Methods of Tryptophan Analysis

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Most amino acids can be determined accurately by ion-exchange or gas chromatographic analysis of protein hydrolysates. Tryptophan, however, is destroyed by acid hydrolysis even under conditions best for other amino acids. Satisfactory alternatives have been sought for many years. Tested procedures include acid hydrolysis, alkaline hydrolysis, biological and enzymatic assays, spectrophotometric and spectrofluorometric methods, and various combinations of these. These methods are generally limited in accuracy and reproducibility, often because of interference by common substances. Complexity of special equipment needed is also sometimes restrictive. Evidently, a fast accurate general method for tryptophan is still urgently needed. One possible strategy is specific chemical modification of tryptophan in proteins to form an acid-stable derivative that can be determined in the hydrolysate in the same way as the other amino acids. Potential instances of scheme and mechanistic rationalizations for several spectrophotometric methods are suggested.

Tryptophan is a vital constituent of proteins. Its destruction is associated with off-flavors in irradiated foods and with yellowing of wool. Various metabolic products are important in normal brain function and in plant growth regulation, and some are potent drugs. It may be even more important in presently unknown functions. It is nutritionally essential for many animals and for man. Because of these known and suspected significances, analytic methods for this amino acid, both free and combined in proteins, have been extensively studied, but without complete success. We review these methods and their results and summarize the data. (A Table in the form of a computer printout summarizing the tryptophan content of about 700 proteins, foods, and feeds will be supplied on request.)

Acid hydrolysis followed by ion-exchange chromatography is generally used to determine the amino acid content of proteins. Acid hydrolysis commonly results in extensive destruction of tryptophan, yielding ammonia as the only recognized product.

Many methods have been tried for determining tryptophan specifically in the presence of other amino acids and constituents of biological products. Such methods have used acid, alkaline, or enzymatic hydrolysis followed by spectrophotometric, spectrofluorometric, or biological assay, and combinations of these procedures.

Colored derivatives from proteins were reported as early as 1831 (Tiedemann and Gmelin, 1831; Bernard, 1856). In the next 70 years many reactions of various proteins were reported to give color and odor similar to those from indole compounds (Vickery and Schmidt, 1931). For example, a characteristic red color forms by reaction with a halogen or nitrous acid (Neumeister, 1890). Neumeister did not know the structure of the compound responsible, but inferred that it probably contained an indole ring; for convenience he named it tryptophan, from the Greek, referring to its occurrence in tryptic digests of proteins. Hopkins and Cole (1901) first isolated tryptophan in a systematic study. For a characteristic test they used the violet color reported by Adamkiewicz (1875) to be produced by reaction of intact protein with glacial acetic acid and a little sulfuric acid. (In fact this test depends on an aldehyde impurity in the reagents.) After the isolation, interest developed rapidly, leading to an avalanche of reports of tryptophan contents of various proteins. Concerning these data Looney (1926) commented, "The multiplicity of methods proposed for the estimation of tryptophan and the inconsistency of the values given are sufficient evidence of the importance attached to the subject and the worthlessness of most of the methods."

Most early methods exploited reactivity of the indole ring to produce colored derivatives by reaction with reagents such as ferric chloride, cupric sulfate, bromine, sodium nitrite, or sodium hypochlorite, usually in acidic solution. Later methods include reaction with potassium nitrite and an aliphatic or aromatic aldehyde in concentrated hydrochloric acid or sulfuric acid. One of the most widely used procedures has been to treat the tryptophan with *p*-dimethylamino-benzaldehyde in sulfuric acid, and then oxidize the product with sodium nitrite.

Unfortunately, these methods are limited in accuracy and tend to be tedious. The microbiological methods are also quite laborious and often less precise than chemical methods, although chemical and biological methods sometimes agree closely. Later workers have formed other colored derivatives, while still others have used the natural characteristic absorption in the near ultraviolet to estimate tryptophan spectrophotometrically and spectrofluorometrically (Udenfriend, 1969; Bernstein *et al.*, 1969; Weinryb and Steiner, 1969; Bhatnagar and Gruen, 1969; Edelhoch, 1967).

