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Prospects for Developing Modifications of Methods for Producing Conjugates for Elisa

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Abstract: It is known that enzyme immunoassay is the most effective and cost-effective in terms of use in the diagnosis of various infectious agents and low molecular weight compounds. Conjugates in the ELISA test systems are obtained by conjugation of the enzyme horseradish peroxidase and proteins. The article provides an analysis of various Saccharomyces cerevisae enzymes as sources for the production of ELISA conjugates.

Key words: enzyme immunoassay, conjugate, affinity, periodate method.

Among the laboratory methods, ELISA is widely used in healthcare, various fields of agriculture, industrial biotechnology, environmental protection, and research. The advantages of ELISA are the possibility of early diagnosis of various diseases, the ability to track the dynamics of the process, speed and ease of operation as an express test system [11].
Enzyme immunoassay – ELISA) is a laboratory immunological method for the qualitative or quantitative determination of various compounds, macromolecules, viruses, etc., which is based on a specific "antigen-antibody"reaction. ELISA involves a number of successive stages, and the result itself can be evaluated visually or by optical density [12].
ELISA is used to diagnose viral, bacterial, fungal, and parasitic infections. Especially the method is indispensable in the diagnosis of viral diseases, where direct methods of detecting the pathogen are difficult. In addition, in some cases, serological studies remain the only method of screening diagnosis of infections, for example, toxoplasmosis, toxocarosis, trichinosis. ELISA is used in two directions: the detection of antibodies in the blood serum of the subject for diagnostic purposes, and the determination of pathogen antigens to establish its generic or species identity [1].
The process of conducting an enzyme-linked immunosorbent assay can be divided into three main stages: the immunochemical process - the formation of an antigen-antibody complex (AG-AT), the attachment of a label to it, and its visualization. The essence of ELISA consists in the specific interaction of an antibody and an antigen, followed by the addition of a conjugate (an anti-species immunoglobulin labeled with an enzyme) to the resulting complex. The enzyme causes the decomposition of the chromogenic substrate to form a colored

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product, which is detected either visually or photometrically. Registration of the reaction results is carried out on special photometers with a vertical beam at a certain wavelength. The result is expressed in units of optical density [1, 8].

Two groups of researchers in the early 70s revealed that antibodies can covalently bind to enzymes such as alkaline phosphatase, beta-glucose oxidase, and horseradish peroxidase, by reaction with glutardialdehyde or other methods. These obtained conjugates were used in light, fluorescence, and electron microscopy for immunohistochemical staining of structures containing the analyzed antigens. These phenomena were observed and used by two groups of scientists in Sweden and the Netherlands to replace a radioactive label with an enzyme one in immunochemical analysis. Swedish researcher Eva Engvall made a great contribution to the development of ELISA, suggesting the use of passive adsorption of antibodies or antigens on the surface of 96-well plates to conduct reactions in the solid phase. It was she who proposed the term ELISA (enzyme-linked immunosorbent assay), in the Russian-language literature "solid-phase enzyme immunoassay" [13, 9].

In all cases, ELISA uses an enzyme conjugate with specific or antiviral antibodies or antigens and a developer (a mixture of the substrate with a chromogen). As a result of the interaction of the enzyme with the substrate, the reaction mixture is colored under the action of chromogen. This allows you to visually or automatically assess the presence of antibodies or antigens in the test material.

Thanks to the success of biotechnology and genetic engineering, it is possible to obtain highly purified proteins-antigens, a variety of poly-and monoclonal antibodies of a given specificity and affinity, marker enzymes and conjugates of enzymes with antigens and antibodies.

Conjugates are artificial structures produced by the chemical crosslinking of two or more different molecules. For ELISA, conjugates containing antibodies, usually antiviral, and an enzyme are used. Sometimes, instead of antiviral conjugates, the A-protein of Staphylococcus aureus is used, which binds to the Fc fragment of the first antibody in the resulting complex.

