8.3 Benzylidene fluorescent probes: a novel approach for albumin detection with applications in health and industry

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Albumin is an important protein present in the body, predominantly found in blood plasma. Serum albumin transports endogenous and exogenous substances, such as amino acids, fatty acids, drugs, and toxins. It is used in medical diagnoses as a biomarker for various diseases, as the concentration of albumin in plasma is closely related to health. Detecting and quantifying albumin is therefore crucial, as it significantly impacts multiple industries, including medicine, biochemistry, and food production. Consequently, there is a demand for selective and sensitive detectors of serum albumin. Bovine serum albumin (BSA) is a well-studied globular protein with a structure similar to that of human serum albumin (HSA), making it widely used as a model protein. The fluorescence of BSA is attributed to tryptophan residues, which can be quenched by various fluorophores. The detection of spectral changes is rapid, selective, and sensitive compared to classical protein detection methods. As a result, fluorescence methods are gaining more attention in the field of biosensor development. The studies were conducted on the quenching of tryptophan fluorescence of BSA in relation to the concentration of the fluorophore (benzylidene derivatives) and the emission of fluorophore fluorescence based on BSA concentration. Selected benzylidene derivatives diluted in DMSO-PBS and BSA dissolved in PBS buffer were examined. Fluorescence changes were investigated at different dilutions of both the protein and the fluorophore, at wavelengths corresponding to or close to the maximum fluorescence of both the protein and the fluorophores. The tested benzylidene derivatives exhibited quenching of the tryptophan fluorescence of BSA while enhancing their own fluorescence, suggesting their potential use as fluorescent BSA probes; however, further research is needed for more detailed analysis.

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8.4 Evaluating the mutagenic risk of benzylidene derivatives through Ames test: ensuring safe integration in biosensor development

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The Ames test is a bacterial reverse-mutation assay designed to identify potential mutagenic chemicals. The test uses modified strains of *Salmonella typhimurium*, which are unable to synthesize histidine and cannot grow on a medium lacking this amino acid. Exposure to a mutagen causes a reversal of mutation, allowing newly mutated bacteria to synthesize histidine and grow on medium lacking it. Substances that can cause mutations have the potential to induce cancer; thus, identifying mutagenic potential is an important step in the safety assessment of chemical compounds. The Ames test is relatively easy and quick to perform and does not require advanced laboratory equipment or high financial outlays, making it a valuable tool for assessing mutagenicity. Benzylidene derivatives show potential as fluorescent sensors for biological applications. Therefore, the studies on the potential mutagenicity of selected benzylidene derivatives were conducted using the Ames test. A series of dilutions of benzylidene derivatives in DMSO was prepared. *S. typhimurium* bacteria were incubated with these dilutions and the controls on an indicator medium containing a pH-sensitive indicator in 384-well plates. If a tested substance exhibited mutagenic properties, the mutation of the bacteria would be reversed, allowing them to grow in the medium, changing its pH and the color of the indicator. After 48 to 72 hours, colony counts were recorded for each well. It is important that potential biosensors are not mutagenic due to their use in detecting biomolecules or visualizing cellular structures. Hence, studies on the mutagenicity of such compounds are essential to ensure their safety for use. The results of the Ames test indicate that the tested benzylidene derivatives are not potentially mutagenic, suggesting that they can initially be considered safe for use as biosensors; however, further and more detailed research on their cytotoxicity is needed.

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