Nanostructure and new properties of hydrolyzed food globular proteins

I A Rogov¹, T N Danilchuk¹, J A Shushkevich², G V Semenov¹, O E Ovchinnikova¹
¹Moscow state university of applied biotechnology, 33, Talalihin street, Moscow, 109316, RU
² Company Base ELEMENT, 30, Rochdelskaya street, Moscow, 123022, RU
E-mail: iar@msaab.ru

Abstract. In the present work we obtained a hydrolysates of food proteins by enzyme hydrolysis, researched the comparative structural and the molecular – mass characteristics of proteins, and compared of hydrolysates particles structural characteristics on nanoscale with their biological properties.

Introduction

Now the requirement of manufacture of pure proteins commercially is rather great. Use of proteins concentrates allows improving and raising value of a foodstuff, improving their functional and medical and biologic properties. Such products are especially claimed at the strengthened physical activities when the norm of consumption of proteins for easy to acquire sharply increases. Among known sources of a protein, which use in food additives, a sports and children's food, the greatest interest is represented by soya proteins and proteins of dairy whey.

Soya protein is well balanced on amino acids. It is the unique protein possessing full of amino acid profile, unique, as a matter of fact, in a vegetative kingdom. After consumption of Soya proteins there is an accurate decrease in level of cholesterol in blood, therefore they are expedient for using in a diet of people with excess weight, and also people of dairy products suffering by intolerance. The main demerit of soya protein is presence inhibitor of digestive enzyme trypsin. For disposal from inhibitor additional processing of fiber with the help of enzyme hydrolysis is necessary [1].

Whey proteins of milk – a perspective and important class of protein products, nutrilites in food and medical appendices. The amino acid structure of milk whey proteins is closest to amino acid structure of a muscular fabric of the person, and under the maintenance of irreplaceable amino acids and amino acids with the branched out chain they surpass all other proteins of a vegetative and animal origin. Proteins of milk whey (α-lactalbumin, β-lactoglobulin, immunoglobulins) possess strongly pronounced antigenic properties, have the highest speed of splitting among integral proteins and high assimilability.
Perspective use of hydrolysates whey proteins is represented. Last years by a number of researchers it is shown that as a result of proteolytic splitting whey milk proteins digestive enzymes form the peptides possessing biological activity. Physiological action of these peptides consists direct participation in regulation mechanisms in gastrointestinal tract or in their ability to an absorption through epithelium intestines, penetration into blood and transportation to peripheral bodies. There is a basis to believe that the spectrum of mechanisms of action of these peptides is wider, than at corresponding protein-predecessor. Depending on physical and chemical parameters of hydrolysis the received peptides can have both different structure, and different biological properties.

The purpose of the present work consisted in obtain of hydrolysis’s of milk whey proteins by enzyme hydrolysis, research of comparative structural and the molecular -mass characteristics of proteins, and also in comparison of structural characteristics of particles of hydrolysis’s on nanoscale with their biological properties.

Materials and methods
As objects of research have been chosen globe food proteins: the natural soya protein (Supro 760), taken directly from raw soybeans; the hydrolyzed soya protein (the experience sample); protein of whey of the cow milk an α-lactalbumin and its enzyme hydrolysis products.

The experience soya sample was received by innovative technology. Its key elements is treatment of initial soya protein by proteolytic enzymes, homogenization at a high pressure and then spray-type drying. For research of soya proteins properties modern methods of the physical and chemical analysis was used.

