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Aminoacid composition of proteins

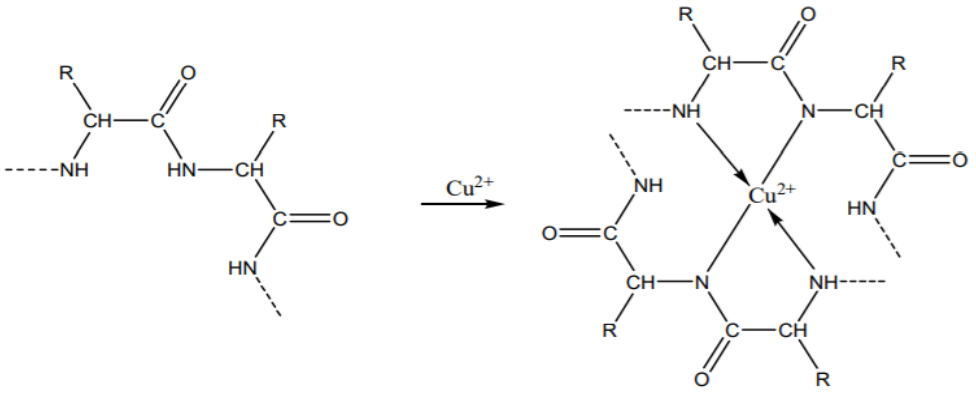
(theoretical part)

Biuret reaction of Piotrowski (the detection of peptide bond)

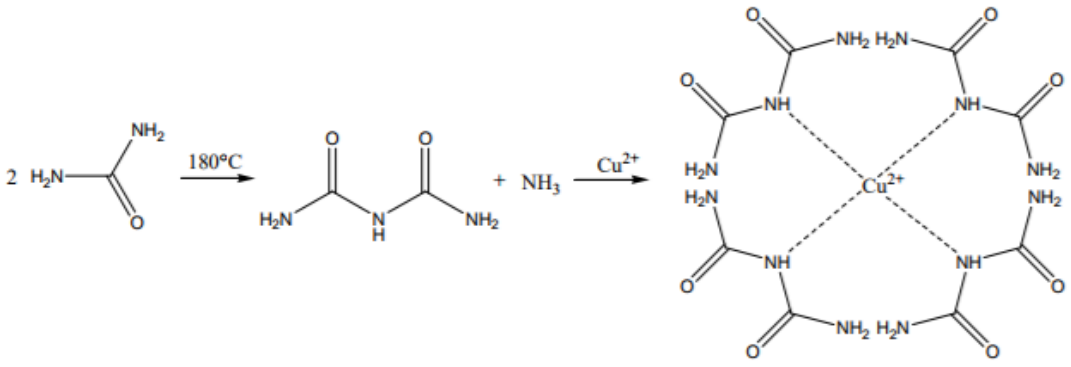
It is one of the most often performed reactions in protein analysis. It allows for the detection and quantitative determination of proteins in solution.

This reaction is positive for molecules containing at least 2 groups –CO-NH-, joint together: directly (in diamide of oxalic acid $H_2N-CO-CO-NH_2$, via nitrogen (in biuret)or via carbon atom (in diamide of malonic acid, succinic acid) or in products of hydrolysis of proteins. The reaction is negative for free aminoacids and dipeptides.

As the result of formation of coordinative junction of cooper with 2 adjacent bonds –CO-NH- coloured product (light violet) of complex salt is synthesized. The intensity of colour depends on the number of peptide bond and the same on the concentration of protein.



*Name is derived from biuret – compound which is formatted during heating of urea up to 180oC. It is also the simplest compound which gives positive result of this reaction

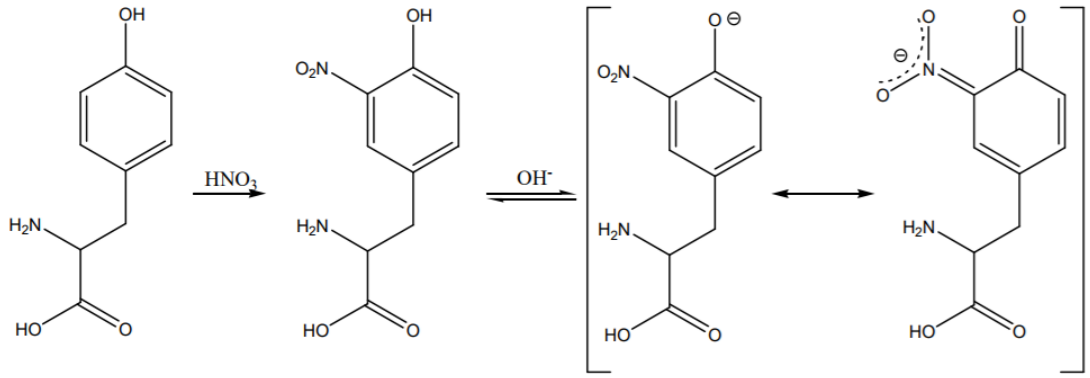


Biuret reaction is used for quantitative determination of proteins in biological samples such as plasma. Linear dependency between the change of colour and the concentration of protein

(the number of double peptide bonds) is used. Colorimetry helps to find the relationship between examined sample and standard curve.