It is noteworthy that tryptophan constitutes about 1% of the amino acid residues in most proteins (Smith, 1966).

ION-EXCHANGE CHROMATOGRAPHY

Tryptophan is eluted from an ion-exchange column as used for standard analysis of the basic amino acids (Figure 1) as a well-resolved peak appearing before lysine. For this reason an ideal protein analysis would allow tryptophan to be released quantitatively by hydrolysis and determined in the same routine used for the other amino acids. If loss of tryptophan cannot be avoided during hydrolysis, a second attractive strategy is the conversion to a derivative that can be recovered quantitatively after hydrolysis and determined, again, as the other amino acids.

Acid Hydrolysis. Although tryptophan is usually destroyed in the usual acid hydrolysis of proteins, Matsubara and Sasaki (1969) discovered that moderate concentrations of thioglycolic acid greatly decrease its destruction. Recoveries of tryptophan ranged from 80–90%. We made similar recoveries when this procedure was applied to keratin

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proteins. Nevertheless, this procedure has several disadvantages: incomplete recovery; elimination of the protective effect of thioglycolic acid by glucose and presumably other carbohydrates; and need for separate analysis for the other amino acids.

It is also noteworthy that the tryptophan content of wool was determined colorimetrically after hydrolysis in 18 *N* H_2SO_4 for 2 hr at 70° C (Cégara and Gacén, 1968). Similarly, Vallés *et al.* (1966) determined the tryptophan content of anchoveta flour colorimetrically with the aid of xanthydrol after hydrolysis for 3 hr in 8 *N* H_2SO_4.

Basic Hydrolysis. For many years, many (but not all) workers have considered tryptophan more stable to alkali than to acid, so that basic hydrolysis of proteins would be preferable for tryptophan analysis.

According to Oelshlegel *et al.* (1970), most methods of alkaline hydrolysis are either time-consuming or inaccurate. They recommend an alkali-resistant centrifuge tube as a liner for an evacuated glass apparatus for protein hydrolysis by sodium hydroxide in the presence of thiodiglycol. They determine tryptophan in the hydrolysate with an automatic amino acid analyzer in which the resin is replaced by potato starch. The procedure appears useful for both pure proteins and for proteins in foods. Recovery of added tryptophan ranged from 89–96.6%.

However, recovery of added tryptophan does not assure recovery of tryptophan combined in proteins. Thus, protein-bound tryptophan gives a greater color yield with *p*-dimethylaminobenzaldehyde than free tryptophan. Shaw and McFarlane (1940) and Graham *et al.* (1947) similarly demonstrated that adding gelatin to free tryptophan before color development gives a color yield different from that of protein-bound tryptophan. These and related observations forcefully demonstrate that recovery of added free tryptophan may not be a valid test of the method, since free tryptophan in alkaline media undergoes side reactions such as racemizations more slowly than tryptophan residues in peptides, and gives a different color yield with *p*-dimethylaminobenzaldehyde. To obtain unequivocal evidence of the merit of alkaline hydrolyses, Spies and Chambers (1949) studied factors affecting the stability of free and peptide-linked tryptophan in 5 *N* NaOH. They devised an hydrolysis procedure in hydrogen in a Parr nickel microbomb after which recoveries of tryptophan were 99.3, 97.7, and 96.9% for 18 hr heating at 100°, 151°, and 185° C, respectively. Spies and Chambers (1949) investigated the effects of time and temperature on racemization of free and peptide-linked tryptophan in 5 *N* NaOH and the relationship of racemization to the stability of tryptophan with respect to protein hydrolytic products. Complete racemization of free tryptophan occurred in 2 and 8 hr at 185° and 151° C, respectively, while after 18 hr at 100° C, racemization of tryptophan was only 11%, if uncombined, 65% in *N*-acetyltryptophan amide, and 100% in proteins. Evidently, during alkaline hydrolysis tryptophan in peptide-linkage is more labile than free tryptophan at the usual temperatures. Hence recovery of free tryptophan added to a protein for alkaline hydrolysis is not a reliable indication of loss of peptide-linked tryptophan. It was shown that losses of tryptophan during alkaline hydrolysis depend on the protein composition.

