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STUDIES ON PROTEIN METABOLISM IN HIGHER PLANT LEAVES—I.

AMINO ACID COMPOSITIONS OF TOBACCO LEAF PROTEINS AT VARIOUS STAGES

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Abstract—The effect of leaf maturity and nutrient treatment on the amino acid composition of tobacco leaf protein was examined. Small but appreciable changes were found in the amino acid composition, especially serine, of total protein during the process of growth. The variation in the composition of proteins of subcellular fractions was smaller than that of the total protein. Fertilization treatment did not affect the amino acid composition either in the total or the individual subcellular fraction. Some differences of the amino acid composition between supernatant and chloroplast proteins were detected. Supernatant protein showed larger contents of basic amino acids, glutamic acid, valine and tyrosine and lower contents of serine, proline, glycine, leucine and phenylalanine compared with chloroplast protein.

INTRODUCTION

THE first studies on the amino acid composition of leaf protein were carried out by Chibnall in the 1930's.[1] Since then, many investigators have carried out similar experiments using many plants under various conditions, such as climate, age of tissue and manurial treatment.[2–4] In general the data showed that the amino acid composition of leaf proteins for any one species probably varies but little with the conditions, although the variation among plant families may be large.

Previous work of our laboratory indicated that free amino acid composition in tobacco leaf varied greatly as the leaf matured.[5] These changes in free amino acids may affect protein metabolism.[2] Therefore, it seemed useful to examine the amino acid composition of leaf protein of tobacco at various stages and to examine the effect of added nutrients.

RESULTS

Acid Hydrolysis of Leaf Protein

The results of experiments carried out to determine the amount of decomposition of the standard amino acids during acid hydrolysis are shown in Table 1. All tryptophan should have been destroyed. As expected, serine was the most labile of the other amino acids and ~12·5 per cent was lost after 24 hr under the standard condition used (see Experimental).

[1] A. C. CHIBNALL, In *Protein Metabolism in Plant*. Yale University Press, New Haven (1939).
[2] J. W. H. LUGG, *Adv. Protein Chem.* 6, 229 (1949).
[3] F. C. STEAWRD and J. F. TOMPSON, In *The Proteins*, Vol. 2, Part A, p. 523. Academic Press, New York (1954).
[4] E. W. YEMM, In *Encyclopedia of Plant Physiology*, Vol. 8, p. 325 (1960).
[5] M. NOGUCHI, K. YAMAMOTO and E. TAMAKI, *Tobacco Sci.* 8, 8 (1964).

Tyrosine, cystine, threonine and phenylalanine were also decomposed considerably during 24-hr heating. Further heating induced destruction of arginine and aspartic acid.[6] Contamination of metal ions not only lead to rapid decomposition of the above labile amino acids in the hydrolysis, but also to decomposition of arginine, aspartic acid, histidine, glycine and alanine producing ninhydrin positive compounds other than ammonia. Similar experiments with acetone powder preparations of leaf protein indicated that the degradation ratios between 24–48 hr of individual amino acids obtained from the acetone powder were similar to those of standard amino acids. For example, the degradation ratios of serine, tyrosine and threonine from the leaf protein were found as 13·8, 6·4 and 9·0, respectively (cf. Table 1).

