DETERMINATION OF PROTEIN IN SOLUTIONS FROM ABSORPTION IN THE ULTRAVIOLET REGION

V. S. Fedenko

UDC 535.341+547.96

A comparison of spectrophotometric methods of determining protein in solutions from absorption in the UV region has shown the advantage of a procedure based on measuring the optical densities at 235 and 280 nm and the possibility of its use for the analysis of plant proteins.

The photometric determination of the concentration of protein in solutions is, as a rule, carried out by Lowry's method [1], the biuret method [2], or the binding of the proteins with dyes [3] or with a color reagent containing silver ammoniate [4]. Spectrophotometric methods are based on measuring the absorption of the protein in the near and far ultraviolet regions [5] or the extinction coefficients after the proteolysis of the proteins to eliminate the influence of their conformational features on differential spectra [6]. An increase in the sensitivity of analysis in the region of absorption of the peptide bond is accompanied by some methodological limitations [7-11]. Absorption at 260 and 280 nm with calculation by means of a formula [12] or a nomogram [13] is usually employed to determine protein in routine analyses. In addition to the procedure mentioned, a method of analysis has been developed which is based on the difference in the absorption at 235 and 280 nm and permits the simultaneous elimination of the influence of the specific extinction of the nucleic acids and of the amino acid differences between proteins [14]. Standard proteins with characterized extinction coefficients were used for the development of this method. However, a considerable number of publications on the determination of proteins is connected with the analysis of solutions of heterogeneous preparations and the problem therefore arises of choosing a spectrophotometric procedure for performing measurements. In the present paper we give a comparative characterization of spectrophotometric procedures based on measuring absorption in the UV region for determining the concentrations of solutions of a number of standard proteins of maize (zeins) isolated from grains of different genotypes.

Protein concentrations (mg/ml) were calculated from the values of the optical densities at 260 and 280 nm by means of formula (1) [12] and from those at 235 and 280 nm by formula (2) [14]:

$$C = 1.45\ D_{280} - 0.74\ D_{260}\ . \quad (1)$$

$$C = (D_{235} - D_{280})/2.51. \quad (2)$$

For a comparative evaluation of spectrophotometric methods we used proteins with different quantitative proportions of tryptophan, tyrosine, and phenylalanine residues, which determine the characteristics of UV absorption. The results of the determinations of the concentrations of solutions of the proteins by the two spectrophotometric procedures in comparison with those of Lowry's method [1] are given in Table 1. They show that the values of the extinction coefficients of proteins (ε 1 mg/ml) at 235 and 280 nm vary within wide limits. The mean value of the numerical coefficient $K = \varepsilon_{235}{}^{1\text{mg/ml}} - \varepsilon_{280}{}^{1\text{mg/ml}}$ for homogeneous proteins is, according to [14], 2.51, while for the heterogeneous preparations used in the present work it was 3.01 (sample coefficients of variations 17.6 and 36.4%, respectively). It must be mentioned that the protein concentrations calculated by means of formula (1) exceed those of the Lowry determination (except for the solution of ribonuclease), and for solutions of chymotrypsin, lysozyme, and peroxidase this difference amounts to a factor of 2-3, which shows a fairly low accuracy of the method. Conversely, for protein concentrations calculated by means of formula (2) the deviation of the results of the analysis from those of Lowry's method decreases.

Scientific-Research Institute of Biology, Dnepropetrovsk State University. Translated from Khimiya Prirodnykh Soedinenii, No. 5, pp. 693-695, September-October, 1989. Original article submitted December 22, 1988.