α-lactalbumin was isolation from bovine milk with use anion-exchange chromatography on DEAE-cellulose and the subsequent clearing by means of a gel-filtration [2]. The protein concentrarions were evaluated spectrophotometrically, using the extinction cjefficient A1% cm 280 nm = 18.2 [3]. The received protein concentrate subjected to enzymatic hydrolysis with use of digestive enzymes: pork trypsin and pepsin (from Sigma). Hydrolysis of α-La was carried out in citrate buffer (pH 2.5) for pepsin and in Tris-HCl buffer (pH 8.0) for the trypsin. α-La (1 mg/mL) were incubated with the enzyme (1:20,enzyme-to-substrate ratio, w/w) for 24 h at 37°C. After incubation, enzymatic reactions were stopped by heating at 95°C for 15 min. Hydrolysates by pepsin were adjusted to pH 7.5 with 1M NaOH. The hydrolysates were centrifuged at 10000 g for 30 min, the supernatants were removed, and an aliquot was frozen and kept at -20°C until use.

The form of particles and their sizes on nanoscale defined by method of the atomic force microscopy (AFM) with use scanning a microscope Solver NEXT. AFM images received in the air environment in a contact mode of scanning (cantilever CSG 01/10). Processing of images made with use of an information technology under program Image Analysis 3.0.

Results
In the process of hydrolysis the natural soya protein (Supro 760) by proteolytic enzymes occurs its structural updating which results are: truncation of molecules of separate polypeptides, formation new steady nanoscale globe structures, exhibiting of hydrophilic groups on a surface of protein substances rupture of cross-section communications, strengthening of superficial activity etc. Thus a protein product gets new properties: increase of solubility of a protein, increase of lathering ability, viscosity decrease, increase of thermo stability at heating (table 1). Besides, hydrolysis leads to occurrence of antioxidant activity and to increase of protein particles stability in the salt medium. Presence of the antioxidant activity in the hydrolyzed food soya proteins is one of key factors of its high biological value.
Table 1. The new properties attached for the product in the process of structural updating.

| Properties                                      | Samples                |
|------------------------------------------------|------------------------|
| The maintenance of a soluble protein (method AOCS) | Supro 760 | The experience sample |
|                                                 | 30                     | 43                     |
| Lathering ability (on Wolf)                      | 30,4 %                 | 100 %                  |
| Dynamic viscosity (small speed of shift)         | 36.40 Pa·s\(^{-1}\)   | 0.48 Pa·s\(^{-1}\)    |
| Dynamic viscosity (high speed of shift)          | 7.72 Pa·s\(^{-1}\)    | 0.09 Pa·s\(^{-1}\)    |
| Part of an insoluble deposit at 50 ºC             | 50 %                   | 22 %                   |

Results of the atomic force microscopy have shown that protein substances as natural, and the hydrolyzed protein of a soya have the globular form. The sizes of Supro-760 protein globules are in range from 100 to 400 nanometers, thus globules with a size 100 nanometers have tendency to agglomeration with a limit of 400 nanometers. During the analysis were able to deduce that agglomerates represent association of three globules (See the figure 1). On the contrary, at the experience sample the average size of visible granules makes 40-50 nanometers. (See the figure 2).

**Figure 1.** AMC image of the natural soya protein (Supro 760). The area of scanning is 2000 * 2000 nanometers.
Figure 2. AMC image of globes the experience sample of soya protein after hydrolysis. The area of scanning is 2000 * 2000 nanometers.

Figure 3. The results of electrophoresis of Soya proteins: a - Supro-760, b - the experience sample (hydrolysate). From above positions of markers of molecular weights in kilo Dalton (kDa) are designated. Below by arrows not hydrolyzed polypeptides are mark. By asterisks are mark hydrolysis products.