TABLE 1. DESTRUCTION OF STANDARD AMINO ACIDS UNDER THE STANDARD HYDROLYSIS CONDITIONS FOR VARIOUS PERIODS

Amino acid	Period of hydrolysis (% Loss)				Factor from 24 to 48 hr-hydrolysis†
	24 hr	48 hr	72 hr	24 hr*	
Lysine	−0·4	1·2	2·2	8·0	1·5
Histidine	0·4	0·4	3·4	18·3	0
Arginine	0·2	1·4	6·8	37·7	1·2
Aspartic acid	3·4	6·8	8·8	23·8	3·5
Threonine	6·5	12·8	18·5	15·8	6·4
Serine	12·3	22·4	31·8	21·4	13·8
Glutamic acid	−0·5	0·0	3·0	3·0	0·5
Proline	0·1	−0·9	−1·1	0·4	−1·0
Glycine	1·5	3·0	2·5	13·4	1·5
Alanine	0·3	0·5	−1·0	10·6	0·2
Methionine	−1·5	2·6	1·0	7·2	3·4
Isoleucine	0·0	1·3	1·3	−0·6	1·3
Leucine	0·3	0·8	0·9	0·6	0·5
Tyrosine	8·5	16·7	19·5	14·2	9·0
Phenylalanine	6·2	8·0	8·4	8·5	1·9

Mixtures of 0·01 μmoles of each standard amino acid was added 2 ml of redistilled 6 N HCl and heated at 100° for each period mentioned in a sealed tube under reduced pressure.

* Distilled HCl not used.

† The factor $= \frac{(\text{recovery of 24 hr}) - (\text{recovery of 48 hr})}{(\text{recovery of 24 hr})}$.

Amino Acid Compositions in Leaf Protein with Different Age

The acid insoluble nitrogen and chlorophyll contents in the chloroplast (200–5000 *g*), small particle (5000–100,000 *g*), and supernatant fractions of the tobacco leaves obtained at different stages are shown in Table 2. Acid insoluble nitrogen compounds are called protein in this paper.

The protein content in the filtrate before centrifugation was found to be 1·3–1·4% of fresh weight for the leaves of 2 and 4 weeks after transplantation and 0·8% for the leaves of 7 weeks. The protein content in the original filtrate was more completely recovered in 200 *g* supernatant in young plants indicating that cells of the leaf tissue of young plants are more easily broken. From the results it appears that cells of the young leaves showed 80–90% per cent destruction, while those of old leaves only 45 per cent by the same homogenation method.

[6] E. L. SMITH and A. STOCKEL, *J. Biol. Chem.* **207**, 501 (1954).

TABLE 2. NITROGENOUS FRACTIONS OF VARIOUS AGED LEAVES

Fractions	Weeks after transplantation		
	2	4	7
	Amount in mg		
Protein in original filtrate	195·4	224·0	120·4
Chlorophyll in original filtrate	13·83	14·75	8·16
Protein in 200 *g* supernatant	176·5	178·5	54·3
Protein in chloroplast	63·5	51·7	15·6
Chlorophyll in chloroplast	10·47	7·57	2·91
Calculated protein in total chloroplasts	87·6	99·6	43·8
Protein in small particles	12·2	15·1	9·1
Protein in supernatant	100·8	111·8	29·6

The values in the Table referred to amounts obtained from a 15-g fresh weight sample of leaves.

The chloroplast protein content as a percentage of total protein in 200 *g* supernatant also decreased with advancing age whereas the protein content in the small particle fraction increased. The small particle fraction contained some chloroplast fragments (grana?) produced by the homogenation. Therefore, these trends in the protein contents of both chloroplast and small particle fractions of the older leaf may, in part, be the result of the preparation procedure. Nevertheless, the calculated values of the chloroplast protein content on the basis of the chlorophyll content were also decreased, therefore, the true chloroplast protein content in a cell seems to decrease with age.

Amino acid compositions of these samples were analyzed and the results are shown in Table 3. The values for the sixteen individual acids detected are shown as a percentage of the total amino acids detected by the analysis. Unusual ninhydrin positive compounds were not detected, except for one which showed a yellowish color after ninhydrin reaction. The compound was observed in hydrolyzates of the acetone powder or chloroplast fraction of all samples. The compound was tentatively identified as levulinic acid from its behavior in the analyzer. It probably was produced from sugars in the process of acid hydrolysis.

Relatively large amounts of aspartic acid (aspargine), glutamic acid (glutamine), glycine, alanine and leucine were contained in the protein fractions obtained from every sample. On the other hand, histidine and methionine contents were found to be very small, and cysteine (cystine) was found only in traces. Unexpectedly low values of cystine may be due to its degradation during the hydrolysis of the samples.