 0009-3130/89/2505-0590$12.50

TABLE 1. Determination of Concentrations of Protein Solutions and of Maize Zeins by Various Methods

Name	^{1} mg/ml ε_{235}	^{1}mg/ml ε_{280}	K	Protein concentration, mg/ml by Lowry	by f-1a (1)	by f-1a (2)	$C_{by\ f\text{-}1a(1)}$ / $C_{by\ Lowry}$	$C_{by\ f\text{-}1a(2)}$ / $C_{by\ Lowry}$
Chymotrypsin	3,70	1,96	1,74	0,46	0,95	0,32	2,07	0.70
Ribonuclease	2,72	0,86	1,85	0,47	0,32	0,35	0,68	0,74
Cytochrome C	5,72	2,32	3,40	0,25	0,44	0,34	1,76	1,36
Pepsin	3,83	1,63	2,20	0,30	0,47	0,26	1,57	0,87
Bovine serum albumin	5,44	1,52	3,92	0,27	0,36	0,42	1,33	1,56
Soybean trypsin inhibitor	3,67	1,17	2 50	0,18	0,20	0,18	1,11	1,00
Trypsin	3,79	1,85	1,94	0,34	0,60	0,26	1,76	0,76
Lyzosyme	6,79	3,05	3,74	0,38	1,16	0,57	3,05	1,50
Ovalbumin	5,20	1,23	3,97	0,35	0,43	0,55	1,20	1,57
Horseradish peroxidase	8,13	3,20	4,84	0,24	0,68	0,46	2,83	1,92
W 64 A+++	2,59	0,73	1,86	0,49	0,32	0,36	0,65	0,73
W 64 Ao2e2o2	3 17	0,97	2,20	0,60	0,46	0,53	0,77	0,88
A 204+++	3,45	1,02	2,43	0,49	0,41	0,47	0,84	0,96
A 204o2o2o2	5,26	2,87	2,39	0,46	0,76	0,44	1,65	0,96
Wf 9+++	4,02	1,48	2,54	0,60	0,55	0,61	0,92	1,02
Wf 9o2o2o2	4,11	1,26	2,85	0,53	0,51	0,60	0,96	1 11
W 155+++	2,33	0,68	1,65	0,69	0,42	0,45	0,61	0,65
W 155o2o2o2	5,26	2,06	3,20	0,46	0,68	$0,5_9$	1,48	1,28

For the alcohol-soluble proteins of maize (zeins), which lack tryptophan, the values of the calculated coefficients K vary to a smaller degree than for the standard proteins (coefficient of variation 20.0%; mean value of K 2.39), a smaller deviation from the results of Lowry determinations is observed (Table 1). At the same time, the accuracy of the determination of protein from the difference in the optical densities at 235 and 380 nm is higher than that obtained in calculation by means of formula (1) using the D_{280} and D_{260} values.

EXPERIMENTAL

Standard proteins were used without additional purification: bovine serum albumin, ovalbumin, lysozyme, pepsin, and cytochrome c from the Olaine Chemical Reagents Factory; chymotrypsin, ribonuclease, soybean trypsin inhibitor, and horseradish péroxidase from Reanal (Hungary); and trypsin from Spofa (Czechoslovakia).

Solutions of the standard proteins were prepared in triplicate (concentration 0.5 mg/ml). The peroxidase preparation was dissolved in an acetate buffer (pH 5), the bovine albumin and the lysozyme in a phosphate buffer (pH 7.9), and the other proteins in water. The zeins were isolated from flours of initial lines (+++) of maize W 64 A, A 204, Wf 9, and W 155 and corresponding lines mutant with respect to the opaque-2 gene (o2o2o2) by extraction with 70% ethanol followed by precipitation with acetone [15]. Solutions of the zeins in 8 M urea (1 mg/ml) were prepared for the measurements.

The optical densities of the solutions were determined on a Specord M40 spectrometer in a 1-cm cell using a program of measurements consisting of three blocks for the values of the analytical wavelengths (235, 260, and 280 nm). The calculation of the protein concentrations by formulas (1) and (2) and the statistical treatment of the results were performed on a SM-4 computer by a program that had been developed.

SUMMARY

It has been established that a spectrophotometric procedure based on the difference in optical densities at 235 and 280 nm permits the determination of protein concentrations with greater accuracy than calculation from the optical densities at 260 and 280 nm. Using zeins as an example, the possibility has been shown of using the procedure for analyzing plant proteins.

