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Inhaltsverzeichnis. — Contents.

Peptides (Bound Amino Acids) and Free Amino Acids[1].

By

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The term "peptide" as distinct from "protein" has come to be reserved for compounds having molecular weight less than, at most, 10,000 or, if of higher molecular weight, having unusually simple amino acid composition. Natural compounds so far shown unequivocally to belong to this class do not exhibit denaturation in its usual sense or, particularly, coagulation by heat.

It should be made clear at the outset that, whilst the amino acids found free in plant tissues are compounds of known or readily ascertainable chemical structure, this is not in general true of the "peptides"; peptides are usually only distinguished from proteins by the means used for separating them and therefore, in the present absence of adequate understanding of the chemical structure of both the proteins and the peptides found in nature the distinction is largely an empirical one. "Peptides" in the sense of much of the literature of plant analysis are no more than chemically bound amino acids found after applying a procedure expected to remove protein. The bonding of the amino acids may not necessarily involve peptide links (see below); indeed, where nothing but an increase of amino nitrogen after hydrolysis of such fractions has been demonstrated, even amino acids may not be concerned.

The least empirical procedures for separating peptides from proteins are dialysis and ultrafiltration with suitable membranes. Thus, there are good grounds for believing that no molecules having molecular weight greater than 10,000 can pass a cellophan membrane. The juice obtained from leaves by the ether-water and similar procedures (cf. CHIBNALL, 1939) has already undergone ultrafiltration through cell walls, and a notably high proportion of the nitrogen found therein passes freely through cellophan (e. g. SYNGE, 1951 b). DANIELSSON (1951, 1952) has used dialysis for studying the nitrogenous fractions of germinating and ripening peas.

Juices obtained after heat coagulation of proteins are likely also to be free from protein, although there are quite sufficient proteins that are not heat-coagulable (e. g. casein, gelatin) to emphasise the need for caution in interpreting fractions thus obtained (p. 24). Heat coagulation (and some protein precipitants) have the great advantage of quickly inactivating proteolytic enzymes. There is thus less chance that peptides and amino acids found in extracts of tissues so treated are artefacts of post-mortem proteolysis etc. Precipitation of protein by boiling has worked well in the hands of MACPHERSON (1952), who used mainly ensiled, already acid materials. 92—98% of the N extracted by him passed through cellophan. In most of the studies referred to below on peptide materials from seaweeds the raw material was initially extracted with boiling or hot water. Boiling has also been used in the isolation of seed "proteoses" (see below).

[1] Determination of total nitrogen cf. Vol. I.

BATHURST (1953), using aqueous extracts of freeze-dried ethanol-extracted grass, precipitated protein by acidification with acetic acid, and commented that this procedure precipitated the same amount of, or more N than conventional deproteinization with trichloroacetic acid. However, he did not subject the filtrates from acetic acid precipitation to treatment with trichloroacetic acid.

In general, there have been few critical studies (e. g. NEUBERGER and SANGER, 1942; BISSET, 1954) of yields of non-protein nitrogen after different deproteinizing procedures and often very little evidence has been adduced that the extracts obtained are free from protein. Students of plant peptides and amino-acids should note and emulate the critical studies by H. N. CHRISTENSEN and colleagues on non-protein fractions of blood and other tissues (e. g. CHRISTENSEN and LYNCH, 1946a, b). The use of protein precipitants such as picric acid, sulphosalicylic acid, trichloroacetic acid, colloidal iron etc. is even more empirical than the procedures referred to above. Early work with such reagents has been reviewed by RONA and STRAUSS (1922). From this period the STUTZER treatment with copper hydroxide has survived by virtue of having been adopted as the official method for determining "true protein" (see Official Methods of Analysis of the Association of Official Agricultural Chemists. 7th Ed^{n}. (1950) p. 345. Washington: Association of Official Agricultural Chemists; Agric. Progress **20**, 51 (1945).

The use of aqueous ethanol for obtaining protein-free extracts can be subjected to the same criticisms. It seems first to have been used to good effect for isolating non-protein nitrogenous fractions by OSBORNE, VICKERY and their colleagues (OSBORNE, WAKEMAN and LEAVENWORTH, 1921, 1922; VICKERY, 1924a, b 1925a, b; VICKERY and LEAVENWORTH, 1925; VICKERY and VINSON, 1925). In recent years it has achieved considerable popularity after its use by DENT, STEPKA and STEWARD (1947) in preparing an extract of potato for chromatography of free amino acids. The slight extraction of inorganic salts by ethanol is an attraction of the procedure. Although BATHURST (1953) has claimed that peptide and protein material are absent from extracts of freeze-dried grasses made with 80% ethanol (w/v or v/v not specified), it would be well to subject each case to individual study in view of the very different results, especially under alkaline conditions, found by OSBORNE, WAKEMAN and LEAVENWORTH (1921, 1922).

