td>24.8</td>
<td>18.3</td>
</tr>
<tr>
<td>Valine</td>
<td>42.5</td>
<td>32.0</td>
<td>10.5</td>
<td>12.4</td>
</tr>
<tr>
<td>Half-cystine</td>
<td>36.9</td>
<td>23.0</td>
<td>13.6</td>
<td>12.6</td>
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<tr>
<td>Methionine</td>
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<td>7.4</td>
<td>0.5</td>
<td>1.7</td>
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<tr>
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<td>41.4</td>
<td>22.0</td>
<td>16.6</td>
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<tr>
<td>Tyrosine</td>
<td>18.0</td>
<td>11.1</td>
<td>6.9</td>
<td>1.6</td>
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<tr>
<td>Phenylalanine</td>
<td>32.2</td>
<td>18.2</td>
<td>14.0</td>
<td>9.9</td>
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<tr>
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<td>63.2</td>
<td>41.3</td>
<td>21.9</td>
<td>14.0</td>
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<tr>
<td>Histidine</td>
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<td>16.7</td>
<td>8.3</td>
<td>7.2</td>
</tr>
<tr>
<td>Arginine</td>
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<td>13.9</td>
<td>10.3</td>
<td>5.0</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Amide nitrogen</td>
<td>36.1</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

a Single hydrolysis for 21 hours.

b From extrapolation to zero time hydrolysis.

c Values for 68 hours of hydrolysis.

d As cysteic acid, owing to oxidative conditions during hydrolysis.

e Poor recovery owing to oxidative conditions during hydrolysis.

f Dashes indicate that no determination was made.

Fig. 3a also shows the optical rotation of the samples as a function of pH, determined before the addition of trypsin. The change in optical rotation corresponds to the well known alkaline transition in this protein (14, 15) and has been interpreted by Leonard, Vijai, and Foster (16) as a decrease in interresidue contacts at increasing pH values. The curve is much steeper than the pH dependence of formation of Fragment L, and its midpoint is at pH 9. While the curves bear little resemblance to one another, there may be a real relationship between the two phenomena. The steepness of the rotatory transition is characteristic of cooperative phenomena, in which the occurrence of each event is facilitated by the preceding one. The primary event which triggers all the subsequent ones in a pH-dependent transition is, necessarily, a change in the ionization of 1 or more amino acid residues. Since the transition is already noticeable at pH 8, i.e. in the region of increased formation of Fragment L, it is possible that the titration of the same group is involved in the initiation of the conformational transition and in the formation of Fragment L.

The curve in Fig. 3b shows the pH dependence of the formation of Fragment A (discussed below), which on cellulose acetate electrophoresis migrates as a thin band between HSA and Fragment L. The curve is similar to the corresponding curve for Fragment L.

It was found that, at constant pH, increasing the concentration of NaCl in the digestion mixture results in decreased yield of Fragment L when digestion is carried out to the same number of bonds hydrolyzed (Fig. 4). That this effect may

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not be caused entirely by the ionic strength per se, i.e. by the general electrostatic effects of high salt concentrations, but may be due to specific chloride binding, is indicated by the observation that 0.1 M borate favors both the rate and the extent of formation of Fragment L.

Pattern of Breakdown in Tryptic Digestion—In order to establish the initial pattern of tryptic fragmentation of HSA, samples were analyzed after 0.5, 1, and 2 min of digestion. These were obtained from a common pool by transfer of aliquots into STI solutions at the indicated times, and were subsequently chromatographed on a Sephadex G-100 column. Fig. 5 shows the elution patterns obtained by measurement of radioactivity in the effluent. Before the addition of trypsin (not shown), the sample contained only the dimer peak and the intact HSA. The patterns clearly show the appearance of Fragment L, culminating in the cleavage of the main peak in the 2-min pattern. All the peaks corresponding to the smaller fragments are already present in substantial amounts at 0.5 min of digestion. Table II shows a quantitative evaluation of the results. The molecular weights of Fragments pre-A, A, and B were evaluated from their mobilities on this column after calibration with substances of known molecular weight (see “Materials and Methods”). The amount of Fragment L, as a percentage of total protein present in the sample, was determined from a quantitative evaluation of electrophoretic patterns on cellulose acetate strips. Fig. 6 shows the results as a function of the time of tryptic digestion. Inspection of the molecular weights in Table II immediately reveals that their sum is much larger than 69,000; thus a single molecule of HSA cannot simultaneously yield all the observed fragments, and no one-stage breakdown mechanism can be postulated. However, the initial equimolarity, the complementary molecular weights of Fragment L (51,000) and of Fragment A (18,500), and the similarity between the calculated and observed amino acid composition of Fragment A (Table I) suggest that the principal mechanism which accounts for the disappearance of 80% of HSA in 2 min is the cleavage of the parent molecule into these two fragments. The remaining 20% of the HSA broken down appears as Fragments pre-A, B, and C. These components either are breakdown products of Fragment L or of Fragment A, or may be the result of a different breakdown mechanism for albumin. That a breakdown mechanism not leading to Fragment L should also be operative under the conditions of this experiment is to be expected, since (a) at pH 8.5 only 70% of the maximum amount of Fragment L can be formed (Fig. 3a), and (b) iodination of albumin always decreases the yield of this fragment. That fragments of a size corresponding to Fragment B can be formed under circumstances in which little or no Fragment L is observed (as in the presence of bound methyl orange (7)) suggests that part of Fragment B results from a direct breakdown of HSA into this fragment. Measurements on samples digested for longer periods of time, however, indicate that the breakdown of Fragment L can also produce component B. If the second mechanism yields predominantly B, then this fragment must be heterogeneous, i.e. it must represent different parts of the original HSA molecule. It is noted that electrophoretically Fragment B is heterogeneous, being distributed in the area between the origin and Fragment L.

