THE DETERMINATION OF GLYCINE IN PROTEINS*

By A. R. PATTON

(From the Division of Agricultural Biochemistry, University of Minnesota, St. Paul)

(Received for publication, June 7, 1934)

Very few methods for the determination of glycine have been published. Fischer's method (1) suffers from the usual inadequacies of isolation methods: requiring a large amount of protein, being extremely long and tedious, and yielding only minimal values. Other isolation methods which have been proposed present equally great difficulties.

Zimmerman (2) has described a color test whereby a violet color is produced through the interaction of o-phthalic dialdehyde and glycine. This test is, however, not specific, since colors are produced with certain other amino acids. Recently Klein and Linser (3) have modified Zimmerman's test, and their modification appears to be specific and quantitative for glycine.

If the o-phthalic dialdehyde reagent is added to a solution of glycine which has been made slightly alkaline and, after standing for a time, this mixture is acidified, a dark green color and precipitate are formed. The precipitate dissolves if a little alcohol is added. Certain other amino acids, if present in the solution, produce red or violet colors. However, the green glycine compound can be quantitatively extracted by chloroform, the colored compounds formed by the other amino acids being left behind in the aqueous solution. Klein and Linser have shown that colors are produced by glycine, tryptophane, cystine, arginine, alanine, asparagine, and ammonium salts, but that only the colors produced by glycine, tryptophane, and the ammonium ion are extracted by chloroform. They found that the following substances produce no color: valine, leucine, tyrosine, serine, phenyl-

* Published as paper No. 1312, Journal Series, Minnesota Agricultural Experiment Station.
serine, asparagine, aspartic acid, glutamic acid, lysine, proline, oxyproline, trans-betaine, trigonelline, nicotinic acid, choline, dimethylamine, diethylamine, dibutylamine, trimethylamine, triethylamine, and tripropylamine. Thus the only protein constituents which interfere with the test for glycine are tryptophane and the ammonium ion, which must be removed from the amino acid mixture before glycine can be estimated.

Before the test of Klein and Linser (3) could be used as a quantitative method for glycine, a method had to be developed for obtaining the protein hydrolysate in suitable form. Details for such a method, lacking in Klein and Linser's paper (3), are presented under "Experimental." Klein and Linser suggested the removal of tryptophane by precipitation with mercuric sulfate. Mercuric sulfate does not remove the tryptophane quantitatively. Furthermore, it is difficult to remove traces of excess mercuric sulfate and hydrogen sulfide, which influence the outcome of the color reaction. I have found that benzaldehyde removes tryptophane quantitatively, does not interfere with the color reaction, and is easily removed by distillation. The final color test is essentially that of Klein and Linser (3), but it is simplified and standardized for greater convenience in use. Original determinations on a number of proteins are presented in this paper.

EXPERIMENTAL

Preparation of Protein Hydrolysate—3 gm. of protein are placed in a 300 cc. Kjeldahl flask, 50 cc. of constant boiling hydrochloric acid are added, a reflux condenser is attached, and the mixture is gently boiled in an air bath over an electric hot-plate. As soon as the protein is dissolved in the acid, 1 cc. of benzaldehyde is added. After boiling for 24 hours, the mixture is washed into a

1 Holm and Gortner (4) have shown that tryptophane is quantitatively removed by hydrolyzing the protein in the presence of benzaldehyde. That benzaldehyde removes the tryptophane, without disturbing the glycine content, is shown by the following experiments. Dilute (0.1 per cent) solutions of glycine and of tryptophane were prepared. Aliquots were boiled in separate flasks for 24 hours with 50 cc. of constant boiling hydrochloric acid and 1 cc. of benzaldehyde. At the end of the time the tryptophane solution was colorless but contained a dark, plastic, insoluble mass. The glycine solution was clear and colorless. The quantitative colorimetric procedure as described in this paper was then applied, both to the original
liter Claisen flask, and vacuum-distilled in a water bath at 65° until a paste remains. This removes the benzaldehyde and most of the free hydrochloric acid. The residue is then diluted with 100 cc. of water, and a few cc. of butyl alcohol, to prevent foaming, and an excess of sodium bicarbonate are added. The resulting alkaline solution is then vacuum-distilled (40-50° and < 30 mm. pressure) to remove ammonia, which interferes with the glycine color reaction. When a volume of about 20 cc. is reached, sodium chloride begins to precipitate. The solution is quickly filtered with suction, and the carbonate and chloride precipitate is washed with a few cc. of 70 per cent alcohol. This filtrate is then neutralized with hydrochloric acid, litmus paper (pH 6 to 8) being used. This neutral solution is again reduced in vacuo to about 10 cc. It is again quickly filtered on a small Witt plate, with suction, and the sodium chloride precipitate is again washed with a few cc. of 70 per cent alcohol. The filtrate is then diluted to 100 cc. with water, and total nitrogen is determined on aliquots by the Kjeldahl method. The solution is now ready for the colorimetric procedure.