The reason that protein-bound tryptophan racemizes more readily than free tryptophan is probably that racemization occurs by reversible abstraction of the proton from the α-carbon atom to yield a carbanion. Reprotonation yields DL-tryptophan because the tryptophan carbanion does not retain asymmetry. Any factor tending to stabilize the carbanion would be expected to enhance racemization.

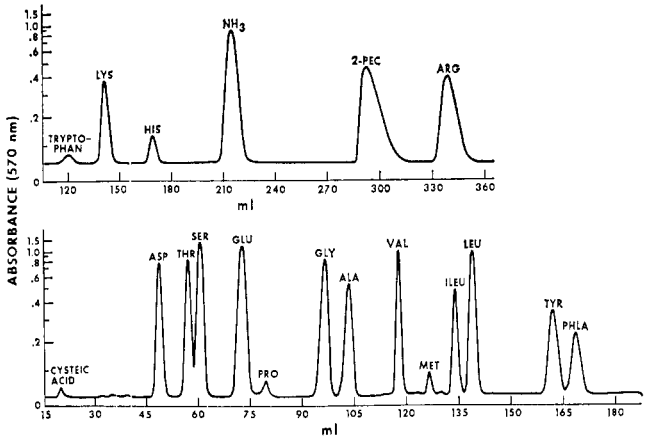

Figure 1. Elution position of tryptophan and other amino acids on ion-exchange columns of an amino acid analyzer. The SH groups in reduced wool were alkylated with 2-vinylpyridine. The *S*-pyridylethyl wool was hydrolyzed in 6 *N* HCl for 24 hr at 110° C in the presence of 1% mercaptoethanol. Upper plot: basic column. Lower plot: acidic and neutral column. Additional analytical details are given in the paper by Friedman and Noma (1970)

Basic hydrolysis by either $Ba(OH)_2$ or NaOH (sometimes in conjunction with enzymatic hydrolysis) of proteins and foodstuffs has been critically examined by many workers, including Spies and Chambers (1949), Dreze (1960), Porath (1960), Gelotte (1960), Lunven (1963), Korfanyi (1964), Babin *et al.* (1966), Spies (1967, 1968), Miller (1967), Robel (1967), Slump and Schreuder (1969), and Knox *et al.* (1970).

SPECTROPHOTOMETRIC METHODS

Hopkins–Cole Reaction (Glyoxylic Acid Method). The earliest quantitative determination of tryptophan in proteins was made by Hopkins and Cole (1901). They demonstrated that glyoxylic acid was an essential reagent in the glacial acetic acid test for indoles first developed by Adamkiewicz (1874, 1875). The tryptophan is precipitated as a mercury salt, then redissolved and treated with glyoxylic acid to take advantage of the reactivity of the indole ring to develop a colored derivative. Several modifications were subsequently suggested (Cary, 1926; Brice, 1933; Winkler, 1934; Shaw

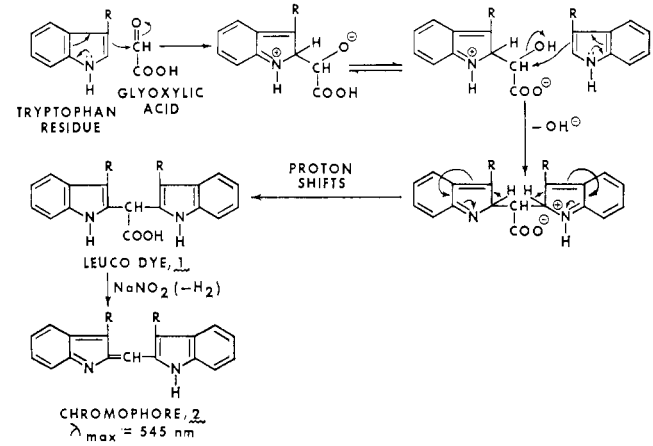

Figure 2. Proposed mechanism of formation of chromophore from 1 mol of glyoxylic acid and 2 mol of tryptophan (Hopkins–Cole reaction)

