PROTEIN DIGESTION, AMINO ACID ABSORPTION AND INTERTISSUE DISTRIBUTION

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Abstract: This article provides information on hydrolysis of amino acids in proteins, preparation of extract from it, determination of its amino acid content by placing samples on chromatography paper from the solution.

Keywords: Protein, amino acid, chromatography, "witness" substances, distribution coefficient.

Proteolytic enzymes involved in the digestion of proteins and peptides are synthesized and secreted as proenzymes in the gastrointestinal tract. They are inactive and do not break down their own proteins. Digestion of proteins in the stomach In the stomach, proteins are digested under the influence of the proteolytic enzyme pepsin, and hydrochloric acid of gastric juice plays an important role in this process. Hydrochloric acid is produced in the accessory cells of the gastric glands and secreted into the stomach. In the head of the stomach, its concentration reaches 0.16 M (about 0.5%). Therefore, the pH of gastric juice is low, that is, it is around 1-2. Young infants who are breastfed contain the enzyme renin, which coagulates milk. In the presence of Ca2+, renin converts the soluble caseins of milk into an insoluble form. It is known that liquids do not stay long in the stomach. The physiological significance of freezing milk is to keep its proteins in the stomach until they are digested. Adult people do not have renin in their stomachs. In them, milk coagulates as a result of the combined action of pepsin with an acidic environment. In the stomach, under the action of pepsin, polypeptides of various sizes and, possibly, a small amount of free amino acids are formed from proteins. Me da sap is a colorless liquid with a strong acid reaction. A person secretes 1.5 liters of gastric juice per day. It contains water, proteins, enzymes (pepsin, gastrixin, renin, mucin, gastrin hormone, hydrochloric acid, acid-forming phosphates and a number of other substances).

Pepsinogenic protein, the precursor of pepsin, is synthesized in the main cells of the gastric glands. Its molecular weight is 40000. Pepsinogen polypeptide chain consists of pepsin (molecular mass 34000), pepsin inhibitor (molecular mass 3100) and residual polypeptide. Pepsin inhibitor is strongly basic because it contains 8 residues of lysine and 4 residues of arginine. In the juice of the gastric glands, the N-terminal part of pepsinogen, which contains 42 amino acid residues, is released. First, the polypeptide residue is cleaved, followed by the release of the inhibitor. As a result of

conformational reconstruction of the remaining part, an active center is formed. The enzyme pepsin is produced. Conversion of pepsinogen to pepsin can go under the influence of hydrochloric acid or pepsin, i.e. autocatalytically

HCI Pepsinogen - pepsin (slowly) pepsin The reaction that occurs on the surface in the presence of hydrochloric acid develops slowly. However, the autocatalytic process is very fast. Thus, a small amount of pepsin formed in the presence of hydrochloric acid causes the rapid conversion of pepsinogen into pepsin shortly after the secretion of gastric juice. Pepsin hydrolyzes peptide bonds away from the ends of the peptide chain, such peptide hydrolases are called endopeptidases. In this regard, as a result of the action of pepsin, the proteins in the stomach are broken down into polypeptides. Pepsin is most active at pH 1-2.5. Pepsin specifically breaks peptide bonds formed from the carboxyl group of aromatic amino acids. It breaks down almost all natural proteins. It acts slowly on peptide bonds formed from aliphatic and dicarbon amino acids. Some keratins, protamines, histones, mucoproteins are exceptions. Pepsin exerts its hydrolytic effect on denatured proteins. Gastricin is close to pepsin in molecular weight (31500). Its pH optimum is about 3.5. Gastricin affects peptide bonds formed from dicarbon amino acids. The ratio of pepsin/gastricsin in gastric juice is 4:1. In case of ulcer disease, it is observed to shift towards the stomach. The combined effect of these 2 proteinases in the stomach adapts the body to different diets. For example: when eating plant and dairy products, it partially neutralizes the acidic environment of gastric juice261 and causes the breakdown of proteins not by pepsin, but by gastrixin. Under the influence of pepsin and gastricin, proteins are broken down into polypeptides (up to albumoses and peptones), and the main breakdown of proteins occurs in the small intestine. Substrate specificity of proteinases.

The mechanism and importance of the formation of hydrochloric acid. Formation of HCI in the stomach.Although the mechanism of its occurrence has not yet been determined, but the available data show that Cl- formed from the dissociation of NaCl in the blood is released from the cell membrane, and in turn, the last products of its metabolism are H2 O and CO2 in the covering cells combines with H+ released as a result of dissociation of synthesized carbonic acid. The HCI produced is then secreted into the gastric mucosa by the lining cells. the balance of ions between the blood and lining cells is achieved by the exchange of negatively charged HCO3- from the cells into the blood instead of Cl- from the blood into the cells. It is assumed that ATF is involved in this process, because the synthesis of hydrochloric acid requires energy.