The difference in amino acid composition in total protein (acetone powder) between young and old leaves was not so distinctive as was that of the free amino acids, shown in a previous report.[5] However, some differences were found in several of the amino acids in the total protein which are beyond the limit of the analytical errors (about 6 per cent of each value tabulated). The contents of proline, glycine, valine, isoleucine and phenylalanine increased with age, whereas, the contents of aspartic acid (aspargine), threonine and serine decreased. However, the differences of amino acid composition in each subcellular fraction between young and old leaves were small, and aspartic acid and serine in the supernatant, and lysine in the chloroplast fraction were the only variable amino acids. Therefore, the differences in the amino acid composition of the total protein may be attributed to the changes in relative amounts of protein in the subcellular fractions at each stage. The amino acid compositions

TABLE 3. AMINO ACID COMPOSITIONS OF LEAF PROTEIN FRACTIONS AT VARIOUS AGES

Fractions	Weeks after transplantation										
	2				4				7		
	A.P.	Super.	Chl.	S.P.	A.P.	Super.	Chl.	S.P.	A.P.	Super.	Chl.
Lysine	5·90	6·60	5·11	6·60	5·98	6·51	5·51	6·15	6·15	6·54	5·66
Histidine	1·83	2·24	1·86	1·99	1·90	2·24	1·81	1·85	1·91	2·12	1·78
Arginine	3·92	5·26	4·26	5·16	4·17	5·14	4·01	4·76	4·16	4·93	4·02
Aspartic acid	10·15	9·14	8·70	8·40	9·68	9·30	8·74	8·74	9·52	9·83	8·76
Threonine	5·25	5·26	5·10	5·24	5·26	5·94	5·16	5·23	4·53	5·14	5·04
Serine	5·79	4·44	5·94	5·58	5·76	4·36	5·77	5·15	4·82	4·01	6·08
Glutamic acid	12·52	11·21	10·07	10·26	12·31	11·17	9·82	10·55	11·91	11·55	9·70
Proline	5·67	5·66	6·55	5·56	5·81	5·83	6·47	5·76	6·16	5·49	6·53
Glycine	9·91	9·57	10·58	9·81	10·02	9·02	10·32	9·98	10·72	9·32	10·28
Alanine	9·41	9·87	9·94	9·70	9·51	9·75	9·89	10·04	9·52	9·95	9·96
Valine	6·76	7·65	6·89	7·25	7·12	7·83	6·95	7·65	7·43	8·18	6·78
Methionine	1·44	1·63	1·44	1·91	1·53	1·72	1·82	1·72	1·51	1·52	1·77
Isoleucine	4·77	5·00	5·29	5·49	4·90	4·92	5·49	5·55	5·22	5·30	5·25
Leucine	9·12	8·94	10·21	9·73	9·23	8·81	10·42	9·85	9·45	8·75	10·35
Tyrosine	2·62	3·68	2·93	2·98	2·71	3·71	2·73	2·70	2·48	3·49	2·76
Phenylalanine	4·09	3·88	5·14	4·39	4·16	3·88	5·12	4·48	4·52	3·99	5·16

Following abbreviations are used: A.P. = Acetone powder; Super. = supernatant fraction; Chl. = chloroplast fraction; S.P. = small particle fraction.
The values are given as a percentage of the content (mole) of each individual amino acid to the total of amino acids detected by the analysis.

of supernatant proteins were considerably different from these of chloroplast protein, as shown in Table 3 and Fig. 1. The contents of basic amino acids (lysine, histidine and arginine), aspartic acid (aspargine), glutamic acid (glutamine), valine, and tyrosine in supernatant protein were larger at any stage than those in chloroplast protein. On the contrary, the contents of serine, proline, glycine, leucine and phenylalanine in chloroplast protein were larger than those in the supernatant protein. The difference in the two proteins was more distinct in the ratio of the content of tyrosine to phenylalanine. This ratio was 0·91 for the supernatant protein and 0·55 in the chloroplast protein. The contents of the other amino acids (threonine, methionine and isoleucine) showed almost the same values in both fractions.