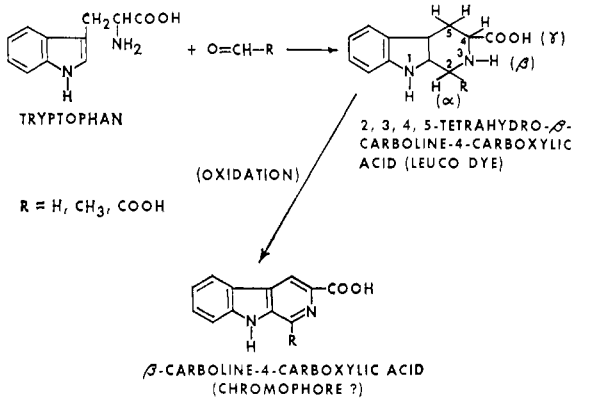

Figure 3. Transformation of tryptophan to carboline derivatives

and McFarlane, 1938). In 1934 Winkler (1934) observed that copper sulfate greatly enhanced color development. Four years later Shaw and McFarlane (1938) further improved the analysis by hydrolyzing the protein with 5 *N* sodium or barium hydroxide, or else dissolving the protein in 10 to 20% NaOH or in 5% formic acid before reaction with glyoxylic acid. In 1960 Fischl (1960) developed a modification with which he was able to determine 5 μg of tryptophan in a sample. In 1962 Polish workers (Opienska-Blauth *et al.*, 1963) described a method in which iron in the presence of H_2SO_4 converts acetic acid to glyoxylic acid that, in turn, effects the Hopkins–Cole reaction.

A possible mechanism for the formation of a chromophore from two tryptophan residues in proteins and one molecule of glyoxylic acid is illustrated in Figure 2. The colorless leuco dye **1** is initially formed and is oxidized to the resonance-stabilized chromophore **2** with $NaNO_2$ or other oxidizing agent. Compound **2** shows a structural resemblance to the ninhydrin chromophore (Ruhemann's purple) which also absorbs visible light (Friedman and Sigel, 1966; Neuzil *et al.*, 1966).

It should be pointed out that simple aldehydes, such as formaldehyde, acetaldehyde, and presumably glyoxylic acid, form tetrahydrocarboline derivatives with tryptophan itself (Figure 3). Since this reaction involves the amino group in tryptophan, it probably does not occur in a protein unless tryptophan is N-terminal. Although the formation of the tetrahydrocarboline derivative has been demonstrated (Harvey *et al.*, 1941; Jacobs and Craig, 1936), the structure of the chromophore as the presumed oxidation product remains to be established.

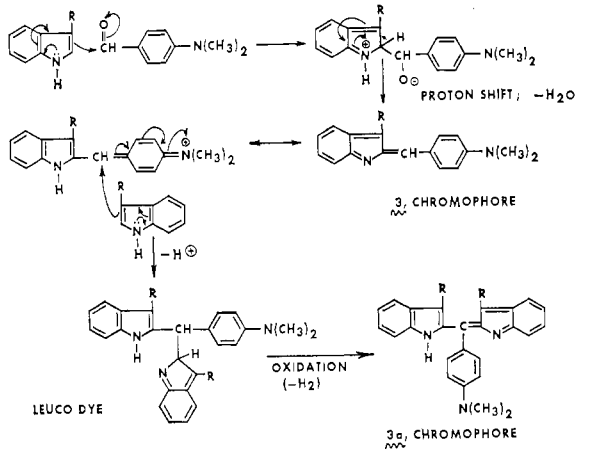

Figure 4. Mechanism of formation of chromophores from tryptophan and *p*-dimethylaminobenzaldehyde

Aldehydes. In 1905 Voisenet (1905) observed that proteins, indole, skatole, and related compounds give highly colored solutions when treated with hydrochloric acid (instead of sulfuric acid), potassium nitrite, and various aldehydes. Voisenet (1905) used formaldehyde, acetaldehyde, benzaldehyde, *p*-hydroxybenzaldehyde, and glucose as aldehydes in the reaction. Rhode (1905) in the same year reported that proteins give a red color in 10% sulfuric acid and 5% *p*-dimethylaminobenzaldehyde (Ehrlich's reagent) when concentrated hydrochloric acid is also added. Other aromatic aldehydes such as vanillin and *p*-nitrobenzaldehyde can be used in place of Ehrlich's reagent. Since that time more tryptophan procedures have been based on reactions with aldehydes than on any other one principle.