Colorimetric Method for Glycine—5 cc. of the amino acid solution to be tested are measured into a 30 cc. test-tube. Into another test-tube are measured 5 cc. of a zein hydrolysate (blank) and 1 cc. of standard glycine solution containing 1 mg. of pure glycine. The contents of each test-tube are treated as follows: 2 cc. of m/15 phosphate buffer of pH 8.0 are added. Immediately 5 cc. of glycine reagent² are added. The contents are thoroughly mixed

² A Kahlbaum preparation labeled "phthalaldehyd, ortho" was used. 2 gm. were placed in 300 cc. of water and the mixture distilled at an atmospheric pressure over a free flame or hot-plate. The first 200 cc. of the distillate are the reagent employed. It should be kept in a brown bottle until used. Experience has shown that this reagent is still satisfactory after several months standing. If this chemical is not readily obtainable, the reagent may be prepared from o-tetrabromo-o-xylene. 10 gm. of the latter and 9 gm. of crystalline potassium oxalate are refluxed with 62 cc. of water and 62 cc. of alcohol for 40 hours, at the end of which time 50 cc. of the alcohol are distilled off. 10 gm. of sodium phosphate and 300 cc. of water are then added, and from the mixture 300 cc. of liquid are distilled, as noted above. This distillate is used as the glycine reagent (Zimmerman (2)).
by shaking, and allowed to stand. At the end of 2 minutes 5 cc. of a mixture of 60 cc. of alcohol and 10 cc. of concentrated sulfuric acid, freshly mixed and cooled, are added. After thoroughly mixing, 10 cc. of chloroform are added. The stoppered tubes are