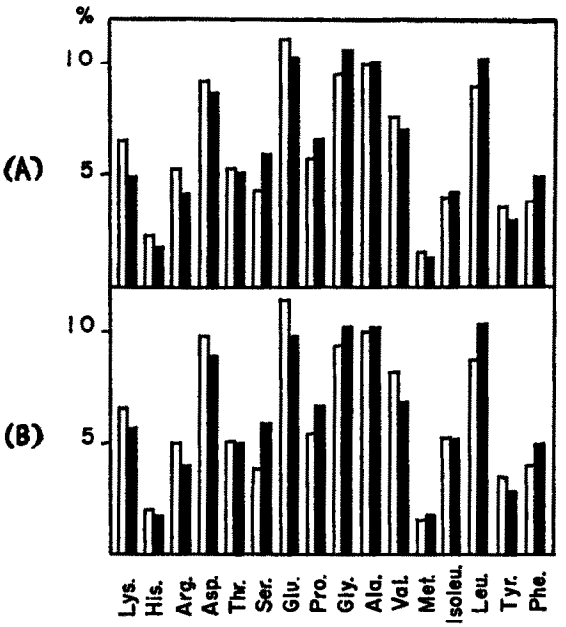

FIG. 1. COMPARISON OF THE AMINO ACID COMPOSITION OF CHLOROPLAST PROTEIN WITH THAT OF SUPERNATANT PROTEIN (FOR DEFINITION SEE TEXT).

Samples of 2 weeks (A) and 7 weeks (B) after transplantation were used. □; supernatant protein. ■; chloroplast protein.

TABLE 4. NITROGENOUS FRACTIONS OF VARIOUS POSITIONED AND NUTRIENT-DEFICIENT LEAVES

	Sample No.				
	1	3	5	7	−Nutrient
Position of leaves from top of plant	1·2	6	10	14	1–8
Average of fresh weight g/leaf	8·1	15·7	21·7	22·5	3·1
Protein in original filtrate (mg)	141·4	148·9	100·8	60·1	55·0
Chlorophyll in original filtrate (mg)	11·2	10·9	8·0	4·4	4·03
Protein in 200 *g* supernatant fraction	122·4	102·0	62·3	35·3	37·2
Protein in chloroplasts (mg)	32·0	24·5	13·3	7·36	14.4
Chlorophyll in chloroplasts (mg)	6·01	3·89	2·44	1·31	2·26
Calculated protein in total chloroplasts	59·6	69·1	43·6	24·7	25·6
Protein in small particles (mg)	11·3	7·07	2·42	0·85	1·78
Protein content in supernatant (mg)	79·1	70·5	46·5	27·0	21·0

−Nutrient; 5 g sample of 1–8 position of three plants grown in the non-fertilized pots were used.