Thomas (1920) adapted the Voisenet–Rhode reaction to samples hydrolyzed for 5 to 7 days with pancreatin. After hydrolysis the hydrolysate was treated with *p*-dimethylaminobenzaldehyde in 20% HCl for 48 hr.

In 1925 Kraus (1925) reported using the vanillin–HCl reaction for tryptophan, including a procedure to separate indole and skatole, thus eliminating high readings due to traces of these compounds.

May and Rose (1937) and Holm and Greenbank (1923) treated intact protein with *p*-dimethylaminobenzaldehyde for several days at slightly elevated temperatures. Many workers have reported modifications of this procedure.

Voisenet (1905) noted that oxidizing agents increase color formation while Thomas (1920) demonstrated the importance of meticulously controlled conditions, not only with respect to reagents but temperature as well. Several others (Furth and Dische, 1924; Rapoport and Eichinger, 1936; Boyd, 1929; Komm, 1926; Tomiyama and Shigematsu, 1934; Sullivan and Hess, 1944) showed the importance of each variable in the reaction independently. In 1940 Shaw and McFarlane compared the *p*-dimethylaminobenzaldehyde method using hydrogen peroxide as oxidizing agent with the Hopkins–Cole glyoxylic acid method.

Graham *et al.* (1947), Spies and Chambers (1948, 1949), and Spies (1950, 1967, 1968) have reported modifications of the *p*-dimethylaminobenzaldehyde method with both hydrolyzed and intact proteins with light or sodium nitrite for oxidation. They also have described the influence of several variables.

In Figure 4 we offer a rational mechanism leading to colored products formed by tryptophan and dimethylaminobenzaldehyde. The mechanistic reasoning is similar to that of Friedman (1965) for interaction of pyrroles and aldehydes to form porphyrin pigments. Current evidence does not permit a choice between structure **3** (derived from 1 mol of tryptophan and 1 mol of aldehyde) and **3a** (derived from 2 mol of tryptophan and 1 mol of aldehyde). However, since formation of **3a** requires oxidation and since the chromophore absorbs in the visible near 600 nm, structure **3a**, which has a more extended cross-conjugation, is the more likely (Ghigi, 1933; Fischer and Nenitzescu, 1925; Strell and Kalojanoff, 1954).

Interfering Substances. Many protein samples containing lipid require solvent extraction before analysis. A more critical question for basic hydrolysis is whether starch interferes with analysis. Dréze (1960) claims that tryptophan is not destroyed in the presence of starch when barium hydroxide is used as the base instead of sodium hydroxide.

Intense light, oxidizing and reducing agents, including

H_2S, bisulfite ions, hydrogen peroxide in low concentrations, and chloride ions, interfere in the Spies dimethylaminobenzaldehyde method (Spies and Chambers, 1948, 1949; Lesuk, 1948; Junéja *et al.*, 1968; DasGupta, 1969). Spies has eliminated interference by H_2S, chloride, and bisulfite ions by appropriate use of silver sulfate, which does not interfere (Spies and Chambers, 1950).

Inglis and Liu (1970) found that during acid hydrolysis in 6 *N* HCl, tetrathionate reacts with tryptophan residues, thereby preventing reaction of this amino acid residue with cysteine. For example, Streptococcal proteinase, with 1 half-cystine residue and 5 tryptophan residues per molecule, yielded only 78% of the expected *S*-sulfocysteine on reduction with dithiothreitol followed by addition of tetrathionate after hydrolysis. The yield was quantitative when a small amount of tetrathionate was added before hydrolysis. Analogous results were noted with two Merino wool samples. Although low tryptophan values are inferred to be due to interactions among sulfur amino acids, tetrathionate, serine, and threonine with the indole ring of tryptophan, the nature of these reactions remains to be established. Thus, serine, threonine, and sulfur-containing amino acids caused partial destruction of tryptophan during alkaline hydrolysis, whereas the other amino acids had a protective effect. None of the amino acids interfered in analysis of intact protein as recommended by Spies and Chambers (1949). However, it was later shown that while the intact protein method gave accurate tryptophan values in many cases, too high values (Spies, 1967) and sometimes abnormal colors were obtained in others (Harrison and Hofmann, 1961). The intact protein method was then replaced by methods involving hydrolysis either in 5 *N* NaOH in nitrogen with the tryptophan stabilized by histidine and basic lead acetate or with Pronase. In either case dimethylaminobenzaldehyde was used to develop color.