Four types of acidity are distinguished in the composition of gastric juice: 1) hydrochloric acid (free HCI) that is not bound to any compound; 2) protein-bound hydrochloric acid (bound HCI); 3) sum of free and bound hydrochloric acid (total HCI);

4) the sum of free, bound and total HCl and the sum of other acidic substances that create an acidic environment from fruit juice (total acidity). These acidities of gastric juice are determined by titration with a 0.1 mol/l solution of NaOH in the presence of an indicator. Total acidity in the presence of the phenolphthalein indicator (pH transition limit 8.2-10) with the amount of 0.1 mol/l NaOH spent to titrate 100 ml of gastric juice (for neutralization of HCI and other acidic substances) measured. The average amount of total acidity is 40-60 mol/l. Free hydrochloric acid262 is measured by the amount of 0.1 mol/l NaOH used to neutralize 100 ml of gastric juice in the presence of a dimethylaminoazobenzene indicator (pH 1.0-3.0). Its average amount is 20-40 mol/l. The bound hydrochloric acid is found by subtracting the total acidity from the free acidity as determined above in the presence of alizaring hydrosulfonate NaOH (pH 4.3-6.3) or using phenolphthalein and dimethylaminoazobenzene indicator. Its average amount is 10-20 mol/l. Increased acidity in gastric juice is called hyperchlorhydria (due to increased HCI). This condition often occurs in gastric and duodenal ulcers and hyperacid gastritis. A decrease in HCI in gastric juice is called hypochlorhydria (hypoacidosis is observed in gastritis and gastric cancer). Absence of only hydrochloric acid in gastric juice is called achlorhydria (stomach cancer and anacid gastritis occur), absence of hydrochloric acid and pepsin enzyme is called achilia (occurs in atrophic gastritis).

In order to determine the amino acids in proteins, it is necessary to hydrolyze the protein first to form free amino acids. For this, it is necessary to prepare whey from plant or animal protein. You can take protein-rich peas or soybeans to make soup. First, 1 g of peas is taken, crushed well and mixed with 10 ml of boiling ethyl alcohol of 75% strength. The dissolved protein is filtered off and the filtrate is evaporated to dryness on a water bath. 1 ml of 1% hydrochloric acid solution is added to the residue. In this case, under the influence of acid, hydrolysis occurs, and the protein is broken down into amino acids. Samples are placed on chromatography paper from the solution and chromatographed with the butanol-acetic acid-water (4:1:1) solvent system. A 1% solution of ninhydrin can be used to reveal amino acids. If there are examples of pure amino acids, witness them.It is not difficult to determine which amino acids are present in the plant. [1] To perform its work, the width of the special chromatography paper (or filter paper) is slightly smaller than the diameter of the cylinder used for chromatography, the length is cut in the range of 30-50 cm, and the top 15-20 cm length of thread is tied to the part.

A horizontal line is drawn with a black graphite pencil 1-2 cm above the lower edge of the paper tape. Then on this line on the paper (with an interval of 2-2.5 cm) four points are marked, and a few drops of the alcohol solution of the tested amino acid mixture and alcohol solutions of "witness" substances - valine, glycine, phenylalanine are dripped (in the above-mentioned method) and then dried in the air. A long cylinder used for chromatography is saturated with phenol in watersolution or a solvent system

consisting of n-butanol-water-glacial acetic acid (4:5:1) is poured. The prepared paper tape is held by the thread and is lowered into the solvent at a depth of 2-3 mm until it is immersed in the solvent. The cylinder is left in a 35-40°C thermostat for 1.5-2.0 hours. During this time, the solvent rises by 10-25 cm. After the specified time, the chromatographic paper is removed from the cylinder, the front line is marked with a pencil, and the chromatogram is dried in a fume cupboard or in a special drying chamber until the phenol or other solvent has evaporated [2].

After the solvent evaporates, the paper tape is taken, hung on a tripod, and sprayed with a 0.1-0.2% alcohol solution of ninhydrin using a pulverizer, or a ninhydrin solution is poured into a cuvette and chromatographic paper is dipped into it. Another 100-110°C dryer is hung in the cabinet for 5-6 minutes. As a result, the place where the amino acid stops on the paper is colored blue, blue-violet. The distribution coefficient of the spots formed from the tested amino acid solution is calculated and compared with the distribution coefficient (R) of "control" substances - valine, glycine, phenylalanine. The coefficient of distribution of these amino acids in the ratio system of n-butanol-water-glacial acetic acid (4:5:1) is Rr, glistin-0.13; 0.36 for valine and 0.46 for phenylalanine. In a saturated solution of phenol in water glistin-0.41; valine-0.76; phenylalanine-0.87. In conclusion, we would like to say that paper chromatography It is more convenient and economical to identify substances using this method.

List of used literature

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