TABLE 5. AMINO ACID COMPOSITIONS OF LEAF PROTEIN FRACTIONS AS A FUNCTION OF STALK POSITION

	Sample No.											
	1			3			5			7		
	Orig.	Super.	Chl.	Orig.	Super.*	Chl.	Orig.	Super.	Chl.	Orig.	Super.	Chl.†
Lysine	6·43	6·87	4·84	6·38	6·66	4·95	5·86	6·79	5·05	5·86	6·39	5·07
Histidine	2·31	2·35	1·79	2·43	2·29	1·93	2·32	2·31	2·11	2·28	2·23	1·94
Arginine	5·00	5·33	4·32	4·73	5·36	4·48	4·65	5·37	4·28	4·08	5·32	4·29
Aspartic acid	8·71	9·10	8·65	8·71	9·30	8·50	9·04	9·51	8·66	8·96	9·66	8·70
Threonine	5·12	5·35	5·25	4·94	5·31	4·67	5·07	5·49	4·74	5·04	5·54	4·75
Serine	5·01	4·74	5·03	4·69	4·68	4·69	4·80	4·75	4·78	5·01	5·08	5·02
Glutamic acid	10·48	11·99	9·94	10·55	11·28	9·90	10·31	11·40	9·84	10·25	11·31	9·70
Proline	6·26	5·66	7·00	6·23	5·71	7·32	5·71	6·19	6·83	5·74	6·11	7·11
Glycine	9·52	9·02	9·89	9·86	9·05	10·21	10·05	9·08	10·32	10·30	9·12	10·51
Alanine	9·12	8·95	9·74	9·31	8·95	9·89	9·45	8·99	9·81	9·66	9·03	9·83
Valine	7·70	7·30	6·94	7·26	7·35	7·05	7·29	7·16	7·13	7·31	7·29	6·94
Methionine	1·81	1·76	1·78	1·82	1·79	1·83	1·83	1·56	1·78	1·94	1·65	1·75
Isoleucine	5·31	5·09	5·87	5·36	5·16	5·68	5·41	4·90	5·73	5·41	4·53	5·64
Leucine	9·93	8·66	11·10	10·15	9·11	10·86	10·21	9·06	11·01	10·15	9·05	10·90
Tyrosine	2·64	3·76	2·22	2·71	3·85	2·19	2·86	3·50	2·12	2·88	3·65	2·25
Phenylalanine	4·73	4·07	5·71	4·97	4·20	5·85	5·25	4·10	5·86	5·30	4·11	5·62

The abbreviations are the same as shown in Table 3 except that "Orig." means the sample of the original filtrate.
* Sample obtained from the leaves of No. 2 (4th position) was used.
† Sample obtained from the leaves of No. 6 (12th position) was used.

Amino Acid Compositions in Protein of the Leaves from Different Position

Both protein and chlorophyll contents in the chloroplast, small particles and supernatant fractions from leaves in different position are shown in Table 4. The total extractable protein content declined as the leaf matured, i.e. at the lower stalk positions. The relative content of chlorophyll to protein was approximately constant regardless of maturity. The percentage of protein in the chloroplasts in the upper leaves were slightly larger than those in the lower leaves. On the contrary, the percentage in the supernatant fraction from the upper leaves was slightly less than the lower leaves. These results may indicate that the relation between the protein and chlorophyll contents of upper and lower leaves on the same plant are similar to those between the largest leaves of young and old plants.

The amino acid compositions of the samples are shown in Table 5. The values of total amino acid compositions shown in Table 3 are different in some degree from those of the total protein in Table 5. The differences may arise from different preparation methods between the two experiments. In the former experiment, acetone powder was used as "total protein", while in the latter experiment the original filtrate was used as "total protein". Nevertheless, the differences between the amino acid compositions of protein in the upper leaves and those in the lower leaves were similar to those between the young and old leaves. The compositions of supernatant and chloroplast protein in the experiment were also closely similar to those of respective proteins in the former experiment.

Comparison of Amino Acid Composition between Normal and Nutrient Deficient Leaves

The largest fresh leaf from plants not treated with fertilizer weighed only 4 g, while the weight of the largest leaf from fertilized plants weighed 22 g. The ratio of chlorophyll to protein in the deficient leaf was nearly the same as in the normal leaf. The chloroplast protein content in the leaves of nutrient deficient plants was larger than that of normal plants.

