Enzyme-Linked Immunosorbent Assay Technology

Subjects: Archaeology

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Mass spectrometry, chromatography, spectroscopy, nuclear magnetic, proteomics, and immunoassay are used to analyse protein materials. Proteomics techniques and enzyme-linked immunosorbent assay (ELISA) technology are two of the most common methods for detecting ancient proteins.

cultural heritage conservation protein enzyme-linked immunosorbent assay (ELISA)

1. Introduction

In the conservation of cultural heritage and archaeological excavations, the materials, and especially the key components, such as the binder of murals and paintings and the organic components of building mortars, are not only closely related to human production life, material life, spiritual life, and all aspects of social life, but they also serve as significant carriers of historical information. In addition, they are the focus of research on traditional building techniques, the prevention and control of the deterioration of cultural relic materials, the elucidation of the physical and chemical causes of material deterioration, and the development of future protective measures based on analysis and testing [1].

Proteins are one of the most significant components of cultural heritage artefacts and are frequently employed as essential additives [2][3][4]. Additionally, proteins can be used to restore and reinforce cultural heritage artefacts [5][6][7]. It is difficult to analyze protein-containing cultural heritage artefacts due to the limited number of available samples or the low protein content of the samples. Moreover, after prolonged exposure to multiple environmental factors, proteins encounter a variety of issues, including ageing, degradation, and contamination [8][9]. The earliest research on protein detection in artefacts of cultural heritage dates back to the 1950s [10]. Archaeologists used mass spectrometry (MS) to detect amino acids, the building blocks of proteins, in archaeological and paleontological artefacts [11]. Since the 1980s, physical chemistry techniques such as mass spectrometry, chromatography, and spectroscopy have been utilised extensively in the field of cultural heritage research and conservation. However, these methods have the disadvantage of requiring a large number of samples, making it challenging to distinguish the complex protein components in cultural heritage objects and lacking species specificity [12]. In recent years, with the development of biotechnology, bioinformatics technology, and MS, proteomics techniques and enzyme-linked immunosorbent assay (ELISA) techniques have been increasingly applied to the study of proteins in cultural relics materials, and these two techniques offer high sensitivity and low detection limits [13].

2. ELISA Technology

2.1. The Concept of Enzyme-Linked Immunosorbent Assay

ELISA is a labelled immunoassay technique that combines the specific binding reaction of antigen–antibody with the color-rendering reaction of enzymes and catalytic substrate to enhance sensitivity [3] by amplifying the signal. In a published article promoting the establishment and development of ELISA [14], Swedish researchers Engvall and Perlmann first proposed the use of ELISA for the quantitative detection of antibodies during the 1970s. The substrate is added after the enzyme is labelled on the antibody, the antigen is immunologically bound to the enzyme-labeled antibody, and then the antigen is specifically bound to the enzyme-labeled antibody. The product of the colour reaction between the substrate and the enzyme is coloured. The colour intensity of the product correlates positively with the quantity of antigen or antibody in the test substance. Using an enzyme marker, the absorbance of the product at a particular wavelength can be measured, and the antigen or antibody can be quantitatively analysed. An antigen is a molecule that can stimulate an organism's immune system, and an antibody is a glycoprotein that can bind to an antigen in a specific manner. Proteins can be used as an antigen to generate antibodies, and for immune experiments to generate specific antibodies, only the protein must be extracted and purified.

