Effect of Hydrolysis Products of Different Proteins of Wheat on Antioxidant Enzymes

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Abstract: *This paper presents a study of the effect of products of enzymatic hydrolysis of various proteins of wheat with a neutral proteinase (neutrase "Novozymes", Denmark) on the activity of peroxidase from horseradish. It is shown that the hydrolysis products of albumin activate peroxidase activity, the constant of activation being 2.3 micromoles. At the same time with increasing the depth of hydrolysis of albumin the activating effect of peptides disappears. Peptides derived from the salt-soluble, alcohol-soluble alkali-soluble proteins had no effect on the activity of peroxidase.*

Keywords: *Wheat proteins, Enzymatic hydrolysis, Peptides, Activation of peroxidase.*

Introduction

Recently, much attention has been paid to deriving and application of natural protein-based antioxidants from plant and animal materials [1, 2, 6]. Active peptide antioxidants and peptides utilizing free radicals have been identified in various protein hydrolysates such as ovalbumin [2], soy protein [1], soluble elastin [3], pig myofibrillar protein [14], milk proteins such as α-lactatalbumin and β-lactoglobulin [7, 12], skin gelatin [10]. One major advantage is that by using different enzymes from a single source of protein peptides with different properties can be obtained. For example, research [8] studied the effect of different enzyme preparations on enzymatic hydrolysis of defatted peanut kernels to obtain antioxidant peptides. Hydrolysates obtained by esperase had high antioxidant activity compared with neutrase, pepsin, protease A and protease N, where the oxidizability of linoleic acid served as a criterion for evaluation. Molecular mass of peptides derived through esperase ranged from 3 to 5 kDa. Antioxidant activity was 3 times higher than that of ascorbic acid.

It should be noted that in living organisms, there is a layered system of protection against oxidative stress, including specialized anti-oxidant enzymes and low molecular weight antioxidants.

Enzyme systems of superoxide dismutase (SOD), catalase (CAT) and peroxidase (PO) form the first layer of protection against oxidative stress by regulating the level and removing the excessive reactive oxygen species (ROS) [9].

SOD carries dismutation of the superoxide radical (O_2^-) to the less toxic hydrogen peroxide, and H_2O_2 is removed by catalase and peroxidase in different ways [5, 11]. CAT destroys the excess of hydrogen peroxide that has formed and PO uses H_2O_2 for oxidation of a wide range of substances, including in detoxification of foreign compounds [5, 13].

The effect of low molecular weight antioxidants is based on their ability to form low-active radicals, thus interrupting the autoxidation reaction.

The purpose of this study is to investigate the effect of hydrolysis products of various proteins of wheat on the activity of antioxidant enzymes (peroxidase).

Materials and methods

Materials

We used proteolytic enzyme preparation from microorganisms of bacteria – *Bacillus amyloliquefaciens* (*Neutrase*, "*Novozymes*", *Denmark*).

As protein substrates were used water-soluble, salt-soluble (10% NaCL), alcohol-soluble (80% ethanol) and alkaline soluble (0.2% NaOH) proteins, isolated respectively from high grade wheat flour.

Peroxidase from horseradish was isolated in laboratory conditions, according to [4].

Enzymatic hydrolysis of proteins

We prepared 0.5-1% solution of the corresponding protein in 0.1 M universal buffer, pH 7.0 and added 0.1% neutrase. The mixture was stirred and kept for some time (30, 60, 120, 240 and 360 min) in a thermostat at 30°C, then 2 ml TCA (trichloroacetic acid) was added to a sample of 2 ml to stop the enzymatic reaction. Then the settled solution was passed through a paper filter and 1 ml of filtrate was taken out and 5 ml 0.5 M solution of sodium carbonate was added. While stirring, 1 ml of working solution of Folin was added. Solutions that are a little-settled become blue in color, whose intensity was determined by a photoelectric colorimeter (at wave length of 670 nm) against the control sample in the cuvette with a layer thickness of 10 mm [3]. The content of hydrolysis products (*P*) was determined by a calibration curve constructed on tyrosine.

Preparation of the sample peptides

During the enzymatic hydrolysis of the proteins, 5 ml samples were collected from the reaction mixture within a certain period of time, then they were heated in a water bath and kept for 5-10 min to inactivate the enzyme. Then they were passed through a paper filter and their inhibitory and activating effects on peroxidase were examined.

Determination of peroxidase activity

Peroxidase activity was determined by the rate of oxidation of benzidine with peroxidase from horseradish in the presence of hydrogen peroxide [4]. The reaction mixture in 0.1 N acetate buffer consisted of 2 ml of benzidine (5 mM), 2 ml enzyme solution, 2 ml of water (quantity of the products of protein hydrolysis added in the water – 0.5 ml) and 2 ml of 3% peroxide hydrogen. The rate of oxidation of benzidine was measured in cuvette 20 mm thick at a wavelength of 670 nm.