In all these separation procedures it should be noted that no control is exercised over the possible retention on the protein of smaller non-protein molecules by adsorption, ion exchange etc. Thus peptides containing residues with basic, acidic, aromatic or higher paraffinic side chains would be expected to be selectively retained on protein, and this may explain the rather simple amino acid composition of the peptide-like materials so far detected in ordinary tissues (see SYNGE, 1953b). Such effects with peptides and amino acids are likely, however, not to give rise to such serious difficulties as those found in extracting large molecules from plant tissues, referred to elsewhere in this work (Vol. I, p. 27). ASTRUP and ØHLENSCHLÄGER (1948) noticed interesting differences in the power of different extracting agents to remove glutathione from yeast.

In agricultural analysis the difference between total N ("crude protein") and N of "true protein" (determined by the STUTZER method referred to above) is often called "amide N". As CHIBNALL (1939, p. 19) has pointed out, this is not because at any time people have believed that only the amides glutamine and asparagine are present in this fraction; other amino acids have been known from the first studies to be present. It is simply that modern chemical nomenclature has been slow to penetrate agricultural analysis and the old name amido-acid (Amidosäure), often shortened to amide, has persisted. These terminological changes should be remembered also when studying the older literature. It has

long been known that the amino-acid composition of the non-protein fraction is less favourable for nutrition, at least of monogastric (non-ruminant) animals, than that of ordinary proteins (see section on amino acids below). The convention of regarding its N as having half the value of the N of "true protein" has thus had some justification in practice.

STREET (1949) has briefly reviewed techniques for determination of some of the main nitrogenous constituents of plants.

A. Peptides (Bound Amino Acids).

It seems clear that among the nitrogenous compounds found in plant extracts deproteinized by the methods referred to above peptides and bound amino acids are not usually present in large amount compared with free amino acids. However, there do not seem to be many cases where the former have been searched for by valid analytical means and not found. The many negative reports based on paper chromatograms coloured with ninhydrin can be ignored since many peptides (especially cyclic peptides) give little or no colour with ninhydrin. (In this connection the reaction of RYDON and SMITH (1952) seems likely to prove very useful for visualizing peptide material on filter-paper chromatograms and in spot tests. It should be noted, further, that peptides containing more than a few amino acid residues per molecule may not give good spots; but only diffuse streaks, on filter-paper chromatography.) However, small open-chain peptides would almost certainly, if commonly present in plant tissues, have been detected, so there are grounds for supposing that such substances, other than glutathione (see below) are usually absent.

It seems useless, in the present state of knowledge of plant peptides, to discuss in detail isolative procedures. Such procedures for peptides in general, as well as methods for further study of their chemical structure, have been well reviewed by SANGER, 1952 (see also BOULANGER and BISERTE, 1952). There is every reason for supposing that plant peptides will prove just as difficult to purify as peptides from other sources, each starting material, as well as each product to be isolated, setting a separate problem.

If isolation or separation is not required, analysis of a protein-free extract for peptide or chemically bound amino acid is comparatively simple. The increase on hydrolysis of free amino acids, determined by the ninhydrin-CO_2 procedure, gives a useful and specific measure of bound amino acids. Changes in other quantities (amino N, titratable basic and acidic groups, extent of Cu complex formation), though less specific, are useful on occasion. The most informative method of analysis, however, is to determine individual free amino acids present in an extract before and after hydrolysis by any of the *specific* methods for amino acids (see below). In applying such procedures account should be taken of the presence of amides.

The variations in "peptide N", determined by one or other of the less specific means just mentioned, or by still cruder difference procedures, have been studied by plant physiologists in a number of materials subjected to varying conditions. While such studies have been useful in throwing light on N metabolism, the variety of methods used prevents comparison of the results of different workers and it would be misleading to draw up comparative tables of total peptide present in different plant materials.

Special caution should be exercised in interpreting such data when there is a possibility of the MAILLARD reaction between sugars and amines having proceeded. This has been the subject of a number of studies in recent years. It proceeds