For these reasons, Spies (1967, 1968) recommends that Procedures U and W supersede those procedures using intact proteins previously recommended. He states (Spies, 1970) that in procedure U, tryptophan is protected during alkaline hydrolysis in nitrogen from destruction by serine, threonine, and sulfur-containing amino acids by using histidine and basic lead acetate. Procedure W uses mild hydrolysis with enzymes before tryptophan assay. Silver sulfate removes interference by sulfur-containing amino acid decomposition products. Light is easily subdued or excluded during reaction. Chloride and bisulfite ions are not common and are easily removable from purified proteins. If present in reasonable concentrations, they would not interfere anyway because of use of silver sulfate. Hydrogen peroxide is not present in purified proteins.

Junéja *et al.* (1968) estimated tryptophan content of wool colorimetrically after hydrolysis with 6 *N* $Ba(OH)_2$ for 5 hr at 125° C followed by removal of liberated H_2S and barium with silver sulfate and sulfuric acid, respectively. The authors evaluate the interference by sulfur–amino acids in detail.

An alternate way to eliminate interference by cysteine and cystine residues is to reduce protein disulfide bonds and alkylate the native and generated SH groups with vinyl derivatives (Friedman and Noma, 1970; Friedman *et al.*, 1970). This strategy permits simultaneous assay of two problem amino acids by ion-exchange chromatography: tryptophan and cysteine or cystine as 2-*S*-pyridylethyl derivatives (2-PEC), as illustrated in Figure 4. The suggested strategy deserves further evaluation with various tryptophan-containing proteins.

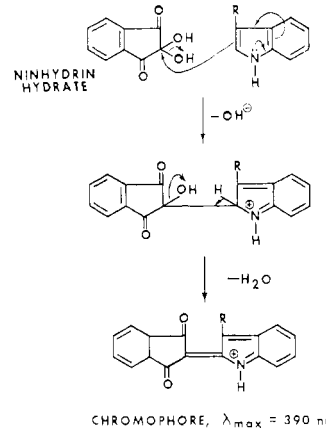

Figure 5. Proposed mechanism of formation of chromophore from tryptophan and ninhydrin hydrate in acid solution

Koshland's Reagent. Koshland and coworkers (Barman and Koshland, 1967; Horton and Koshland, 1965) have used 2-hydroxy-5-nitrobenzylbromide as a specific reagent for tryptophan free and in proteins. Although treatment of model indole derivatives with 2-hydroxy-5-nitrobenzylbromide leads to two types of tetracyclic indoline derivatives (Witkop, 1968; Loudon *et al.*, 1969) similar compounds may not result from tryptophan residues in proteins inasmuch as the postulated indoline derivatives would be expected to absorb near 300 nm rather than near 410 nm as observed by Koshland with proteins (Spande *et al.*, 1968; Ohno *et al.*, 1970).

Horton and Tucker (1970) showed that dimethyl(2-hydroxy-5-nitrobenzyl)sulfonium salt can be used for spectrophotometric estimation of tryptophan in proteins at pH 3. This water-soluble sulfonium salt may, in some applications, offer a potential advantage over the water-insoluble 2-hydroxy-5-nitrobenzyl bromide. Although Koshland's reagent is now widely used for soluble proteins, it may not always be sufficiently specific and accurate. Thus, Burton *et al.* (1970) obtained low and inconsistent results for the tryptophan content of *E. coli* pyrophosphatase.