TABLE 6. COMPARISON OF AMINO ACID COMPOSITIONS OF THE PROTEINS OF NORMAL LEAVES WITH THOSE OF NUTRIENT DEFICIENT LEAVES

	Normal condition*			−Nutrient		
	Orig.	Super.	Chl.	Orig.	Super.	Chl.
Lysine	6·13	6·18	5·00	5·75	6·53	5·10
Histidine	2·34	2·30	1·94	2·33	2·41	2·08
Arginine	4·37	5·35	4·34	4·61	5·16	4·68
Aspartic acid	8·86	9·39	8·63	8·95	9·60	8·62
Threonine	5·04	5·42	4·83	5·20	5·44	4·99
Serine	4·88	4·81	4·88	4·99	5·14	4·77
Glutamic acid	10·40	11·50	9·85	10·60	11·22	9·61
Proline	5·99	5·92	6·99	6·06	6·13	6·72
Glycine	9·93	9·07	10·33	10·12	9·00	10·50
Alanine	9·39	8·98	9·77	8·90	8·94	9·90
Valine	7·39	7·28	7·02	7·40	7·00	6·87
Methionine	1·85	1·69	1·79	1·82	1·64	1·79
Isoleucine	5·37	4·92	5·73	5·36	5·00	5·62
Leucine	10·11	8·97	10·97	10·42	9·06	10·92
Tyrosine	2·77	3·69	2·20	2·46	3·61	2·47
Phenylalanine	5·06	4·12	5·76	5·15	4·22	5·64

The abbreviations are the same as shown in Tables 3 and 5.

* The values of normal were obtained as the mean values of samples No. 1, 3, 5, and 7 of the Table 5.

Amino acid compositions of proteins in the nutrient deficient leaves were compared with those in the normal leaves. The results are shown in Table 6 and the values show little difference in both fractions.

DISCUSSION

There have been many reports on changes during the hydrolysis of protein.[7,8] It is generally claimed that the only ninhydrin positive destruction product of amino acids during acid hydrolysis is ammonia. However, our data showed that many decomposition products appeared during hydrolysis under certain conditions, such as using non-distilled HCl. These products also appeared when an unwashed chloroplast fraction was used. These results lead us to suspect that metal ions contained in commercial HCl or in unpurified chloroplast fraction may cause the formation of these products. An increase in the decomposition products correlated with a decline of the content of arginine, glycine and alanine, although these amino acids are normally stable to acid hydrolysis. It is concluded that it is necessary to wash particulate fractions in order to obtain reliable values.

It has been found that many leaf proteins vary during leaf growth[9] and the variations were mostly investigated by following changes in enzyme activities. However, in this study, the changes in the amino acid composition of leaf protein during growth is small in comparison with that found in free amino acids.[5] Recently, Wilson[10] found that the amino acid composition of leaf proteins from different species was remarkably similar even at various stages of growth, although differences in maturity and nitrogen fertilization caused a marked change in the non-protein nitrogen. Similar results were found by Kanazawa[11] during the algal life cycle. Nevertheless, it cannot be concluded from this that there is no correlation between the free amino acids and the compositions of protein. Earlier studies of Lugg[2] indicated differences in the incorporation of radioactive sulfate into cystine and methionine in various plant proteins which had fairly similar contents of the sulfur-containing amino acids. From these results, it may be supposed that the turnover rates of individual amino acids in leaf proteins may differ from each other, although different aged leaf protein has similar amino acid composition, and the turnover rate may correlate with the content of individual free amino acid.

Sisakyan *et al.*[12] showed that the amino acid contents in chloroplast protein isolated from sugar beet leaves of different ages were considerably different from each other. However, such large changes were not found in our experiments with tobacco during growth, although the appreciable variation in the amino acid composition in chloroplast protein was found in the process of yellowing stage of the leaf.[13] On the other hand, as mentioned above, small but appreciable change was observed in the amino acid composition of total leaf protein during growth, especially in the content of serine. The change may be induced by the change in the relative proportion of subcellular fractions (supernatant, chloroplast, etc.) which differ in amino acid composition from one another. Comparison of the amino acid composition between cytoplasmic and chloroplastic proteins was also carried out by Chibnall[1] and Yemm

[7] M. W. REES, *Biochem. J.* **40**, 632 (1946).
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[10] R. F. WILSON, *Exptl. Progr. Grassl. Res. Inst. (Hurley)* **11**, 65 (1958).
[11] T. KANAZAWA, *Plant Cell Physiol.* **5**, 333 (1964).
[12] N. M. SISAKYAN, E. N. BEZINGER, N. A. GUMILEVSKAYA and N. F. LUKYANOVA, *Biochemistry (USSR)* **20**, 368 (1955).
[13] N. KAWASHIMA, H. FUKUSHIMA and E. TAMAKI, **6**, 339 (1967).

and Folkes [14] with spinach and barley leaves, respectively. Their data indicated that only lysine content showed significant differences of the eighteen amino acids estimated in spinach and barley leaf proteins. Compared with these results, our experimental data showed more distinct differences in thirteen amino acids of sixteen estimated. These characteristic qualities in chloroplast protein may be useful for future studies of chloroplast formation or degradation.