Results and discussion

The system we studied, i.e. the protein composition of wheat grain consists of albumin, globulins, prolamins and glutelins. These proteins were subjected to varying degrees of enzymatic hydrolysis with neutral protease. The tests we performed showed that under these conditions the enzymatic hydrolysis of alcaline soluble proteins proceeds more rapidly than other proteins. For example, during fermentation for 60 minutes (at 30°C in 0.1 N Na-acetate buffer) the formation of hydrolysis products not precipitated by trichloroacetic acid was 0.212, 0.130, 0.128 and 0.086 mmol·ml^{-1} for glutelin, globulin, prolamin and albumin, respectively (Fig. 1).

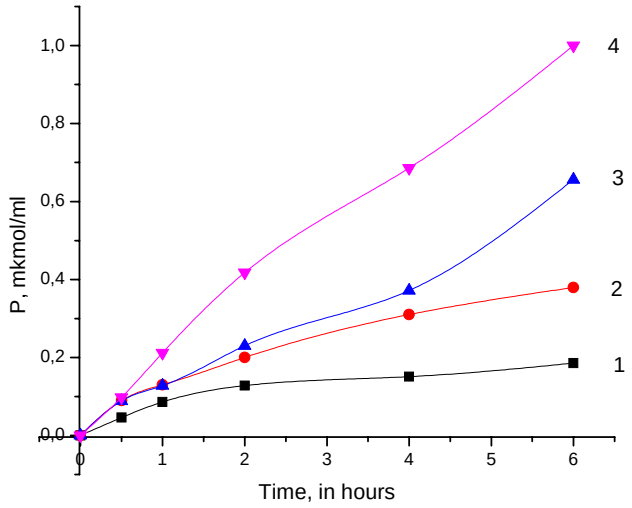

Fig. 1 Enzymatic hydrolysis of various proteins of wheat
with neutral protease from *Bacillus amyloliquefaciens*
1 – albumin (0.5%), 2, 3 and 4 – salt-soluble, alcohol-soluble
and alkaline soluble protein (1%), respectively

We should also note that peptides with molecular weight 3.5 kDa derived from plants are stable and not denatured by boiling. Given these features, after fermentation of proteins, we separated the liquid part of the reaction mixture by centrifugation and subjected it to boiling to denature the enzyme (of protein). Then we passed it through a paper filter and determined the activating and inhibiting action of the products of hydrolysis of proteins on antioxidant enzymes in the filtrate.

Fig. 2 shows the effect of hydrolysis products of wheat proteins on the activity of peroxidase. The data presented shows that the peptides derived from different proteins of wheat have a different degree of influence on the activity of peroxidase. Our tests showed that the hydrolysis products of albumin promoted the activation of peroxidase. In this case the depth of the hydrolysis of proteins is of significant importance. At low degrees of hydrolysis the activating effect of hydrolysis products of proteins was high. For example, for 1 hour the hydrolysis products of albumin enhance the activity of peroxidase by 3.5-4 times (Fig. 2, curve 1). With increasing time of fermentation of proteins, i.e. with increasing degree of hydrolysis of proteins the activating effect of the peptides decreases.

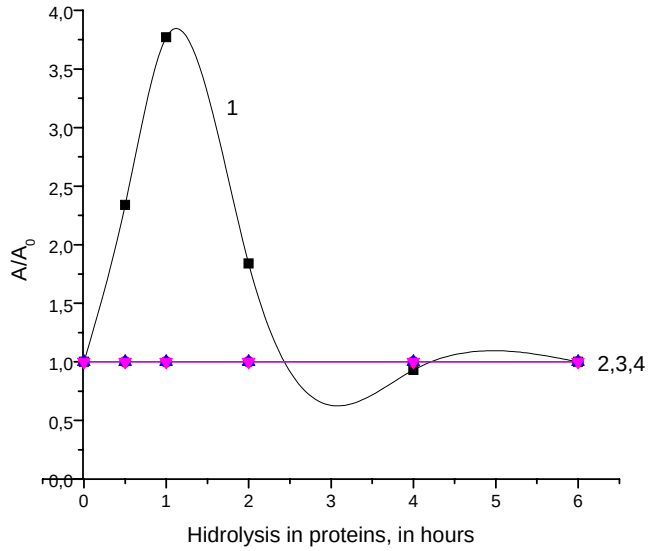

Fig. 2 Effect of hydrolysis products of various proteins on the activity of peroxidase from horseradish depending on their degree of hydrolysis
1 – albumin, 2, 3 and 4 – globulin, prolamin and glutelin, respectively
(A_0 – peroxidase activity in the presence of proteins at the beginning of fermentation, A – peroxidase activity in the presence of proteins after a certain time of fermentation)

Peptides obtained by hydrolysis of other proteins such as salt-soluble, alcohol-soluble and alkaline soluble protein does not affect the activity of peroxidase (Fig. 2, curves 2, 3 and 4).

