Determination of Tryptophane in Whale Meat

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Since the discovery in 1906 by Rose, Willock, and Abderhalden, that tryptophane was an indispensable amino acid in human body, many reports have been published regarding its change and mechanism in the living body. The authors, in order to determine the content of this amino acid in whale meat and to determine whether whale meat could be utilized as resource for peptone production, carried out comparative tests on quantitative determination of tryptophane and thereby found a novel revised method.

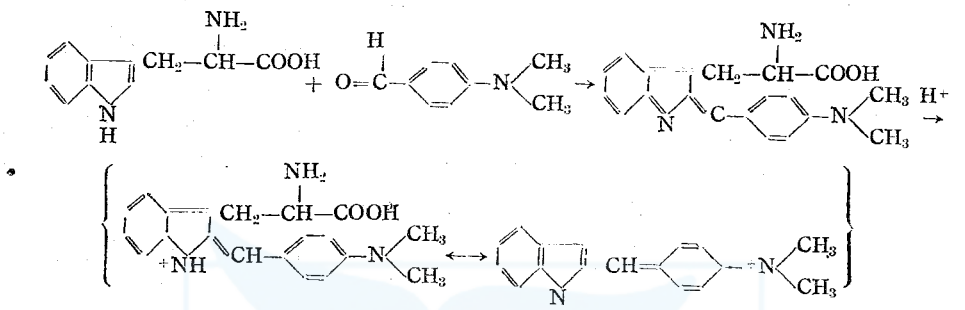
Theoretical

There are already numerous methods of determining tryptophane, which, unlike other amino acids, possesses indole nucleus in its structure so that there are qualitative, quantitative and colorimetric methods for its determination. Homer[1] led tryptophane to its bromo compound and then titrated excess amount of bromine by iodometry. Homer maintains that tryptophane gives octabromo-tryptophane by bromination. However, Levner says that it forms tribromo compound and this point has not been settled as yet. If this point can be settled, bromination methhod is excellent and simple because the reaction by halogen is limited to free tryptophane except ablin (N-methyltryptophane). All other tryptophane derivatives and addition products do not react to this reagent.

Quantitative determination by Hopkins-Cole reagent of mercury-tryptophane method and its modification by Onslon[2] by the determination of N-value in mercury-tryptophane are some of other methods but the most ordinary method is to use colorimetric measurement. Colorimetric reagent employs, chiefly, glyoxylic acid, formaldehyde and p-dimethylaminobenzaldehyde, which possess carboxyl radical. Others employ xanthoprotein[3] and Pauly's diazo reactions but these are also positive to amino acids having benzene ring, such as tyrosine and phenylalanine, so that a more complicated processes are required and cannot, moreover, be taken as a specific reaction for tryptophane. It follows, therefore, that colorimetric determi-

nation of tryptophane should employ reagents possessing carboxyl radical, especially the Ehrich reagent recommend by Neuber and Rohde, Hohm[4], May and Rose[5].

Ehrlich reagent employs p-dimethylaminobenzaldehyde which is condensed to tryptophane in H_2SO_4-acidity and the dyestuff is thus formed. Chemical mechanism of the dyestuff formation can be explained as follows:



Colouring

The above reaction necessitates the presence of H^+ so that, if OH^- is present, the reagent aldehyde does not condense to the nucleus but with the amino radical in the side chain. Ehrlich reagent, therefore, colors only under strong acidity.

If tryptopnane itself undergoes change only under this condition, this reaction would become unsuitable for the determination of tryptophane. The authors, therefore, observed the change in tryptophane, with considerations of the effects of reaction time and temperature, and found, as is generally known, that tryptophane resisted acids. In other words, no change could be observed when tryptophane was boiled in aqueous solution of mineral acids. However, when carbohydrates, beside tryptophane were present, these reacted under strong acidity and formed the so-called Fumine substance of unknown structure. In these cases, there is no sense in colorimetric determination of tryptophane. However, if this reaction is carried out under 40°C, Fumine substance is not formed, even under strong acidity. This leaves the question of reaction time. As a result of determining minimum time required for total tryptophane in protein to be isolted by hydrolys it was found that, although it differed slightly according to the proteins used, the hydrolysis was completed in 5—10 days with proteins from whale meat and casein. Side reactions occurred when the reaction time was prolonged over two weeks and the amount of tryptophane found was decreased considerably. These experiments were conducted under the

presence of p-dimethylaminobenzaldehyde. The reason for this is that, even if carbohydrates are isolated from proteins by hydrolysis, tryptophane will have condensed with p-dimethylaminobenzaldehyde before it had time to bond with carbohydrates to form Fumine substances. Rose determined the amount of tryptophane in protein by using Ehrlich reagent and 20% HCl, with the reaction at 35—37° for 24 hours and at room temperature for 48 hours, determining the coloration here formed. The authors learned that, uder these conditions, complete hydrolyses of protein cannot be expected and therfore, determineed the time where coloration by Ehrlich reagent was at its maximum by determing amino form nitrogen by Van Slyke method by which the degree of protein hydrolysis would be measured. As a result, the afore-mentioned reaction time of 5—10 days, temperature of 35°C, and degree of protein hydrolysis of 85—90% were obtained.

Reaction above these figures would give discoloration which eventually gave decreased amount of tryptophane.

Results obtained by Boyd[6], Hohm and Konm[7], on pure tryptophane coincided with the results obtained by the authors.

Experimental

Colorimetric Standard and Test Solution:

Preparation of Standard Solution: For the preparation of this solution, Protiens not containing tryptophane, such as gelatine or zein, are used. 3a (a=50 mg) of this protein is added to 1 mg of pure tryptophane.

Test Solution: (1—2) a of sample protein is added to (1—2) a of gelatine bringing the amount of total protein to 3a. The object of the addition of gelatine is to shorten reaction time somewhat and to control the coloration.

Method:

100 g of the sample protein and 50 mg of gelatine are placed in 100c.c. measuring flask. On the other hand, standard substance is prepared by placing 150 mg of gelatine and a determined quantity of pure trytophane in 100 c.c. measuring flask. 1 c.c. of Ehrlich reagent (5% p-dimethylamino-benzaldehyde in 10% H_2SO_4 solution) is added to each of the above flasks, shaken well, and bring the amount of solution to 100 c.c. by adding 20% HCl. This is left in a thermostat at 35°C and the coloration observed every day. Colorimetric determination with Dubosqui's colorimeter is made when the coloration seems to be at a maximum. In the present experiment, the

reaction required 5—10 days. Following are the results obtained:

Content of tryptophane in whale meat.

After dehydration of whale meat by acetone, degreasing and drying, the sample is taken of this dried whale meat. Crude protein contained in the sample was 66.3% as determined by Kjeldahl method. Content of tryptophane in whale meat.

Sample	Tryptophane (%)	Average (%)
A	0.97	0.95
B	0.91	
C	0.96	
D	0.94	

Content of Tryptophane in Casein not purified.

Sample	Tryptophan (%)	Average (%)
A	1.66	1.79
B	1.83	
C	1.89	

References

1) Homer: J. Biol. Chem. (1915) XXII 369.
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3) Tillmann: Biochem. Z. (1928) 198 3719.
4) Hohm: J. Am. Chem. Soc. (1923)XIV 1788.
5) May and Rose: J. Biol. Chem. (1922) 54 213.
6) Boyd Biochem: J. (1929) 23 78.
7) Komm: J. Physiol. Chem. (1922—23) CXXIV 287.