3 It may be noted that part of the discrepancy between the calculated and the observed values of the amino acid composition of Fragment A (Table I) is probably due to the sensitivity of the predictions to the correct assumptions for the molecular weights of the three species involved in the calculation. The larger differences (leucine, lysine, arginine) may be due partly to actual release of smaller peptides during the conversion of HSA to Fragment L and Fragment A.
Table II
Quantitative summary of gel filtration data

<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.5 min</td>
<td>1 min</td>
<td>2 min</td>
<td>0.5 min</td>
<td>1 min</td>
</tr>
<tr>
<td>Dimer</td>
<td>138,000</td>
<td>53.0</td>
<td>40.6</td>
<td>27.2</td>
<td>0.800</td>
<td>42.5</td>
</tr>
<tr>
<td>HSA</td>
<td>69,000</td>
<td>618.0</td>
<td>694.8</td>
<td>580.9</td>
<td>0.292</td>
<td>88.3×</td>
</tr>
<tr>
<td>Fragment L</td>
<td>51,000</td>
<td>15.0</td>
<td>16.8</td>
<td>20.6</td>
<td>0.262</td>
<td>83.2×</td>
</tr>
<tr>
<td>Pre-A</td>
<td>25,000</td>
<td>18.50</td>
<td>41.4</td>
<td>43.6</td>
<td>0.666</td>
<td>27.6</td>
</tr>
<tr>
<td>A</td>
<td>15,000</td>
<td>23.0</td>
<td>31.9</td>
<td>43.3</td>
<td>0.064</td>
<td>15.0</td>
</tr>
<tr>
<td>C</td>
<td>&lt;10,000</td>
<td>16.6</td>
<td>20.3</td>
<td>27.0</td>
<td>0.764</td>
<td>12.7</td>
</tr>
</tbody>
</table>

*a Ratio of ninhydrin to radioactivity. Multiplication of radioactivities (Column 1) by the factors eliminates the differences in specific activity, and renders the figures in Column 3 proportional to amounts of protein. For the determination of factors, see "Materials and Methods."

*b Percentages of each component, calculated from Column 3.

c Obtained by division of the figures in Column 3 by the molecular weights.

d Partition between HSA and Fragment L was evaluated from electrophoresis on cellulose acetate strips.

![Graph](https://via.placeholder.com/150)

**Fig. 6.** Time-course of fragment formation; data from Fig. 5. Calculations are shown in Table II.

The cessation of formation of Fragment L before its theoretical maximum extent is an interesting feature of the tryptic breakdown. Were all HSA molecules to possess a bond (or bonds), the cleavage of which would be determinant for the formation of Fragment L, and the susceptibility of which to trypsin would be greater than that of all other bonds, one would expect that an increase in trypsin concentration would result in preferential hydrolysis of these bonds. This, however, is not the case; beyond a final concentration of 0.06% trypsin, no further increase in the yield of Fragment L is observable. Neither can rapid inactivation of trypsin account for the phenomenon, because addition of a second dose of trypsin, 1 min after the first dose, fails to produce further amounts of this product. The possibility was also considered that during the initial breakdown of HSA a low molecular weight product is formed which is adsorbed to unattacked HSA and prevents the cleavage of bonds necessary for the formation of Fragment L. However, addition of digested HSA to intact albumin, before the addition of trypsin, failed to inhibit the formation of Fragment L. Finally, it was considered that the "intact HSA" remaining after the formation of Fragment L is, in effect, not intact but has undergone tryptic attack which rendered it unable to yield this intermediate. To investigate this possibility, material collected from a partial digest of radioactive HSA was separated on Sephadex G-100; tubes containing material of the mobility of intact HSA were pooled, and the solution was concentrated by pervaporation. The following tests were carried out. (a) An aliquot of this material was further digested with trypsin and analyzed by electrophoresis on cellulose acetate strips. Only one major band was observed with a mobility corresponding to that of intact HSA; no Fragment L was detectable. (b) A sample was precipitated with 1.2 M perchloric acid, and the radioactivity in the supernatant was measured. The count was 6 times larger than that obtained from a sample of undigested HSA of identical protein concentration, indicating the release by acid of low molecular weight peptides. (c) The disulfide bonds of this material were reduced with 0.2 M 2-mercaptoethylamine in 8 M urea and subsequently alkylated with a 5-fold excess of iodoacetate. The reduced, alkylated protein, as well as a similarly treated sample of undigested HSA, was separated on a Sephadex G-100 column. The elution pattern of the control HSA showed a single sharp peak of radioactivity, while the test material indicated the presence of breakdown products of heterogeneous size distribution, in addition to some apparently intact HSA. These experiments suggest that the fraction of HSA which does not yield Fragment L also suffers tryptic attack, which, however, in the neutral pH range results in no apparent change in molecular size or electrophoretic mobility. Release of smaller peptides, unattached by disulfide bonds to the bulk of the molecule, does occur in acid solution.
while the larger fragments are released only after reductive cleavage of these bonds. Thus, the inability of HSA to yield the theoretical amount of Fragment L may be due to the accumulation of random hits on the molecule, some of which may fall within the region of the molecule corresponding to Fragment L, resulting finally in the release of breakdown products of this piece in the form of Fragment pre-A or B.