There are several different methods for covalent binding of an indicator enzyme to an antibody. Now the most popular are various modifications of the method of periodate oxidation. This method is suitable for enzymes containing carbohydrate residues, such as peroxidase.

One of the most important advantages of ELISA methods over radioimmune analysis is the high stability of conjugates containing the enzyme. In the presence of stabilizers, the conjugates are able to maintain their activity for 1-2 years.

An important condition for successful ELISA is the appropriate concentration of the conjugate. One of the main tasks of production is to determine the optimal concentration of the conjugate. If the conjugate concentration is too high, its excessive nonspecific binding to the carrier is observed, which significantly increases the optical density value. At very low conjugate concentrations, the sensitivity of the assay can be markedly reduced as a result of the delayed conversion of the substrate into the product [10].

It is necessary to choose an enzyme that can maintain its activity for a long time, not lose it during the operation of binding to an antigen or antibody, and have a high specificity to the substrate. Horseradish peroxidase, alkaline phosphatase, and Escherichia coli beta-

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galactosidase are widely used. The activity of enzymes is recorded by changing the optical density, fluorimetrically and electrochemically. For example, peroxidase catalyzes the reaction:

$AH_2 + H_2O_2 \rightarrow A + 2H_2O$

Different compounds can be used as AN2. Thus, the reduced colorless o-phenylenediamine is oxidized by peroxidase to form a colored product with a maximum absorption at 435 nm.

The enzyme must be bound to the antibody or antigen so that the enzyme retains its activity, and the properties of the antibody and antigen are not disturbed. Therefore, there are three groups of methods: biochemical, immunological, and genetic engineering. In biochemical methods, the crosslinking of the enzyme E with an antibody or antigen is used with the participation of free reactive groups: - NH2 -, COOH, - SN, - OH. For example,

$E\text{-}NH^{2-} + HOOC\text{-}A\Gamma \rightarrow E\text{-}NH\text{-}CO\text{-}A\Gamma + H_2O$

If the direct interaction cannot be realized, bifunctional crosslinking agents are used: glutaricdialdehyde, n-benzoquinone.

Immunological methods for the production of antigens or antibodies labeled with enzymes are based on the use of antibodies or their components as cross-linking links.

The genetically engineered method for obtaining labeled antigen is based on the synthesis of hybrid proteins with the help of microorganisms. By this method, using transgenic E. coli, hybrid proteins containing the complete amino acid sequence of bacterial β-galactosidase and a specific protein sequence from the human immunodeficiency virus or hepatitis B virus were obtained [4].

Among the biological objects, yeast - saccharomycetes are the leaders. This interest in them is related to the peculiarities of their metabolism. The presence of two ways of energy metabolism in yeast-anaerobic (glycolysis) and oxidative, formed the basis for the production of fermentation products, in particular beer, and the biomass of baking yeast.

To create highly effective food technologies based on the cultivation of yeast, it is necessary to know the features of their metabolism and physiology. Based on these skills, it is possible to realize the potency of yeast in order to increase the efficiency of the biomass accumulation process in any branch of biotechnology where saccharomycete yeast is used, in particular in the production of baker's yeast, brewing, winemaking and ethanol biosynthesis [7].

Recently, in the food industry, the most important species is Saccharomyces cerevisiae, which includes yeast that is used in baking, brewing, winemaking, in the production of kvass and alcohol production. Studies of yeast by hereditary traits allow us to conclude that the yeast that causes the fermentation process, belonging to the species Saccharomyces cerevisiae, are mutants with partially lost traits, selected under certain conditions or on certain substrates.

Currently, these plants usually use dry yeast as a seed material, which is produced in France, Canada, the United States and a number of other countries [5].