Sulfenyl Halides. Boccu and associates (1970) developed a procedure for estimating tryptophan in proteins by reaction of tryptophan residues with either 2- or 4-nitrobenzenesulfenyl halides (Nps–Cl) in aqueous acetic or formic acid. Tryptophan is estimated in the sulfenylated protein by spectrophotometric determination at 365 and 328 nm of the 2- and 4-nitrobenzenesulfenyl chromophores.

Sulfhydryl groups of cysteine residues also react with sulfenyl halides to form mixed disulfides that absorb at 450 nm. Consequently, it is necessary to remove the yellow label (the mixed disulfide) from the cysteine residues by dissolving the modified proteins in 0.1 *N* NaOH before spectrophotometric determination of tryptophan. However, the method of Boccu *et al.* (1970) suffers from the disadvantage that after reaction of the protein with Nps–Cl, the condensation product has to be separated by Sephadex filtration, the product isolated and dissolved, and the concentration of protein determined indirectly from the average recoveries of leucine, arginine, and alanine in the amino acid analyzer. This procedure is not very convenient or straightforward from the standpoint of quantitative technique.

Ninhydrin Reaction. Gaitonde and Dovey (1970) and Gaitonde (1967) discovered an acid ninhydrin reagent for determining both cysteine, which gives a very low ninhydrin color yield under the usual conditions of reaction (Friedman

and Sigel, 1966), and tryptophan. Treating either free or protein-bound tryptophan with ninhydrin in a mixture of formic acid and hydrochloric acid for 10 min at 100° C results in a chromophore, with λ_{max} at 390 nm. This method is particularly useful for determining tryptophan in biological fluids and tissue proteins. Thus, a mean tryptophan content of 11.25 μmol/100 mg of protein (2.1% of the amino acid residue) was found in rat brain during development from 1 to 82 days after birth.

Although the extinction coefficient of the chromophore varied somewhat with the nature of the model compound and reaction conditions, we propose the mechanism illustrated in Figure 5 for the acid–ninhydrin reaction of tryptophan. The reasoning on which this mechanism is based is like that described for the ninhydrin reaction at pH 5.5 (Friedman and Sigel, 1966; Friedman, 1967).

Direct Ultraviolet Methods. The spectrophotometric methods for tryptophan determination take advantage of the different absorption maxima of tryptophan and tyrosine in 0.1 *N* sodium hydroxide. First reported by Holiday in 1936 and later by Goodwin and Morton (1946) and Bencze and Schmid (1957), the procedure of determining the absorbance at two wavelengths and calculating tryptophan and tyrosine in an intact protein offered great promise.

Edelhoch (1967) made a significant improvement by dissolving the protein samples in guanidine hydrochloride. This technique improved solubility by denaturing the protein in solution, but unfortunately many proteins were still not soluble under these conditions.

Edelhoch's procedure is based on spectral examination of a protein solution in 6 *M* guanidine hydrochloride and the solution of two simultaneous equations derived from studies of the absorption spectra of trysoine- and tryptophan-containing model compounds. The small absorption due to cystine has to be subtracted from the calculated tryptophan value. The author stresses that anything that contributes to absorbance at 280 and 288 nm (contaminants, cofactors, turbidity) will interfere with the spectral analysis. He further suggests that a good method for checking for absorbing impurities and opalescence is to examine the absorbance of the protein in neutral 6 *M* guanidine hydrochloride at 315 nm where tyrosine and tryptophan do not absorb.

MICROBIOLOGICAL METHODS

Greene and Black (1943) have described special features of determining tryptophan with microorganisms. The tryptophan must be available to the test organism, and substitutes for the tryptophan must be eliminated from the system (Greene and Black, 1944). *Lactobacillus arabinosus* 17-5 was found very sensitive to L-tryptophan and would not grow on closely related compounds.

Subsequently the same workers compared a colorimetric method with the microbiological one (Smith *et al.*, 1946), The two methods agree quite well. Microbiological assays have been used extensively since the middle 1940's with mutants of *E. coli* 113-3 (Verbina and Konova, 1966), *Bacillus subtilis*, and *Streptococcus zymogenes* (Verbina, 1965). It is not necessary to use microorganisms for bioassay. Harwood and Schrimpton (1969) used chick growth as an index of tryptophan present in intact protein.

