POTENTIOMETRIC TITRATION OF PEPTIDES, THE STARTING MATERIALS FOR THEIR SYNTHESIS, AND INTERMEDIATES. II. ACIDIMETRIC MICRODETERMINATION OF PEPTIDES

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UDC 543.257.1:547.466

The possibility has been shown of the microdetermination of the weight proportion of the main substance in chromatographically pure peptide preparations. The results of quantitative determinations of an analog of luliberin have been compared with the results of analyses obtained by the methods of high-performance liquid chromatography and UV spectrophotometry. The method developed has also been used in analyses of preparations of oxytocin, angiotensin, and vasopressin.

The ever wider practical use of peptide preparations in biochemical investigations and medicine is raising demands on their quality characteristics. This has led to the search for methods of determining the weight fraction of the main substance. Existing methods either do not ensure adequate reliability of the results, or their use is limited by the absence of standard samples of peptides in which the weight fraction of the main substance is known with the maximum accuracy. Titrimetric methods, which are successfully used in the analysis of starting materials for, and intermediates in, peptide synthesis, have not found wide use in determinations of peptide preparations. In particular, the method of potentiometric acid-base titration of aqueous solutions has been used for the solution of research problems [1]. The method of high-frequency titration with perchloric acid in acetic acid medium is unpromising because of the relatively large (up to 40 mg) amounts of the samples to be analyzed [2].

In preceding papers we have described the microdetermination of a glycopeptide [3] and of 5'-tritylated N-acyl-2'-deoxyribonucleotides [4] by the method of potentiometric acidometric titration. Our aim was to investigate the possibility of using this method for determining the weight proportion of the main substance in preparations of an analog of luliberin (H-Glu-His-Trp-Ser-Tyr-D-Ala-Leu-Arg-Pro-NHEt), angiotensin (H-Asn-Arg-Val-Tyr-Val-His-Pro-Phe-OH), oxytocin (H-Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly-NH_2), vasopressin (H-Cys-Tyr-Phe-Gln-Asn-Cys-Pro-Arg-Gly-NH_2), vasopressin (H-Gln-Asn-Cys(Bzl)-Pro-Leu-Gly-NH_2), and the oxytocin hexapeptide (H-Gln-Asn-Cys(Bzl)-Pro-Leu-Gly-NH_2). We have also investigated individual samples of the tripeptides H-Arg-Trp-Glu-OH and H-Glu-His-Pro-NH_2 and the pentapeptide Boc-β-Ala-Trp-Met-Asp-Phe-NH_2.

TABLE 1. Results of the Potentiometric Titration of Peptides

Peptide	Mol. mass	Groups titrated	Weight of samp., mg	Proportion of the main substance by weight, %
Luliberin analog	1167	Amino groups of the arginine and histidine residues	1,898	77,6
Hexapeptide of oxytocin	720	Terminal amino groups	1,954	90,3
Oxytocin	1007	Terminal amino groups	2,775	91,0
Vasopressin	1084	Terminal amino group and arginine residue	2,153	88,8
Angiotensin	1038	Terminal amino group and arginine and histidine residues	1,609	87,5

All-Union Scientific-Research Institute of Applied Biochemistry, Biolar Scientific-Industrial Association, Olaine. Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 83-86, January-February, 1990. Original article submitted January 31, 1989.

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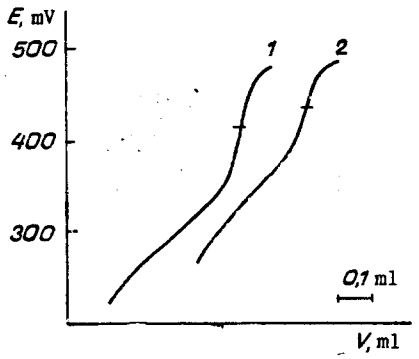

Fig. 1. Potentiometric titration curves of angiotensin (1) and the trifluoroacetate of the luliberin analog (2) with a 0.05 M acetonitrile solution of perchloric acid in acetic acid–acetone (1) and acetic acid–nitromethane (2).

TABLE 2. Results of Comparative Determinations of the Content of the Main Substance in Preparations of the Luliberin Analog

Arbitrary number of the samp.	Potentiometric titration $X \pm S_r$, % (at n = 5, α = 0.95)	HPLC, %	UVS, %
1	88,6±1,2	90	88
2	76 5±1,4	—	74
3	77,7±1,5	79	—
4	86,7±1,1	85	87
5	90,8±1,2	—	91

As titrant, however, we have successfully used ~0.005 M acetonitrile and nitromethane solutions of perchloric acid. The titration medium was a mixture of acetic acid with acetone, acetonitrile, or nitromethane in volume ratios 1:5-10. The mixtures of solvents for the analyses were selected in such a way that only the amino groups possessing the highest proton-accepting properties underwent titration, and the amide groups and peptide bonds were not affected (so as not to complicate the interpretation of the results of the titration).