Relevance of Data to Subunit Hypothesis—As mentioned above, current studies on the proteolytic fragmentation of serum albumin suggest that this molecule may consist of several compact regions connected by short, easily digestible peptide sequences. Unlike the case of γ-globulin, in which proteases of differing specificities yielded nearly identical fragments, the pattern of fragmentation of serum albumin depends strongly on the protease used, and, depending on the conditions of digestion, even the same protease may yield a different family of fragments. Thus, in the studies of Schlamowitz, Peterson, and Wissler (17), pepsin at pH 1.7, at a BSA to pepsin ratio of 10,000, released breakdown products ranging in molecular weight from 56,000 to 5,200; in contrast, in the studies of Weber and Young (4) with a BSA to pepsin ratio of 1,000 to 3,000, at pH 3, only fragments of mol wt 30,000 and 12,500 were observed in significant quantities. A fragment comparable in size to the latter was not encountered in the studies of Schlamowitz et al.; the nearest neighbors were 19,000 and 5,200. In the studies of Adkins and Foster (6) on the subtilisin digestion of BSA, a 29 X 8 fragment was obtained, in addition to smaller fragments, when digestion was carried out above pH 9. (This sedimentation constant is compatible with a molecular weight of 30,000.)

When, however, albumin was digested in the presence of 100 moles of sodium dodecyl sulfate per mole of albumin, rapid conversion to fragments of mol wt 30,000 to 33,000 was observed, indicating cleavage of the molecule into two approximately equal halves, without observable release of small fragments. The relationship of these fragments to the one obtained in the absence of detergent, or to the corresponding peptic fragments, has not yet been studied. The present results on the tryptic fragmentation of albumin differ from the aforementioned studies primarily in the size of the largest fragment obtained. This is unexpected, since in several respects the tryptic breakdown pattern resembles that obtained by subtilisin digestion in the absence of detergent. Thus, in both cases, digestion at pH 7 yields only small fragments, while digestion at pH 9 results in the liberation of larger intermediates. Also, the tight binding of 12 molecules of dodecyl sulfate results in both cases in the release of only small fragments. The difference in the size of the largest fragments obtained by digestion with the two proteases appears to be real. When HSA was digested with subtilisin (without detergent) under the conditions used by Adkins and Foster, no electrophoretic component comparable to the tryptic Fragment L was obtained. This cannot be due to the species difference between the two albumins used, since BSA readily yields Fragment L upon tryptic digestion, which behaves like the human fragment in both electrophoresis and gel filtration.

These considerations can in no way rule out the possibility that serum albumin consists of several well defined, compact regions; they simply indicate that the molecule possesses more than one proteolytically vulnerable region, and scission in these regions by different proteases produces fragments which may themselves consist of several compact regions. The major difficulty one encounters in the interpretation of the results of fragmentation is the relatively large uncertainty in the size of the fragments. Added to this is the uncertainty in the molecular weight of albumin itself; the difference between 65,000 and 69,000 becomes important in deciding, for example, how many of the 12,500 peptic fragments can be assigned to a single albumin molecule. That the structure of albumin may not be a rigid one has been proposed also on the basis of physical measurements. Fluorescence depolarization (19, 1) and low angle x-ray scattering (20) have both suggested the possibility of the presence of subunits in this molecule. On the basis of low angle x-ray diffraction, electron microscopy, and viscosity measurements, Bloomfield (21) recently proposed a model consisting of three linearly arranged spheres and correlated these with the peptic fragments of Weber and Young (4, 5). We feel that, while there is little doubt concerning the existence of subdivisions within the albumin molecule, it may be premature to attempt to identify these with fragments obtained by the use of any single proteolytic enzyme. This identification will only be possible on the basis of comparative amino acid analyses and peptide mapping on fragments obtained by the use of several proteolytic enzymes.

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REFERENCES


Recently, Pese, Fahey, and Weber (18) reported the formation of a 50,000 mol wt peptic fragment of bovine serum albumin, obtained at pH 3.6. (The value of 45,000 given in the abstract has been revised.)