

2989

Errors in Amino Acid Analysis Due to Formaldehyde and Decolorizing Carbon

Frequently biological materials are preserved in formaldehyde or are exposed to aldehydes during their production, purification, modification, or analysis. It is generally assumed that the hydrolysis procedure will remove bound formaldehyde and the correct amino acid composition will be obtained on analysis of the hydrolyzate. Baudouy (1) in 1942 showed that formaldehyde could not be recovered completely from proteins containing histidine and tryptophan, and Fraenkel-Conrat and Olcott (2) showed that a Mannich reaction forming acid-resistant methylene bonds could account for the disappearance of the formaldehyde. Also, Alexander, Carter, and Johnson (3) recovered reduced amounts of tyrosine from wool that had been treated with formaldehyde. These observations did not definitely establish whether formaldehyde liberated or produced during hydrolysis could react with freshly liberated amino acids. Therefore, the effect of small amounts of formaldehyde upon the recovery of amino acids from hydrolyzates of a standard amino acid mixture and a sample of skin from a hair sheep has been determined.

There also appears to be a strong tendency to decolorize samples containing appreciable amounts of humin or other colored products before submitting them for amino acid analysis. However, Block and Bolling (4), in their description of the early attempts to separate amino acids, show that the aromatic amino acids are completely retained on activated carbons and other amino acids are partially retained. To establish more fully the magnitude of the errors produced the effect of two different levels of activated carbon upon the recovery of amino acids from hydrolyzates has been determined.

Procedure. A standard solution containing 2 μ moles/ml of each of 17 amino acids, 4 μ moles of proline and 8 μ moles of hydroxyproline was used.

A mixture composed of 10 ml of the amino acid standard, 4 ml of a solution containing 2.5 mg of formaldehyde, and 14 ml of concentrated hydrochloric acid was hydrolyzed at boiling for 16 hr.

The hydrolyzate was evaporated to dryness, dissolved and made to 10 ml with 0.1 N HCl. *Control hydrolyzates* were made by hydrolyzing a similar mixture in which the 4 ml of formaldehyde was replaced with 4 ml of distilled water.

Additional 10 ml portions of the standard amino acid solution (0.1 N

in HCl) were mixed with 0.1 or 0.5 gm of activated carbon (Norit FQP¹), brought to boiling, and filtered. The residual carbon was washed five times with 10 ml portions of boiling water. The fifth wash gave a negative ninhydrin test for amino acids. The sample and washes were combined and evaporated to dryness, dissolved, and made to 10 ml with 0.1 N HCl. The standard solution processed in the same manner without activated carbon served as a control sample.

Approximately 200 mg, MFB,² samples of pickled skin from a hair sheep were hydrolyzed in boiling 6 N HCl for 16 hr. The hydrolyzates were evaporated to dryness, dissolved in 10 ml of water, and treated with activated carbon as described above. Other samples of the skin were hydrolyzed in the presence of various amounts of formaldehyde.

Results. The various amino acid solutions produced above were analyzed for their amino acid content by the Piez-Morris (5) system. The standard deviation calculated for the latest three standard runs through the amino acid analysis system was less than 2% of the average value for each of the amino acids except hydroxyproline, proline, and hydroxylysine, for which the standard deviations were less than 4% of the average value. Heating of the standard solution to boiling and filtering as was done for the decolorizing experiments did not cause deviations greater than the standard deviation of the unheated standard. Therefore, there is no change produced by this short boiling in the absence of charcoal.

When the standard amino acid solution was subjected to the conditions of protein hydrolysis, boiling 6 N hydrochloric acid for 16 hr, the deviations from the expected value were less than 5% for all the amino acids except for losses of 11% for hydroxylysine, 13% for hydroxyproline, 16% for serine, and 8% for threonine.

To obtain the data given in Table 1 the average content of each amino acid found in two determinations on the treated samples was subtracted from the content of the same amino acid in the corresponding control to obtain the decrease due to the treatment. This decrease as a per cent of the control value is given in Table 1. Only differences greater than 5% have been considered significant.

The presence of formaldehyde in the hydrolyzing mixture has completely prevented the determination of tyrosine by the ninhydrin reagent. Approximately one-third of the cystine and histidine and small amounts of glutamic acid and lysine have also been affected by the formaldehyde and are no longer determinable by the ninhydrin reaction. The 2.5 mg

¹Mention of trade names does not imply endorsement by the Department of Agriculture over other products which may be suitable.

²MFB, moisture free basis.

TABLE 1
Per Cent Decrease in Recovery of Amino Acids from a Standard Solution

Amino acid	Treatments		
	Decolorized with charcoal		Hydrolyzed in presence of formaldehyde
	0.1 gm	0.5 gm	
Arginine	5.0	9.6	
Aspartic acid	5.4	8.2	
Cystine	17.5	29.9	34.6
Glutamic acid	15.7	16.4	9.3
Glycine	5.1	5.8	
Histidine	3.2	8.2	38.7
Hydroxylysine	4.4	8.8	
Hydroxyproline	5.5	8.4	
Isoleucine	3.4	9.0	
Leucine	4.4	10.2	
Lysine	2.6	5.6	7.0
Methionine	12.9	35.1	
Phenylalanine	82.3	100.0	
Proline	8.6	8.0	
Serine	13.9	14.6	
Threonine	6.6	9.4	
Tyrosine	76.4	100	100

of formaldehyde used is only twice the molar concentration of the tyrosine and histidine combined. Therefore, it appears that traces of formaldehyde can affect the recovery of tyrosine and higher concentrations can affect the recoveries of cystine, histidine, glutamic acid, and lysine.

Only 0.1 gm of decolorizing carbon in 10 ml of solution reduces the recovery of 12 amino acids by 5% or more, and 0.5 gm of carbon affects 17 of the 19 amino acids present. Only the alanine and valine recoveries were not affected. Tyrosine and phenylalanine are completely removed and substantial amounts of serine, glutamic acid, cystine, methionine, and leucine have been absorbed by the charcoal.

Similar results were obtained with the amino acids from the skin of a hair sheep. Formaldehyde completely removed tyrosine and cystine and decolorizing carbon completely removed the tyrosine and phenylalanine and reduced the amounts of the other amino acids recovered.

Thus it is clear that the presence of formaldehyde or the use of decolorizing carbon will reduce the validity of an amino acid analysis.

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Received January 16, 1968*