AMINO ACID DETERMINATIONS ON CRYSTALLINE BOVINE AND HUMAN SERUM ALBUMIN BY THE ISOTOPE DILUTION METHOD*

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(Received for publication, April 18, 1945)

In this paper there is reported the determination, by the isotope dilution method (1), of glutamic acid, aspartic acid, tyrosine, and glycine in both bovine and human serum albumin, and of lysine in bovine serum albumin. A summary of the analytical results is given in Table I and the data for the individual experiments are given in Tables II and III.

The isotope dilution technique was also applied to the problem of the conversion of serine into glycine under conditions existing during the acid hydrolysis of proteins. It was found that although serine is partially destroyed (3) it is not converted to glycine.

EXPERIMENTAL

The preparation and analytical data of the standard isotopic amino acids (4) and the isolation and purification of the L-amino acids from the protein hydrolysates (5, 6) were essentially the same as those already described in reports from this laboratory. Weighed samples of the proteins, corrected for moisture, were hydrolyzed for 15 hours with 20 times their weight of 20 per cent HCl and known amounts of the different isotopic L-amino acids were then added, after which the solution was boiled for 1 hour. Amino acids isolated from the hydrolysates were recrystallized under conditions known to lead to the pure l isomers (5, 6). Their purity was established by the nitrogen content, the constancy of isotope concentration at successive stages of recrystallization, the specific rotation, and, in the case of glycine, by the melting point of the p-toluenesulfonyl derivative.

Tyrosine—The hydrolysate containing the added amino acids was evaporated to dryness in vacuo and the residue dissolved in water and treated with cuprous oxide to remove cystine, as recommended by Bailey, Chibnall, Rees, and Williams (7). The filtrate, after being freed of copper

* The present analytical studies of plasma proteins have been carried out under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Columbia University. The preparations examined were kindly supplied by the Department of Physical Chemistry, Harvard Medical School.

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with hydrogen sulfide, was at pH 3.5; it was concentrated to a small volume and cooled. Tyrosine crystallized as a mixture of the l and dl isomers (8). This was converted into the copper salts by treatment with basic copper carbonate in a volume calculated to be just sufficient to dissolve all

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Bovine</th>
<th>Human</th>
<th>No of residues per mole, mol. wt. = 70,000</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>per cent</td>
<td>per cent</td>
<td>Bovine</td>
</tr>
<tr>
<td>Glutamic...</td>
<td>16.95</td>
<td>17.03</td>
<td>81</td>
</tr>
<tr>
<td>Aspartic...</td>
<td>10.25</td>
<td>9.77</td>
<td>54</td>
</tr>
<tr>
<td>Tyrosine...</td>
<td>5.53</td>
<td>4.73</td>
<td>21</td>
</tr>
<tr>
<td>Glycine...</td>
<td>1.96</td>
<td>1.60</td>
<td>18</td>
</tr>
<tr>
<td>Lysine...</td>
<td>12.42</td>
<td></td>
<td>60</td>
</tr>
</tbody>
</table>

**TABLE II**

**Analysis of Crystalline Bovine Serum Albumin**

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Amount of protein hydrolyzed (B)</th>
<th>Amino acid added</th>
<th>Amount of l-amino acid (A)</th>
<th>N\textsuperscript{3} \text{H excess in compound at successive stages of recrystallization (C)}</th>
<th>Amino acid in protein ((\text{C} - 1) \times 100)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>gm.</td>
<td>mg.</td>
<td>atom per cent</td>
<td>atom per cent</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>4.925</td>
<td>Glutamic acid</td>
<td>39.9</td>
<td>18.69</td>
<td>0.840, 0.857, 0.866</td>
</tr>
<tr>
<td>4</td>
<td>10.10</td>
<td>&quot; &quot;</td>
<td>94.7</td>
<td>18.69</td>
<td>1.091, 1.071, 1.079</td>
</tr>
<tr>
<td>1</td>
<td>4.925</td>
<td>Aspartic &quot; &quot;</td>
<td>36.75</td>
<td>11.82</td>
<td>0.790, 0.800</td>
</tr>
<tr>
<td>4</td>
<td>9.10</td>
<td>&quot; &quot;</td>
<td>90.60</td>
<td>11.82</td>
<td>1.057, 1.051, 1.056</td>
</tr>
<tr>
<td>9</td>
<td>7.12</td>
<td>Tyrosine</td>
<td>63.37</td>
<td>6.85</td>
<td>0.954, 0.949, 0.945</td>
</tr>
<tr>
<td>1</td>
<td>4.925</td>
<td>Glycine</td>
<td>307.1</td>
<td>3.13</td>
<td>2.38, 2.43, 2.37</td>
</tr>
<tr>
<td>4</td>
<td>9.10</td>
<td>&quot; &quot;</td>
<td>300.7</td>
<td>3.01</td>
<td>1.92, 1.87, 1.87</td>
</tr>
<tr>
<td>9</td>
<td>7.12</td>
<td>Lysine</td>
<td>49.89</td>
<td>9.09</td>
<td>0.483, 0.488, 0.485</td>
</tr>
</tbody>
</table>

* Preparations “17” (used in Experiment 1) and “C. B. 25” (used in Experiments 4 and 9), from the Department of Physical Chemistry, Harvard Medical School (cf. (2)).

of the copper dl-tyrosine at 0° (6). The l salt which crystallized was freed from copper and the resulting l-tyrosine purified by recrystallization until the specific rotation of each sample conformed with the data of Stein, Moore, and Bergmann (8). The tyrosine values for bovine and human serum albumins, 5.53 and 4.73 per cent respectively, found by the isotope
dilution method are in very good agreement with those of Brand et al. (2), 5.49 and 4.66 per cent respectively, determined by their photometric method (9).