The direct method, double antibody sandwich method, indirect method, and competition method are typical ELISA detection techniques used for protein analysis of cultural relic materials [15] (**Figure 1**). The direct method involves adsorbing the antigen onto a solid-phase carrier, incubating it at an appropriate temperature and relative humidity, and then washing away any unbound antigens and impurities. To bind other unbound sites on the solid-phase carrier, a high concentration of nonspecific proteins and enzyme-labeled specific antibodies are added. At an optimal temperature and relative humidity, the antigen and antibody react completely, and the sample is then rinsed to remove unreacted antibodies. Finally, the chromogenic substrate is added, and within a certain amount of time, the enzyme-catalyzed colour develops. The experimental operation requirements for the labelling reaction of antibodies are high; each antigen detected by the direct method must be labelled with a specific antibody that interacts with it, resulting in a high cost of detection. Using the sandwich method, a known antibody is affixed to the surface of a solid-phase carrier, followed by the test sample. If the sample contains an antigen, specific binding will occur; then, an enzyme-labeled antibody is added to produce an antibody–antigen–enzyme-labeled antibody "sandwich" structure. After the addition of the substrate, the antigen in the sample is detected and analysed using a color-generating reaction. Double antibody sandwiching is a common technique for detecting macromolecular antigens [16]. However, when using the double antibody sandwich method to detect each antigen, the specific antibody that reflects that antigen must be labelled; when combined with indirect methods, each antigen requires two antibodies, making the double antibody sandwich method more expensive. The indirect technique can be used to detect antigens. The principle is to adsorb the antigen to be tested onto the solid phase, followed by the addition of specific antibodies and enzyme-labeled antibodies. Alternately, after the antigen has been adsorbed in the solid phase, the antibody to be examined and the enzyme-labeled antibody are successively added. In the indirect method, the colour intensity is directly proportional to the concentrations of the coated antigen and the primary antibody. The binding between the first and second antibodies is based on the principle that cross-reactions can

occur between distinct subclasses of the same antibody. Therefore, specific antibodies can be applied directly to ELISA without being labelled, preserving the high activity of the specific antibody, reducing the number of experimental steps, and saving time and money. Consequently, indirect methods are the most popular. It is also the most popular method for detecting proteins in cultural relics [17]. The competition method is distinguished by the interaction between the antigen being tested and the enzyme-labeled antigen and the solid-phase antibody. The more the enzyme-labelled antigen binds to the solid-phase antibody, and the darker the colour, the lower the antigen concentration in the test sample. The intensity of the colour of the competitive solution is directly proportional to the antigen concentration in the mixed solution and inversely proportional to the antigen concentration on the solid carrier. This method is primarily used to determine small-molecule hapten [18][19][20].

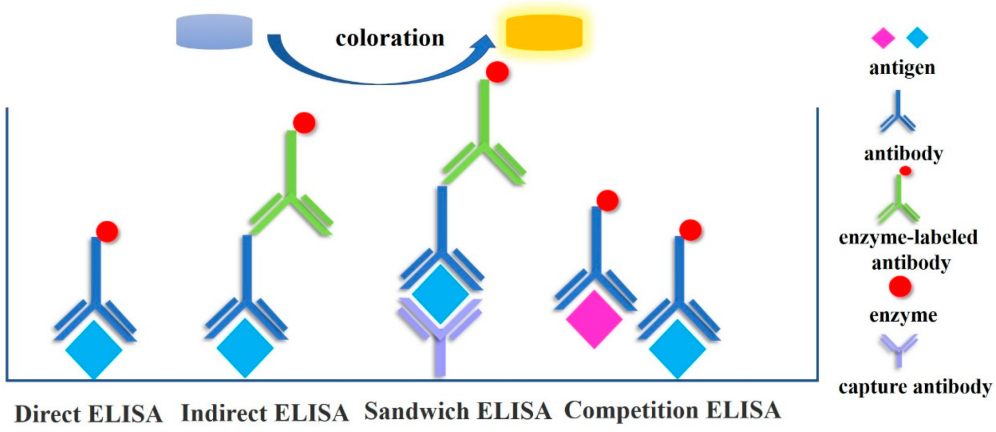

Figure 1. Common detection methods of ELISA.

2.2. Application of Early ELISA in Protein Detection of Cultural Relics Materials

Since the introduction of ELISA technology, it has been widely used for the detection of viruses, antibiotics, heavy metal ions, disease-related proteins, residues, etc., due to its high sensitivity, easy and rapid operation, the small amount of sample required, low cross-reactivity, and high specificity. The development of ELISA technology has also piqued the interest of archaeologists, who have started applying it to the detection of proteins in materials from cultural relics. Earlier studies on the application of ELISA in archaeology were numerous. In 1990, for instance, ELISA was used to detect albumin protein in ancient corpses and animal remains [21]. It was also used to determine that the pigment deposits of the Chumash Indians in the United States consisted of both animal and human blood. Cattabeo [22] utilised ELISA to analyse ancient human bone extracts in 1992. Albumin was discovered in 23 of the 31 skeletons, whereas only one skeleton contained IgG. Therefore, it has been demonstrated that albumin is a significantly superior target molecule for long-term survival in ancient bones. The results demonstrated that there is no cross-reaction between human and animal materials when using ELISA to detect albumin in bones, and that as little as 10 ng of protein is detectable, thereby expanding the possibilities for bone archaeology. In 2002, Schweitzer [23] used ELISA to identify immunologically active polypeptides in 100,000 to 300,000-year-old fossilised skulls. This demonstrates that ELISA is sensitive enough to detect proteins that are extremely aged.

