LITERATURE CITED

1. F. C. Czygan, Planta Med., Suppl., 169-185 (1975).
2. B. D. Patterson and D. P. Carew, Lloydia, 32, 2 (1969).
2. G. H. Svoboda and J. S. N. Neuss, J. Amer. Pharm. Assoc., Sci. Ed., 48, 11 (1959).
4. G. H. Svoboda and J. S. Johnson, J. Pharmac. Sci., 51, 8 (1962).
5. N. J. Cone, R. Miller, and N. Neuss, J. Pharm. Sci., 52, 7 (1963).

DETERMINATION OF THE NUMBER OF METHIONINE RESIDUES IN PEPTIDES BY THE METHOD OF PARTIAL SUBSTITUTION

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Methionine is one of the few amino acids the quantitative determination of which is not infrequently made difficult through its loss during the isolation and hydrolysis of peptides because of its ease of oxidation to the corresponding sulfoxide or sulfone [1-4].

In our opinion, it may be better to determine the number of methionine residues (or, in general, the number of sulfide groupings) in natural materials by a method based on partial substitution in combination with paper electrophoresis, the principle of which has been described in detail previously in papers dealing with the determination of the number of free NH_2, -SH, -COOH, and OH groups in polypeptides and aminocarbohydrates [5-9].

In contrast to residues of diamino or dicarboxylic amino acids, methionine residues present in a polypeptide chain are electroneutral, and therefore the electrophoretic variant of the method of partial substitution can be used only if the sulfide grouping of methionine is alkylated with a suitable alkyl halide. It is known [10] that the acylation reaction forms a sulfonium salt derivative of the sulfide bearing a charge of +1:

$$\begin{matrix} CH_3 \\ R^1 \end{matrix}\!\!>\!S + X - R^2 \longrightarrow \left[\begin{matrix} CH_3 \\ R^1 \end{matrix}\!\!>\!\overset{+}{S} - R^2\right] X^-,$$

where X is I, Br, Cl.

Of the alkyl halides that we have tested, the most suitable proved to be iodoacetamide (IAA), which in the pH range below 4.0 reacts strictly specifically with methionine residues to form the S^+-carbamoylmethyl derivative of methione (S^+-CAM-Met), which has an additional positive charge (+1) in a wide pH range [11, 12]. Because of the acquisition of the additional charge, the S^+-CAM derivative of methionine (or a peptide containing a methionine residue or another compound with a sulfide group) will migrate to the cathode on paper electrophoresis at a greater rate than the initial compound. If the peptide analyzed contains one methionine residue (or one-SR group), the electrophoretogram should show one additional spot migrating to the cathode faster than the initial peptide, and if the peptide contains two methionine residues there should be two additional spots, and so on. The number of methionine residues in the peptide N=n-1, where n is the number of spots on the electrophoretogram including the spot of the initial peptide. The degree of substitution is regulated by the time of reaction of the IAA, taken in excess, with the peptide. The method has been checked on a number of model compounds and peptides (Fig. 1), and this has shown its reliability. The number of methionine residues in peptides can be determined with a high accuracy using microamounts (2-10 μmole) with molecular weights between 100 and 3000 daltons.

The reaction of 2-10 μmole of a peptide with 20-30 μmole of IAA is performed in 0.5 ml of 85% HCOOH-CH_3COOH-H_2O (28:20:52) [6] at 37°C for 3-5 h with the periodic removal (every 1 h) of aliquots and their de-

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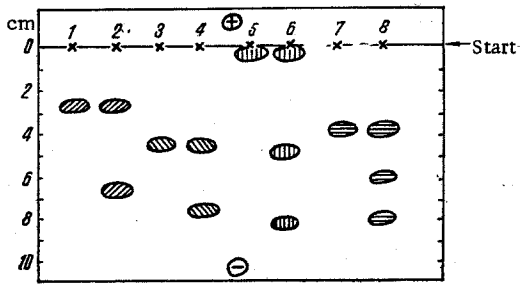

Fig. 1. Electrophoretic analysis of the products of partial substitution of model compounds: 1) methionine; 2) methionine + IAA; 3) Ala-Met; 4) Ala-Met + IAA, 5) diketopiperazine derivative of methionine; 6) diketopiperazine derivative of methionine + IAA; 7) Met-Met; 8) Met-Met + IAA. Electrolyte – 2 N CH_3COOH, 600 V, 2 h.

position on "Filtrak" FN 2 chromatographic paper. The electrophoretic analysis of the products of partial substitution is performed either in 2 N acetic acid (600 V, 2 h) or in an electrolyte with the composition given above at 300 V for 2.5-3 h in instruments working on the principle of the Durrum moist chamber [13]. After drying, the substances are revealed on the electrophoretogram with the aid of a 0.5% solution of ninhydrin in ethanol or by the iodine-azide reagent [14]. In the latter case, sulfur-containing compounds appear in the form of white spots on a brown background.

During the determination of the number of methionine residues in peptides by the method of partial substitution, we detected no cleavage of the peptide bond at the COOH group of methionine which, as is known, takes place highly specifically under the action of cyanogen bromide in an acid medium on methionine-containing peptides [15].

LITERATURE CITED

1. W. J. Ray, Jr., and D. E. Koshland, Jr., J. Biol. Chem., 237, 2493 (1962).
2. L. R. Njaa, Acta Chem. Scand., 16, 1359 (1962).
3. N. F. Floyd, M. D. Cammaroti, and T. F. Lavine, Arch. Biochem. Biophys., 102, 343 (1963).
4. L. Baratova and L. P. Belyanova, in: Methods of Biochemical Experimentation [in Russian], Moscow (1974), p. 3.
5. L. A. E. Slyterman, Biochem. Biophys. Acta, 17, 169 (1955).
6. V. M. Stepanov, A. B. Silaev, and G. S. Katrukha, Biokhimiya, 25, 749 (1960).
7. V. M. Stepanov and G. L. Muratova, Izv. Akad. Nauk SSSR, Otd. Khim. Nauk, No. 9, 1677 (1961).
8. A. B. Silaev and G. S. Katrukha, Biokhimiya, 27, 608 (1962).
9. A. B. Silaev, G. S. Katrukha, and S. P. Katrukha, Biokhimiya, 29, 873 (1964).
10. S. Veibel, Identification of Organic Compounds, Copenhagen (1954).
11. B. B. Lawson, E. Gross, C. M. Foltz, and B. Witkop, J. Amer. Chem. Soc., 83, 1509 (1961).
12. J. Tang and B. S. Hartley, Biochem. J., 102, 593 (1967).
13. E. L. Durrum, J. Amer. Chem. Soc., 72, 2943 (1950).
14. I. M. Hais and K. Macek, Paper Chromatography, Academic Press, New York (1963).
15. E. Gross and B. Witkop, J. Biol. Chem., 237, 1856 (1962).