Research by method of electrophoresis has shown that in sample Supro-760 protein substances are presented, basically, by globules with molecular weights around 40 and 70 kDa. This in a linear scale corresponds to the sizes more than 100 nanometers (See the Fig. 3a). In the experience sample predominates polypeptides with the molecular weights in a range 5 and 11 kDa. The maintenance of these short polypeptides is estimated in 60-65 % from weight of fiber. Other products of hydrolysis (17, 22, 24, 28 and 40 kDa) make an order 1/3 from all quantity of protein (See the Fig. 3b). In a linear scale this distribution corresponds to fractions with the sizes from 4 to 10 nanometers (2/3 of general protein) and around 40-50 nanometers (1/3 of general protein). Globules of the second fraction are well identified by method AFM.
Proteins of the first fraction are not defined on AFM images because of the insufficient permission used cantilever, not allowing completely to scan objects with the sizes less, than 10 nanometers. Proteins the experience sample (hydrolysate) have globular form and are steady, not subject to agglomeration. Stability of experience sample globules can be connected with change of tertiary structure of protein as a result of rupture cross-section bisulphide S-S communications between cysteine molecules. Rupture cross-section S-S communications occurs in the process of protein homogenization under high (to 800 atmosphere) pressure which is a component of applied technology. Besides, stability to agglomeration influences the surface activity, obtained by protein structures in the biocatalysis process. Thanking nanosize of peptides and their stability the experience sample has additional properties of solubility, thermostability, stability in the salt medium and very low viscosity.

The great number of biologically active peptides, or directly present at foodstuffs, or isolated of proteins containing in these products by the use of enzymatic or chemical hydrolysis is revealed. In the course of natural processing of food proteins in a digestive system such peptides are liberated from structure of proteins and start to operate as independent regulation units possessing activity, similar to the hormonal. Now public recognition the leading part endogenous peptides in regulation of various systems of an organism. The direction of creation of parapharmaceutical preparations on a basis regulation peptides successfully develops [4].

The basic source of biologically active peptides of milk is its protein part, namely casein and whey proteins. α-lactalbumin (α-LA) is one of the basic components of protein fraction of milk whey (20-25 %), plays an important role in biosynthesis of lactose and it is capable to fixed ions of such metals calcium and zinc that causes a number of its biological functions. In the present work influence of conditions enzymatic hydrolysis on structural changes α-LA has been investigated.

Results of analysis AFM-images of particles an alpha-lactalbumina and its hydrolysates show that having the form of rigid balls globules an alpha-lactalbumin in the course of hydrolysis by proteolytic enzymes turn to the developed extended structures which length makes 1-2 microns (figure 1-4).

Figure 1. The AFM-images of the peptide fragments derived from α-LA digestion by trypsin
**Figure 2.** The AFM-images of the peptide fragments derived from α-LA digestion by pepsin.

**Figure 3.** Profile peptide fragments derived from α-LA digestion by pepsin.
Results electrophoresis researches of the received hydrolyzates, testify to presence of proteins particles since the sizes from 1.4 to 10 kDa. Except, the analysis of particles of hydrolysis’s by AFM method shows presence of friable structures of the roughest form, having the nanoscale size, that can serve as the proof of that in the process of hydrolysis there is a formation disorder "fused globes ", which truncation leads to formation of the developed extended structures. Thus there are the new properties shown, first of all, in increase of physiological activity of an protein preparation: antimicrobial action, anti-oxidative properties, ability to stimulate proliferation cages of an animal organism, etc.

Existence fused globes of α-lactalbumin was observed at studying of influence of denaturad (guanidinibichloride) on a protein solution [5]. The facts of formation of linear nanotubes are known at partial hydrolysis of α-LA by proteolytic enzymes after entering of calcium ions into solutions of hydrolysis’s. The length of such tube makes thousand nanometers, external diameter is equal 20 nanometers, internal - 8 nanometers. These structures are stable enough, capable to transfer pasteurization, freeze- drying and are unique nanotubes, made from food protein. The internal cavity of such tubes can be used for capsulation various substances, for example, vitamins or enzymes, for the purpose of their masking or protection against destruction.

Thus, in the process of structural updating globe food proteins break up to particles, having the nanoscale size and forming a product possessing new unique properties, directly depending on their size and atomic structure of a surface. Use in the operated biocatalysts process of the technological influences, directly turned to macromolecular structure globe proteins, in aggregate with use of tools of nanotechnologies allows expanding possibilities nanobiotechnologies in creation of the preparations possessing preventive and medical effect.

References

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