2.3. ELISA Detection of Proteins in Cultural Relics Materials in a Complex Environment

With the ongoing development and enhancement of ELISA, a large number of research results for protein analysis and detection in materials from cultural relics have emerged, and the sensitivity and specificity of detection have improved continuously. In the protein processing method, ELISA employs the trypsin and other prior procedures of aged proteins so that the protein's antigen determinant is exposed and can react with antibodies. This method detects ageing and denatured proteins in cultural relic materials without the need for a complex protein separation and purification procedure [24]. Protein components that must be detected in cultural relic materials frequently exist in complex environments and are difficult to separate; for instance, protein-cemented materials in mural layers are frequently mixed with inorganic materials, and cations of lead, copper, calcium, and iron are present in pigment layers. When amino acid analysis is performed using mass spectrometry, it will interfere with the derivatization process and alter the results [25]. In the presence of impurities, such as inorganic materials, active cations, and organic polymers, ELISA is superior to other detection methods for identifying proteins in cultural relic materials. In 2010, Palmieri et al. [26] analysed proteins in samples of cultural relics materials containing inorganic matrices using ELISA. After testing samples of 13th-century murals, it was determined that casein could still be detected in common mural substrates, such as carbonated stucco or gypsum, with a detection limit as low as 1 nanogram, indicating that these inorganic matrices will not affect the detection ability and sensitivity of ELISA. Similarly, the use of ELISA technology for determining the age of protein binders in pigments containing distinct metal ions will not affect the test results. In 2015, Lee et al. [25] modified the colour portrayal of Alkaline Phosphatase (AP), a commonly used enzyme in ELISA, to horseradish peroxidase (HRP), which is more sensitive than AP and has a greater magnification range. Animal cements containing pigments such as lead white and ultramarine are mined for proteins. The ELISA signal remains unaffected, and the protein type can still be identified. Similarly, a sample was extracted from a 12th- to 13th-century Peruvian feather robe. The ELISA results revealed that the sample's adhesive was arabino-galactose gum, not animal glue as was previously believed. Type I collagen was detected in paper samples obtained from 19th-century to early 20th-century watercolour paintings. Ultimately, type I collagen and ovalbumin were discovered in tempera paintings on canvas from the 14th century. These results demonstrate that ELISA is capable of detecting various classes of proteins containing distinct metal ions.

2.4. ELISA Detection of Trace Samples

ELISA can detect and identify proteins in cultural relic materials that are stored in adverse environments, severely damaged, ageing, or even completely denatured, as well as those that are difficult to distinguish visually. It can also differentiate between protein types, determine their biological origin [27] and detect and identify even trace concentrations of proteins. Liu [28] used indirect ELISA and indirect competitive ELISA to detect and identify samples of wool and leather from cultural relics. Cowhide was identified as the variety of leather found in samples from dry regions of ancient Xinjiang. The ELISA indirect detection threshold for keratin was 10 ng/mL. Additionally, the collagen type I in the three ancient leather samples was identified, and the results of various proportions were obtained, thereby completing the species identification. Wu [29] laboured in 2017 to detect lacquer in samples of cultural relic material. Due to the complex composition of the remaining samples, which were contaminated and

contained only trace quantities, the FTIR and Py-GC/MS results for lacquer phenol were insufficient. The glycoproteins in the dried lacquer films were analysed using ELISA, and the results were applied to eight samples collected from six remnants or ancient structures in different Chinese cities. This study's detection limit reached 106 g/mL, and muddy samples were also analysed. In 2021, Weng [30] used ELISA to analyse samples of Chinese building mortars from 4300 years ago (approximately 2300 BC) to determine if they contained traces of commonly used binders, such as glutinous rice, tung oil, sugar, and animal adhesive. The results indicated that the samples contained components of animal adhesive. ELISA was able to detect protein components in old, exceedingly low-content mortars.

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