If we enter the data obtained from the hydrolyzate of albumin in the coordinates A/A_0 of the concentration of peptides, where A_0 – is the velocity of oxidation of benzidine in the absence of hydrolyzate, and A – peroxidase activity in the presence of various amounts of hydrolyzate of albumin, then the calculated concentration of peptides required for activation by 2 times is 2.2-2.3 mmol (Fig. 3).

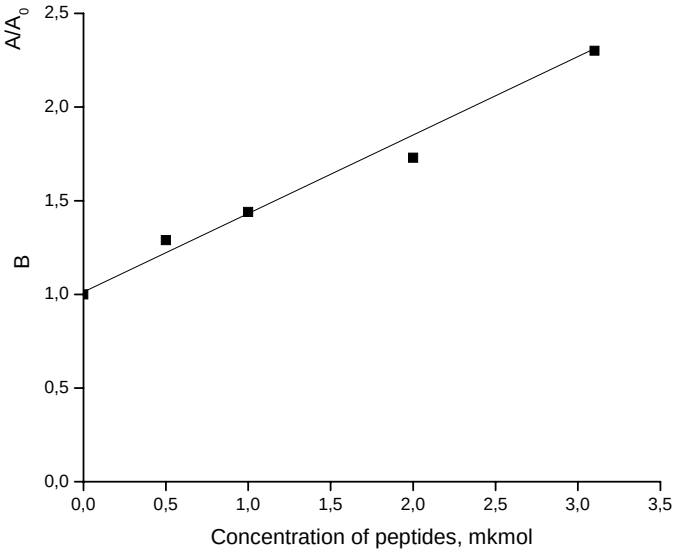

Fig. 3 Effect of peptides on the rate of oxidation of benzidine with peroxidase from horseradish

Dependence is linear and this means that the peptide has an effect only on the enzyme molecule.

The activation rate of oxidation of benzidine is accompanied by changes in the catalytic constants of peroxidase.

Fig. 4 shows the effect of concentration of benzidine on the rate of oxidation in the coordinate of Lineweaver-Burk. The data presented shows that in the presence of peptides Michaelis constant does not change. Activation with peptides has noncompetitive i.e. substrate and peptide independently binds with the active center of the enzyme.
At low concentrations of substrate the oxidation rate is higher than in the absence of peptides.

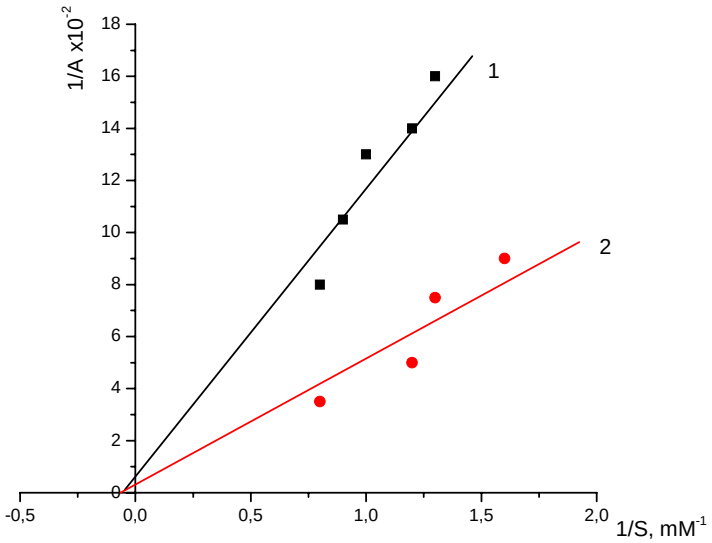

Fig. 4 Effect of concentration of benzidine on the rate of oxidation
in the coordinate of Lineweaver-Burk
1 – in the absence of peptides, 2 – in the presence of peptides (2 mkmol)

Thus, the products of enzymatic hydrolysis of different proteins not only have antioxidant activity, but to a certain degree hydrolysis obtain the activating properties of antioxidant enzymes (PO). We can assume that in living organisms, the products of protein decompositions may also play a significant role in protecting against oxidative stress and in regulating the level of excessive active oxygen species as well as removing them.

Conclusions

Hydrolysates of various proteins of wheat affect the activity of PO in varying degrees. Hydrolysis products of albumin with neutral proteinase activate peroxidase activity while the hydrolysis products of salt-soluble, alcohol- soluble and alkaline soluble proteins do not affect the activity of peroxidase. The activating effect is observed at low degrees of albumin hydrolysis. With increasing the depth of protein and peptide hydrolysis the activating effect of peptides disappears. The constant of the activation of peptides obtained for 1 hour of fermentation is 2.3 mkmol.

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