EXPERIMENTAL

Materials

Tobacco plants, 50-day old, (*Nicotiana tabacum*, L. "Bright Yellow") obtained from a seedplot at Utsunomiya Tobacco Experiment Station were transplanted in pots on April 23. Nitrogen was supplied in the form of farm-manure and urea. For the experiments on nitrogen deficiency, these nutrients were omitted. Of thirty plants, the largest leaves of five plants in each sampling were taken 2, 4 and 7 weeks after transplantation. For experiments using the leaves in different positions, plants 9 weeks after transplantation were used.

Samples

Fifteen g of deribbed leaves of each sample was homogenized in a Waring blender for 1 min with 60 ml of 0·1 M phosphate buffer, pH 7·0, containing 0·4 M sucrose and 0·05 M sodium ascorbate. The homogenate was squeezed through gauze and centrifuged at 200 *g* for 5 min to remove large cell debris. The supernatant was centrifuged at 5000 *g* for 20 min. The precipitate was collected and called "chloroplast fraction". The supernatant was recentrifuged at 100,000 *g* for 120 min. The precipitated fraction and light yellow supernatant were designated as "small particle fraction" and "supernatant fraction", respectively. Chloroplast and small particle fractions were suspended with 0·1 M phosphate buffer, pH 7·0, containing 0·02 M sodium ascorbate, immediately after fractionation.

Separation of Protein Fraction

To a suitable volume (containing 50–100 mg acid-insoluble nitrogen) of each fraction was added the same volume of 20% trichloroacetic acid (TCA) solution. After standing at room temperature for 2 hr, resultant precipitates were collected by low speed centrifugation. The precipitated cake was washed twice with 1 ml of 7% TCA solution. The cake was dissolved or suspended in 1 ml of 0·1 N NaOH, and then reprecipitated by addition of 2 ml of 20% TCA solution. The precipitate was washed with 85% acetone until colored substance (mainly chlorophyll) was removed. The white or gray precipitate was hydrolyzed and used for amino acid analysis.

Analytical Methods

The most suitable conditions for acid hydrolysis of the protein fractions were found to be as follows; each protein fraction for the amino acid analysis was transferred into distilled hydrochloric acid (azeotrope mixture, about 6 N, b.p. 108°) of 200–500 volumes and hydrolyzed at 110° for 24 hr in a sealed glass tube under reduced pressure (20 mm Hg at room temperature). All the amino acids obtained after the hydrolysis were stable at −20° in the sealed tube for about a month. Insoluble materials produced during the hydrolysis were removed by filtration just before the analysis. The filtrate was dried *in vacuo* and the residue

[14] E. W. YEMM and B. F. FOLKES, *Biochem. J.* **55**, 700 (1953).

was dissolved in 5 ml of 0·1 M citric acid buffer, pH 2·2. The solution was used for amino acid analysis with a Beckman/Spinco Model 120 amino acid analyzer.

The nitrogen content in TCA insoluble fraction was determined by the method of Johnson.[15] The protein content was calculated by multiplying the nitrogen values by 6·25.

Total chlorophyll determinations were carried out according to the procedure described by Arnon.[16]

Acetone Powder

Five g of each leaf was homogenized with 100 ml of acetone at −30°, and the homogenate was filtered quickly by suction. The residue was resuspended in 60 ml of chilled acetone and 40 ml of ether. The residue was dried in vacuo and used as acetone powder.

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