<table>
<thead>
<tr>
<th>Protein</th>
<th>Source</th>
<th>Glycine per 100 gm. protein</th>
<th>Colorimetric determination</th>
<th>Isolation Values by other investigators</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Experiment 1</td>
<td>Experiment 2</td>
</tr>
<tr>
<td>Zein hydrolysate</td>
<td>Corn</td>
<td></td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>0.66% glycine</td>
<td></td>
<td></td>
<td>0.655</td>
<td>0.655</td>
</tr>
<tr>
<td>Zein hydrolysate</td>
<td></td>
<td></td>
<td>1.203</td>
<td>1.312</td>
</tr>
<tr>
<td>1.33% glycine</td>
<td></td>
<td></td>
<td>22.2</td>
<td>22.2</td>
</tr>
<tr>
<td>Bacto-Gelatine</td>
<td></td>
<td></td>
<td>0.50</td>
<td>0.51</td>
</tr>
<tr>
<td>Hordein</td>
<td>Barley</td>
<td></td>
<td>1.60</td>
<td>1.76</td>
</tr>
<tr>
<td>Casein</td>
<td>Milk</td>
<td></td>
<td>2.05</td>
<td>2.05</td>
</tr>
<tr>
<td>Edestin</td>
<td>Hempseed</td>
<td></td>
<td>0.47</td>
<td>0.46</td>
</tr>
<tr>
<td>Arachin</td>
<td>Peanut</td>
<td></td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Alcohol-soluble</td>
<td>Milk</td>
<td></td>
<td>0.68</td>
<td>0.00</td>
</tr>
<tr>
<td>&quot;</td>
<td>Sorghum</td>
<td></td>
<td>1.75</td>
<td>1.75</td>
</tr>
<tr>
<td>&quot;</td>
<td>Wheat</td>
<td></td>
<td>2.64</td>
<td>2.64</td>
</tr>
<tr>
<td>Lactalbumin</td>
<td>Corium</td>
<td></td>
<td>6.5</td>
<td>7.2</td>
</tr>
<tr>
<td>&quot;</td>
<td>Soy bean</td>
<td></td>
<td>1.53</td>
<td>1.56</td>
</tr>
<tr>
<td>Rat fetus</td>
<td>Milk</td>
<td></td>
<td>10.6</td>
<td>10.6</td>
</tr>
<tr>
<td>Egg, whole (fat extracted)</td>
<td>Blood</td>
<td></td>
<td>0.81</td>
<td>0.81</td>
</tr>
<tr>
<td>Fibrin</td>
<td>Wheat</td>
<td></td>
<td>0.81</td>
<td>0.76</td>
</tr>
<tr>
<td>Glutelin</td>
<td>Oat</td>
<td></td>
<td>1.09</td>
<td>1.10</td>
</tr>
<tr>
<td>&quot;</td>
<td>Maize</td>
<td></td>
<td>0.75</td>
<td>0.74</td>
</tr>
<tr>
<td>Vitellin</td>
<td>Egg</td>
<td></td>
<td>0.81</td>
<td>0.85</td>
</tr>
<tr>
<td>Albumin</td>
<td>Egg</td>
<td></td>
<td>1.75</td>
<td>1.75</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>Blood</td>
<td></td>
<td>0.41</td>
<td>0.41</td>
</tr>
</tbody>
</table>
shaken vigorously for half a minute. The chloroform layer is allowed to settle. With a dry pipette, 5 cc. of the green chloroform solution are removed from the bottom layer and placed in a colorimeter cup. To this is added 1 cc. of alcohol, and the solution is stirred with a small glass rod until all turbidity has disappeared. The standard solution is treated in the same manner, and the colors are compared in the colorimeter. The amount of standard glycine solution is adjusted on subsequent tests so that with the standard at 20 mm. the unknown reads between 15 and 25 mm.

Comments—A much smaller sample of protein than 3 gm. may be used if necessary. The hydrolysate must be neutralized with great care, since the ensuing color test is very sensitive to differences in reaction of the hydrolysate. If the hydrolysate is allowed to remain alkaline for a time before the buffer is added, the amount of green color produced is considerably weakened. If, on the other hand, the solution remains acid after the buffer solution has been added, no color is produced by the glycine reagent. The omnipresent straw to brown color found in protein hydrolysates does not interfere with the color test, because it does not dissolve in the chloroform layer which dissolves the green glycine compound. It was found that using the practically colorless zein hydrolysate in the blank with added standard glycine solution gave a color which matched most proteins better than did standard glycine alone.

Analyses of Proteins—In Table I are shown analyses of a representative series of proteins. Values for total nitrogen, humin nitrogen, and ammonia nitrogen were available from previous analyses of the same samples. To correct the glycine values for losses of humin nitrogen and ammonia nitrogen, as well as for mechanical losses (see second term in the equation), the following calculation was employed.

\[
\left( \frac{\text{Weight of glycine in final hydrolysate}}{\text{Weight of protein taken}} \right) \times \left( \frac{\text{total N in protein taken} - (\text{humin N} + \text{ammonia N})}{\text{total N in final hydrolysate}} \right) \times 100 = \text{gm. glycine per 100 gm. protein}
\]
SUMMARY

A method is presented for preparing a protein hydrolysate for the colorimetric determination of glycine, including an improved method for the quantitative removal of tryptophane.

The colorimetric test of Klein and Linser (3) is simplified and standardized for greater convenience in use.

Original determinations of the glycine content of a number of proteins are presented.

BIBLIOGRAPHY

THE DETERMINATION OF GLYCINE 
IN PROTEINS

A. R. Patton


Access the most updated version of this article at http://www.jbc.org/content/108/1/267.citation

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/108/1/267.citation.full.html#ref-list-1