Saccharomyces cerevisiae yeast is used in the production of alcoholic beverages and bakery products, and it is also widely used in scientific research. S. Cerevisiae became the first eukaryotes whose genome was completely sequenced. The same species served as one of the model objects in the study of the ability of microorganisms to react ahead, i.e. to anticipate

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changes in environmental conditions. The mechanisms of gene expression, the role of heat shock proteins, and many others are being studied in the culture of S. Cerevisiae. [6,20].

Yeasts of the genus Saccharomyces are heterotrophic microorganisms. They use only the binding energy of organic carbon compounds, which are essential for the biosynthesis of components, as a source of energy for their vital activity. Such compounds include various carbohydrates contained in malt wort and beet molasses, which are mainly used in modern fermentation industries as nutrient media for yeast [7,19].

The cells of S. cerevisiae have a rounded, ovoid or ellipsoid shape; their size ranges from 2.5 to 10 microns across and from 4.5 to 21 microns in length. The size and shape of cells of the same strain are determined genetically and can vary within certain limits depending on the conditions of cultivation and subsequent operations for the production of commercial yeast (dehydration).

In living cells, there are many biochemical processes and successive enzymatic reactions, in which the product of one reaction is the substrate for the subsequent reaction. According to studies, they include 156 reduction reactions, 21 – decarboxylation, 17 – deamination, 14 – oxidation, 10 - esterification, 9 – condensation, 5 – hydrolysis, 1-amination. [3,18].

Among the enzymes, special attention should be paid to invertase, acid phosphatase and trehalase. These enzymes are located both in the CS and in the periplasmic space and can be secreted by yeast cells outside into the environment.

Invertase, or phosphofructosidase, hydrolyzes sucrose. Sucrose is the main carbohydrate of molasses – for glucose and fructose. Alkaline and acid phosphatases are known. Acid phosphatase has the maximum activity, since the vital processes of yeast in fermentation production occur at a pH of less than 5.5, while the optimum pH for alkaline phosphatase is 7.0. Acid phosphatase hydrolyzes various phosphoric acid ether bonds, in particular, releasing orthophosphate from ATP molecules. Yeast has two main forms of acid phosphatase – repressed, the synthesis of which is inhibited by the orthophosphate of the medium, and constitutive. Its level does not depend on the concentration of PO4-3 in the nutrient medium.

Trehalase is an enzyme that hydrolyzes the spare disaccharide trehalose to form two glycoside residues.

The most essential components of the yeast cell are water, nitrogen-containing compounds, carbohydrates, fats and minerals [7,17].

The yeast Saccharomyces cerevisiae is a eukaryotic microorganism that is genetically more complex than bacteria. A yeast cell contains 3.5 times more DNA than an Escherichia coli cell. As an experimental object, yeast has many of the technical advantages that have enabled rapid advances in the molecular genetics of prokaryotes and their viruses. In this regard, we should mention their ability to multiply rapidly, the possibility of manipulating individual yeast cells, the ease of simultaneous transfer of multiple yeast cultures to selective media and isolation of mutants, the detailed study of yeast as a genetic system and, perhaps most importantly, the possibility of versatile use of the system of genetic transformation of yeast. Unlike many microorganisms, yeast cells can remain viable if multiple genetic markers are present in their genotype. Yeast is not pathogenic, so working with it does not require extreme precautions.

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The optimal temperature for growing yeast cultures is 30 °C. The standard full-fledged nutrient medium is called YEPD and consists, in addition to water, of yeast extract, peptone and glucose. For cultures of normal haploid yeast cells growing exponentially at the optimal temperature in the YEPD medium, the generation period is about 90 minutes. When the culture is grown in a synthetic medium, the generation period of normal cells increases to about 140 minutes. In many mutant strains, the growth rate on synthetic media is much lower.When cultured in YEPD, the maximum density of the culture is usually 2 x 108 cells/ml, but with the use of special techniques, it can be increased by an order of magnitude. The maximum density of cultures grown in a synthetic medium is usually about 107 cells / ml [2,16].

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