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On the Determination of the Peptide-Amino-Nitrogen by the Copper Method

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Pope and Stevens [1] have developed a method for the determination of amino-nitrogen based on the formation of soluble copper compounds between the amino-acid or digested protein and the excess of copper present in the form of copper phosphate. In this laboratory we have used the same method, commonly called the copper method, since 1940, and found it to give correct figures with amino-acid-mixtures and with completely hydrolyzed proteins. In partially hydrolyzed proteins, on the other hand, the values are too high indicating that the soluble copper compounds of peptides formed in this reaction are not similar to those of the amino-acids as suggested by Pope and Stevens.

In order to investigate this question some determinations were carried out with synthetic peptides and with hydrolysates of zein and zein plastein. In the following, the results of these experiments are briefly given.

EXPERIMENTAL

Solutions of each synthetic peptide (Amino Acid Manufactures, University of California) were prepared containing about 10—20 mg total nitrogen per 25 ml. In each of these solutions the amino-nitrogen was determined by the copper method and by Van Slyke's volumetric nitrous acid method (5 min shaking). The total nitrogen was estimated by the micro method of Kjeldahl. Table 1 shows the results.

Table 1. Amino-nitrogen content of different synthetic peptides.

Peptide	Total N mg	Copper method		Van Slyke	
		N mg	N %	N mg	N %
Glycyl-*l*-leucine	12.19	11.85	97.2	6.15	50.5
	10.12	9.92	98.1	5.07	50.8
Glycyl-glycine	12.61	12.38	98.1	6.24	49.5
	16.44	16.14	98.2	8.17	49.7
l-Leucyl-*l*-tyrosine	12.29	12.10	98.3	6.10	49.6
	12.30	12.12	98.5	6.08	49.4
l-Leucyl-glycyl-glycine	17.75	11.82	66.6	6.00	33.8
	19.72	13.15	66.7	6.69	33.9

The mixture of the synthetic peptides mentioned in Table 1 dissolved in 25 ml water gave the values shown in Table 2.

Table 2. Amino-nitrogen content of the peptide mixture.

	Amino-N mg
Copper method	11.46
Van Slyke, 5 min. shaking	5.74
» » 30 » »	7.07
Free α-amino-N, calculated	5.75

The relation between the results obtained by the copper method and by Van Slyke's method in an enzymic digest appears from the following experiment. A sample of 200.6 mg purified zein (Corn Products Refining Company, U. S. A.) was suspended in 10 ml of 0.175 *N* hydrochloric acid in a Pyrex test tube, 9.8 mg crystalline pepsin (The Armour Laboratories, U. S. A.) and 0.5 ml toluene were added, the tube was sealed by melting up, and the ampul thus prepared was incubated at 37° C for seven days. Thereafter the hydrolysate was filtered off, and the ammonia nitrogen of the clear solution was determined according to Pucher *et al.*[2] The remaining solution was transferred with distilled water into a 25 ml volumetric flask, adjusted slightly acid and made up to the volume. Aliquots of this solution were then analyzed for total nitrogen (total soluble nitrogen was calculated by adding the value obtained in the estimation of ammonia nitrogen to that of total nitrogen),

Table 3. Amino-nitrogen content of the peptic digest of zein.

	Nitrogen	
	mg	% of total peptide-N
Total soluble N	24.82	—
Ammonia N	3.74	—
Free amino-acid N	0.52	—
Total peptide N	20.56	—
Van Slyke amino N	5.25	—
Peptide amino N	4.73	23.0
Copper amino N	9.86	—
Peptide amino N	9.34 (4.67) *	45.5

for amino-nitrogen according to Van Slyke and by the copper method, and for »ninhydrin nitrogen» (free amino-acid-N) according to Van Slyke *et al.*[3] The total peptide nitrogen can approximately be calculated by subtracting the »ninhydrin nitrogen» and the ammonia nitrogen from the total soluble nitrogen, and the peptide-amino-nitrogen by subtracting the »ninhydrin nitrogen» from the total amino-nitrogen. The results obtained are listed in Table 3.

Another experiment with an acid digest of zein plastein [4] gave the results shown in Table 4. 2 g of zein plastein were hydrolyzed with 0.175 *N* HCl

Table 4. Amino-nitrogen content of the acid digest of plastein.

	Nitrogen	
	mg	% of total peptide-N
Total soluble N	18.72	—
Ammonia N	0.297	—
Free amino-acid N	0.95	—
Total peptide N	17.47	—
Van Slyke amino N	4.82	—
Peptide amino N	3.87	22.1
Copper amino N	8.66	—
Peptide amino N	7.71 (3.86) *	44.2

* The number in parentheses — half of the value obtained by the Cu-method — represents the real NH_2-N in peptides.

for 43 days at 37° C, the clear hydrolysate was filtered off, and the same determinations were carried out as in the previous experiment.

In later experiments with pentaglycine ethyl ester the amino-nitrogen value obtained by the copper method was 3.82 mg (calculated 1.96 mg). When using the factor 0.14 instead of 0.28 the value 1.91 mg (97.5 %) should be obtained. Another determination with glycyl-tyrosine methyl ester gave by the copper method the value 4.16 mg (calculated 1.935 mg), by using the factor 0.14 2.08 mg (107.5 %).

DISCUSSION

The experiments described above show that the amino-nitrogen values obtained by the copper method (calculated in the same way as in the case of amino-acids) are twice as high as those obtained by Van Slyke's method. Woiwod [5], using a slightly modified copper reagent, has recently shown that the ratio α-amino-N/copper for a number of amino-acids and related compounds (hydroxyproline, aspartic acid, serine, threonine, valine, leucine, *iso*-leucine, glutamic acid, methionine, asparagine, glycine, tyrosine, arginine, phenylalanine, cystine, tryptophan, glutamine, alanine, proline) varies from 0.38 to 0.48, but the same ratio for the dipeptide alanyl-glycine is 0.22. For the formation of the complex A_2Cu the theoretical ratio should be 0.44 showing that alanyl-glycine binds twice as much copper as an amino-acid theoretically does. This finding is in good agreement with the results reported in this paper.

Unfortunately there was no possibility to determine the amino-nitrogen in synthetic peptides containing proline. It is well known that proline does not react in Van Slyke's method. We could decide this way whether proline occupies the end position in a peptide chain or not.

Pope and Stevens [1] have shown that in iodometrical titration of the copper compounds of amino-acids each ml of 0.01 *N* thiosulphate is equivalent to 0.28 mg amino-nitrogen. Now, when titrating the peptide-copper-compound in the same way, each ml of 0.01 *N* thiosulphate is equivalent to 0.14 mg amino-nitrogen. When calculating the results by using this factor instead of 0.28 correct values for the peptide-amino-nitrogen can be obtained.

SUMMARY

The copper method of Pope and Stevens has been applied to the determination of the free amino-nitrogen of the peptide chains. It has been found that the peptides bind twice as much copper as the amino-acids, consequently, in calculating the results the factor 0.14 instead of 0.28 should be used.

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REFERENCES

1. Pope, C. G., and Stevens, M. F. *Biochem. J.* **33** (1939) 1070.
2. Pucher, G. W., Vickery, H. B., and Leavenworth, C. S. *Ind. Eng. Chem., Anal. Ed.* **7** (1935) 152.
3. Van Slyke, D. D., Mac Fadyen, D. A., and Hamilton, P. *J. Biol. Chem.* **141** (1941) 671.
4. Virtanen, A. I., and Kerkkonen, H. K. *Nature* **161** (1948) 888.
5. Woiwod, A. J. *Biochem. J.* **42** (1948) xxviii.

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