As the investigation showed, value of the potential jumps on the potentiometric titration curves (Fig. 1) amounting to ~80-150 mV are sufficient for finding the equivalence points and the corresponding volumes of titrants consumed in a satisfactory manner. The least-well-defined potential jumps (under otherwise the same circumstances) appeared on curves of the potentiometric titration of the trifluoroacetate of the luliberin analog (curve 2). Analogous salts of stronger acids (for example, hydrochloric acid and p-toluenesulfonic acid) are not titrated by perchloric acid in the above-mentioned mixtures of solvents (there are no potential jumps on the titration curves) and, consequently, cannot be analyzed by direct acidimetric titration.

In a study of the conditions for quantitative determinations, in addition to comparing the results of comparative analyses performed by different methods we evaluated the changes in the stoichiometry of the titration reactions according to the amino acid compositions of particular peptides. The results showed that the terminal amino groups of peptide chains where the terminal fragments are asparagine, cysteine, glutamine, arginine, phenylalanine, or proline undergo direct titration. The simultaneous binding of two or sometimes three moles of perchloric acid by each molecule of luliberin analog, vasopressin, and angiotensin (see Table 1) is due to the presence in the molecules of these peptides of arginine or histidine residues or both (in the case of angiotensin). Apparently, residues of other basic amino acids of the corresponding peptides will participate in titration processes similarly.

In the preliminary evaluation of the results of the quantitative determinations only the analysis of the tri- and pentapeptides mentioned caused no doubts, since the weight content of the main substance in a number of the samples was 98-101%. As can be seen from Table 1, which gives as examples individual results of titrimetric analysis, the proportions of the luliberin analog, angiotensin, vasopressin, and oxytocin in the preparation differed from those theoretically possible and ranged within wide limits (for example, in Table 1, from 77.6 to 91.0%). To substantiate the reliability of the lowered (according to the titration results) purities of the peptides we performed preparative analyses of the samples by the Karl Fischer method (to

determine moisture and water of crystallization), by gas chromatography, by elementary analysis, etc. It was established that for chromatographically pure samples (containing not more than 1% of impurities of peptide nature) the difference between the weight fraction of the main substance (determined by potentiometric titration) and the theoretically possible 100% content frequently corresponded to the sum of the percentages of water of crystallization and acetic or trifluoroacetic acid.

A more convincing proof of the reliability of the potentiometric method was the fairly good agreement of the results of a comparative analysis of the weight content of the main substance by the methods of high-performance liquid chromatography (HPLC) and UV spectrophotometry (UVS). Table 2 gives the results of comparative determinations of the amounts of the luliberin analog, which confirmed the possibility of using potentiometric titration in the analytical control of peptide preparations.

EXPERIMENTAL

In the investigations and analyses we used chromatographically pure (characterized by a purity of 99% according to the HPLC method) samples (5-7 of each substance) of peptides that had been synthesized under laboratory conditions in the Biolar Scientific Production Combine (luliberin analogs) or in the experimental factory of the Institute of Organic Synthesis of the Academy of Sciences of the Latvian SSR.

Quantitative determinations by the HPLC method were performed on a Milikhrom microcolumn chromatograph (USSR) using salicylic acid as internal standard with a 2 × 60 mm column containing the support Silasorb C18 (7.5 μm) with, as the mobile phase, phosphate buffer solution (pH 3.7)—acetonitrile (80:20); UV detection at 210 nm.

For UV-spectrometric analysis we took 1-mg samples, dissolved each in 25 ml of distilled water, and measured the optical densities (in relation to distilled water) at 278 nm. In the calculations we used a value of the coefficient of molar extinction for the luliberin analog, $\varepsilon = 6900$, which was determined on solutions of artificial mixtures of tyrosine and tryptophan at pH 7).

The equipment for potentiometric titration, the preparation of the electrodes, and the determination of the concentration of the titrant have been described in preceding papers [3, 4]. Quantitative determinations by titration were carried out by dissolving a weighed sample (1.5-3 mg) in 2 ml of glacial acetic acid and adding 15 ml of acetone, acetonitrile, or nitromethane. If the samples dissolved slowly at room temperature, the solution was heated to 50-60°C. Titration with an 0.005 M acetonitrile or nitromethane solution of perchloric acid was continued until the appearance of the potential jump on the curve. If a precipitate appeared during the titration process, in a repeat analysis the amount of acetic acid in the titrated solution could be increased to give a solvent ratio of 1:5.

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