LITERATURE CITED

1. O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, J. Biol. Chem., 193, 265 (1951).

2. R. K. Scopes, Protein Purification, Principles and Practice, Springer, New York (1985), p. 265.
3. I. A. Gorina, "Critical review of modern methods of determining protein," [in Russian], Moscow (1979); Paper Deposited in VINITI [All-Union Institute of Scientific and Technical Information], No. 1803-79 from Vestnik Mosk. Univ.
4. K. Gerald, M. Chirs, and M. Brad. Anal. Biochem., 148, 451 (1985).
5. A. P. Demchenko, Ultraviolet Spectrophotometry and Protein Structure [in Russian], Naukova Dumka, Kiev (1981), p. 153.
6. T. A. Beweley, Anal. Biochem., 123, 55 (1982).
7. V. S. Asatiani, New Methods of Biochemical Photometry [in Russian], Nauka, Moscow (1965), p. 134.
8. M. K. Gil'manov, O. V. Fursov, and A. P. Frantsev, Methods of Purifying and Studying Plant Enzymes [in Russian], Nauka, Alma-Ata (1981).
9. N. P. L'vov, G. D. Erygin, and O. I. Kamenskii, Prikl. Biokhim. Mikrobiol., 7, 99 (1971).
10. L. V. L'vova, Selekts. Semenovod., No. 60, 60 (1986).
11. D. Maruhn, V. Wessel, and D. Paar, J. Clin. Clin. Biochem., 19, 764 (1981).
12. J. L. Bailey, Techniques in Protein Chemistry, Elsevier, Amsterdam (1962) [Russian translation, Mir, Moscow (1965), p. 265].
13. G. A. Kochetov, Practical Handbook on Enzymology [in Russian], Vysshaya Shkola, Moscow (1971), p. 312.
14. J. R. Whitaker and P. E. Granum, Anal. Biochem., 109, 156 (1980).
15. Yu. V. Peruanskii and V. N. Savin, Fiziol. Biokhim. Kul'turn. Rast., 17, 390 (1985).

COMPARATIVE INVESTIGATION OF ZEIN ISOLATED FROM GLUTEN BY VARIOUS METHODS

A. N. Vinnichenko, V. S. Fedenko, N. P. Kotsyubinskaya, L. V. Shupranova, V. S. Struzhko, and N. G. Zinchenko

UDC 547.96.7

A comparative study has been made on zein preparations isolated from gluten by extraction with 70% ethanol and precipitation under various conditions; by dialysis, and by the addition of a 0.1 M solution of sodium sulfate or of acetone. An investigation of absorption spectra and of electrophoresis in polyacrylamide gel has shown the advantage of the method using acetone for precipitating the protein preparation.

Zein is the alcohol-soluble protein of maize [1]. Industrially, it is obtained by extraction with aqueous alcoholic solutions from gluten – a by-product of the maize starch industry [2]. The degree of purification and the characteristics of the zein depend on the conditions of extraction and precipitation [3]. There have been limited physicochemical investigations of preparations obtained by different methods [4].

In the present paper we give comparative characteristics of zein preparations isolated by extraction with 70% ethanol from gluten followed by precipitation under various conditions. Precipitation was performed by dialysis of the aqueous alcoholic extract obtained and also by the addition of 0.1 M NaCl solution or of acetone.

As an index of the degree of freeing of the zein from nonprotein substances it is possible to use the ratio of the optical densities at 277 and 255 nm (D_{272}/D_{255}) and also those at 277 and 320 nm (D_{277}/D_{320}) [5-7]. The value of 255 nm corresponds to the position of the minimum and that of 277 nm to the maximum in the UV spectrum of zein after purification [5], while at ~320 nm there is an inflection in the main absorption band connected with the pres-

Scientific-Research Institute of Biology, Dnepropetrovsk State University. Translated from Khimiya Priorodnykh Soedinenii, No. 5, pp. 695-699, September-October, 1989. Original article submitted January 2, 1989; revision submitted March 20, 1989.

 0009-3130/89/2505-0592$12.50