Xantoprotein reaction

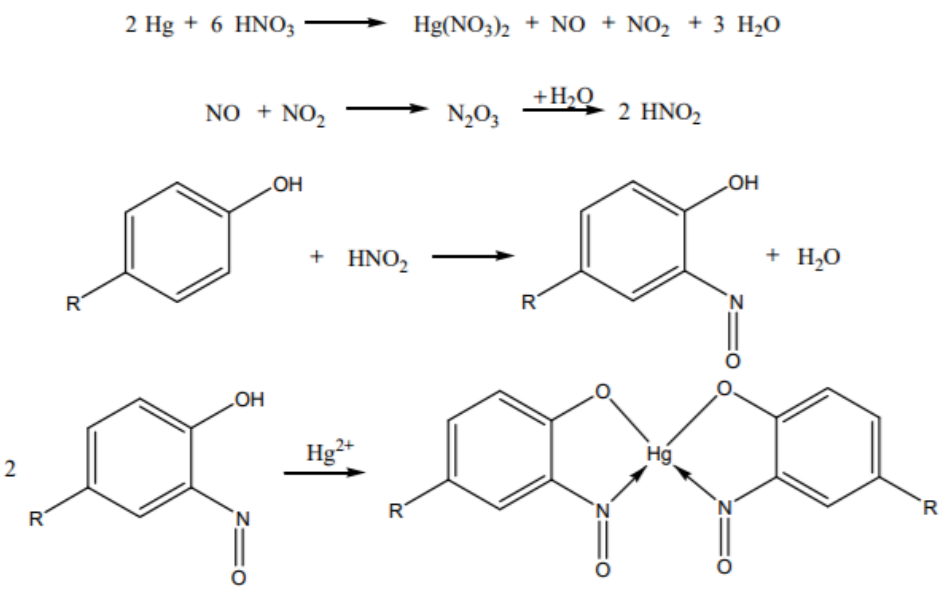
Positive xantoprotein reaction is detected with aromatic aminoacids: **tyrosine, tryptophan, phenyloalanin** and proteins that contain these aminoacids. These compounds undergo the nitration reaction during heating with concentrated solution of HNO_3. In result yellow nitro derivatives are formatted (phenyloalanin for nitration requires the addition of H_2SO_4). After alkalinization nitro-derivatives become orange:



*Xantoprotein reaction is the example how to distinguish between aromatic and non-aromatic compounds. It is positive with all compounds containing aromatic ring (in case of only one substituent in the ring or its lack, the addition of sulfur acid(VI) to nitrate mixture is indispensable.

Millon reaction

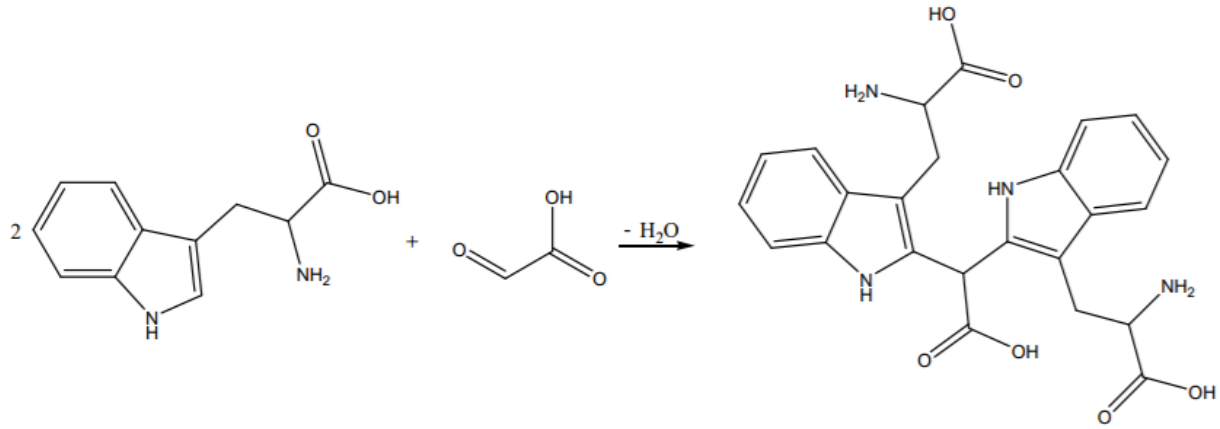
It serves for the detection of **tyrosine** and proteins which contain it. Tyrosine undergoes nitration reaction in orto position of aromatic ring. -o-nitrozo-derivatives which are formatted create coloured complex with mercury ions Hg^{2+}.



*Millon reaction is characteristic for phenols with at least one not substituted orto position. It is used in organic chemistry for the detection of phenols with free orto position and substituents in para position (as it is in tyrosine) which do not give positive results in other reactions for the detection of phenols.

Adamkiewicz-Hopkins reaction

It serves for the detection of **tryptophan** and proteins which contain it. In acidic environment indol rings of two molecules of tryptophan condense with glyoxylic acid and coloured product is formatted:



Cystein reaction

Compounds that contain hydrosulfide groups (oxidised or reduced) as cystein or cystin during heating in basic environment undergo disintegration. Their Sulfur undergo the liberation as sulphide ions which react with ions of Pb^{2+} and black sediment of PbS is formatted. Other sulfur aminoacid methionin gives negative result of this reaction as sulfur of methionin is blocked by methyl group.

The concept of protein excellence

Proteins which are the compounds of human food have diverse aminoacid composition. Generally they can be divided into 3 groups:

1. wholesome proteins – contain all essential aminoacids in ratio that assure appropriate covering of demands of the body. The examples are milk, eggs, chees ect.
2. partially defective proteins – contain all essential aminoacids but at least one is in insufficient amount.
The examples are cereal products (flour, groats).
3. defective proteins – contain little amounts of essential aminoacids or even can not contain one or few of them.
The examples are tryptophan free gelatin or proteins of tendons which do not contain tryptophan and contain only trace amounts of branched aminoacids.

References:

1. Crystallographic Studies of the Biuret Reaction, Freeman, H. C.; Smith, J. E. W. L.; Taylor, J. C.,
Nature, Volume 184, Issue 4687, pp. 707-710 (1959).