Glutamic Acid—The barium salts of glutamic and aspartic acids were precipitated from the tyrosine filtrate. The glutamic acid, isolated as the hydrochloride from the dicarboxylic acid fraction, was recrystallized from 20 per cent hydrochloric acid under conditions such that at least twice the weight of dl-glutamic acid hydrochloride added to the protein hydrolysate would have remained in solution (5). The specific rotation, based on free glutamic acid, was $[\alpha]_b^{25} = +31.0^\circ \pm 0.2^\circ$ (2 to 3 per cent in 2.5 N HCl) for all samples.

The necessity of removing cystine prior to the precipitation of the barium dicarboxylates was demonstrated by the following observation. In the purification of glutamic acid hydrochloride from a hydrolysate of human serum albumin which had not been treated with cuprous oxide, the isotope content at successive stages of recrystallization was constant (0.736,
0.719, and 0.735 atom per cent $N^{15}$ excess) and the nitrogen content was 7.7 per cent, from which it might have been concluded that the substance was pure l-glutamic acid hydrochloride. However, the specific rotation, even after five recrystallizations, was $-4.3^\circ$ instead of $+31^\circ$. The contaminant proved to be cystine dihydrochloride, the identity of which was established by the isolation of crystalline cystine by precipitation at pH 4 to 5. Its presence is explained by the high cystine content of serum albumin (2). It was found that the solubility of cystine dihydrochloride in 20 per cent hydrochloric acid at 0° is considerably lower than that of glutamic acid hydrochloride in the same solvent.1

Aspartic Acid—The filtrate from the glutamic acid hydrochloride was taken to dryness in vacuo and from the residue aspartic acid was isolated as the copper salt. l-Aspartic acid was regenerated from the recrystallized copper aspartate and repeatedly recrystallized. The specific rotation, based on free aspartic acid, was $\left[\alpha\right]_D^{25} = +25.3^\circ \pm 0.2^\circ$ (1.5 to 3 per cent in 2.5 N HCl) for all samples.

Glycine—From the filtrate of the barium salts of the dicarboxylates, freed from barium and alcohol and concentrated in vacuo, glycine was isolated as the trioxalatochromiate (10) and converted into the $p$-toluenesulfonyl derivative, m.p. 147-148°. The values for glycine are not as precise as those for the other amino acids, for the ratio of the isotope concentration of the added glycine to the isotope concentration of the isolated glycine is low (1). As the proteins contain but little glycine, it was necessary to add comparatively large amounts of glycine in order to isolate enough of the pure amino acid for the $N^{15}$ determinations at successive stages of recrystallization.

Lysine—Lysine was isolated as the $\epsilon$-monobenzoyl derivative according to the method of Kurtz (11) from a hydrolysate of bovine serum albumin from which cystine, tyrosine, and the leucine-phenylalanine fraction had been removed. The purity of the $\epsilon$-benzoyl-$l$-lysine was established by nitrogen analysis and by constancy of isotope concentration and specific rotation on successive recrystallization. $N = 11.1$ per cent (calculated 11.2 per cent); $\left[\alpha\right]_D^{25} = +18.8^\circ \pm 0.2^\circ$ (2.3 per cent in N HCl).

The specific rotation in N HCl of the isolated $\epsilon$-benzoyl-$l$-lysine was constant during five recrystallizations and the same as that of a sample prepared from pure $l$-lysine. The value observed for the reference sample in 50 per cent acetic acid was $+11.4^\circ$, in contrast to $+27.2^\circ$ reported by Goldschmidt and Kinsky (12). The reason for this discrepancy is not clear.

1 Solubility determinations were carried out on cystine dihydrochloride and glutamic acid hydrochloride in 6.05 N hydrochloric acid at 0°; the solubilities were 0.51 and 0.90 gm. per 100 gm. of solution of cystine dihydrochloride and glutamic acid hydrochloride respectively. The solubility of glutamic acid hydrochloride is the same as that found by Graff, Rittenberg, and Foster (5).
Non-Conversion of Serine to Glycine—The possibility that glycine might be formed, by a decomposition of serine in acid solution analogous to that known to occur in an alkaline medium (13), was investigated by the isotope dilution method. To 990 mg. of serine, 500 mg. of isotopic glycine containing 1.095 atom per cent N15 excess were added and the mixture was refluxed for 17 hours with 100 cc. of 20 per cent hydrochloric acid. From this mixture glycine was precipitated with trioxalatochromiate and purified as the p-toluenesulfonyl derivative. The isotope value of the isolated glycine was found to be 1.091 atom per cent N15 excess. Since no significant dilution of the added glycine had occurred, it was concluded that none of the glycine in the protein hydrolysate was derived from serine.

SUMMARY

The isotope dilution method was employed to determine the amounts of glutamic acid, aspartic acid, tyrosine, and glycine yielded by crystalline bovine and human serum albumins, and of lysine yielded by bovine serum albumin.

It was found that cystine dihydrochloride is much less soluble in 20 per cent hydrochloric acid than glutamic acid hydrochloride, and in proteins containing appreciable amounts of cystine glutamic acid hydrochloride is very likely to be contaminated unless the cystine is first removed.

Conversion of serine to glycine does not occur under the conditions for acid hydrolysis of proteins.

BIBLIOGRAPHY

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