FUTURE STUDIES

Most procedures mentioned in this paper have inherent disadvantages with respect to accuracy, reproducibility, sensitivity, convenience, and general applicability to the determination of tryptophan present in a variety of soluble and insoluble proteins, feeds, foodstuffs, and tissues. Original authors cited in this review often discuss these disadvantages in more detail than given here. A need still exists for a fast, accurate, generally applicable method for determining tryptophan. A possible solution that we propose is specific chemical modification of tryptophan residues in proteins to form an acid-stable derivative that can be determined in an acid hydrolysate simultaneously with the other amino acids by ion-exchange or gas–liquid chromatography, or individually by mass spectroscopy. [Such a solution was successfully developed for the analysis of cystine residues in soluble proteins, keratins, and seed meals (Friedman *et al.*, 1970; Friedman and Noma, 1970).] Several attempts of this kind, including known and potential chemical photochemical transformations that may lead to the desired objective, are briefly mentioned in this section. We are currently exploring some of the cited reactions.

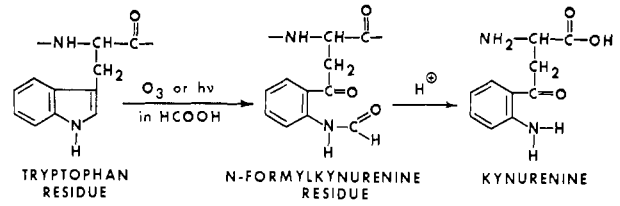

Figure 6. Chemical and photochemical oxidation of tryptophan to kynurenine residues

Oxidation to Kynurenine. Tryptophan residues are oxidized to N^1-formylkynurenine by ozone (Previero and Coletti-Previero, 1967, 1964) and by proflavin-sensitized photooxidation to kynurenine (Galiazzo and Scoffone, 1968; Lapuk *et al.*, 1968) (Figure 6). Kynurenine has an absorption maximum near 360 nm and is eluted from the column of an amino acid analyzer as a discrete peak before lysine, as shown in Figure 7 (Friedman and Finley, 1970). Conceivably conditions could be devised for quantitative transformation of tryptophan to kynurenine residues to soluble and insoluble proteins. The resulting acid-stable kynurenine could be estimated spectrophotometrically before protein hydrolysis and by ion-exchange chromatography after hydrolysis, with suitable modification of the ion-exchange procedure.

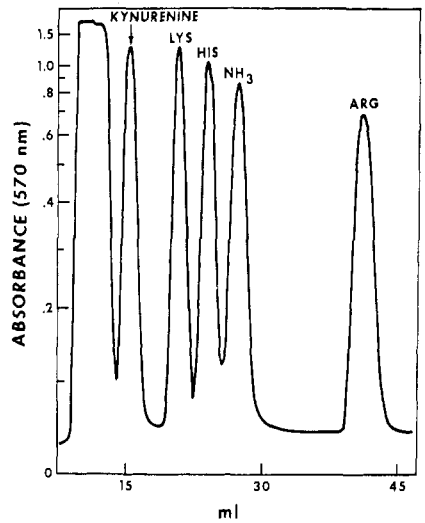

Figure 7. Elution position of kynurenine on a basic ion-exchange column of an amino acid analyzer

Acid Degradation Product of Tryptophan. Another strategy for tryptophan determination would be to determine and measure quantitatively the acid degradation products of tryptophan. At present, the identity of these products is not known. If they were isolated and characterized, a suitable analytical procedure could very likely be devised for their estimation.

CONCLUSIONS

Because of uncertainties in any of the described methods in response to an unknown sample, tryptophan analyses should be rechecked by different methods to assure reliable results. For an initial survey, we suggest acid hydrolysis in the presence of either thioglycolic acid (Matsubura and Sasaki, 1969) or mercaptoethanol (Friedman and Finley, 1970) because it is relatively quick and simple. The results can then be improved by careful use of Spies (1967) procedures U and W for pure proteins; procedure W (Spies, 1968) for foodstuffs; or the ninhydrin method (Gaitonde and Dovey, 1970) for biological fluids.

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