- 1P338 酵素反応へのマイクロ波照射の効果
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The effect on the enzymatic reaction under microwave irradiation
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The effect of microwave irradiation on various organic synthetic reactions is well known, but the reaction mechanism is not understood at the present time. The general explanation is that microwave irradiated reaction system generate the thermal energy by rotation of molecules with dipole moment. However, these proposed mechanism could not be understood some typical high yields and quick conversions reported in the literature. Recently, microwave effect have been proposed on the characteristic phenomena of microwave irradiated chemical reactions. Therefore, we researched the microwave effect on the enzymatic reaction under microwave irradiation. The lipase, esterase, aldorase, and nuclease were studied as the model enzyme. The reaction was also demonstrated under the various conditions, microwave power energy, aqueous or organic media, and thermal control. The reactivity of almost enzymes was accelerated by microwave effect.

- 1P340 TOF-SIMS による電極固定化リゾチウムの表面構造測定
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Surface structure measurement of lysozyme immobilized on electrode by means of time-of-flight mass spectrometry
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Lysozyme molecules immobilized on electrodes were measured with time-of-flight secondary ion mass spectrometry (TOF-SIMS) in order to evaluate their orientation. TOF-SIMS provides chemical information of the upper surface part of a macromolecule. Surface structure of a particular protein immobilized on a substrate can be determined based on analysis of fragment ions obtained with TOF-SIMS. In this study, different types of the lysozyme-immobilized samples were prepared. All samples were immobilized on indium tin oxide (ITO) coated glass plates, and were dried with freeze dryer before TOF-SIMS measurement. The samples were measured with TOF-SIMS with the cluster ion sources such as Au³⁺ and Bi³⁺. The TOF-SIMS spectra of each sample were analyzed to select fragment ions specific to each sample. Chemical formulas of secondary ions specific to each sample were determined with searching all possible chemical structures generated from proteins. Based on the specific fragment ions, the most upper surface structures of the immobilized protein molecules were determined, and then they were compared with the amino acid sequence and the 3D structure. As a result, it is indicated that the surface part of the protein molecules immobilized in the different ways have different structures. Thus differences depending on the immobilization processes are evaluated by means of TOF-SIMS analysis.

- 1P339 飛行時間型二次イオン質量分析法によるタンパク質分子層の構造評価法の開発
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Development of structural characterization method of protein monolayer by means of time-of-flight mass spectrometry
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It is difficult to structurally analyze a protein molecule reconstituted as a monolayer because it requires ultra high sensibility. Time-of-flight secondary ion mass spectrometry (TOF-SIMS) enables measurement of upper surface of one layer of molecules, and provides detailed information on chemical structures. In a previous study, analysis method of TOF-SIMS spectra for biomaterials based on mutual information and chemical formula determination programs considering all possibilities had been developed. In this study, our novel measurement method was employed to characterize self-assembly monolayer of cytochrome b5, driven by the chemisorption of the sulfur onto gold surface, in order to assure availability of this method. Biophysical characterizations have been engaged to investigate this thin biological layer and good correlations in protein coverage were obtained between Surface Plasmon Resonance (SPR) and Atomic Force Microscopy (AFM) data. The sample was also measured with TOF-SIMS (ION-TOF) with the bismuth cluster ion source, and then specific peaks of fragment ions related to the surface part of cytochrome b5 were selected comparing with control sample without cytochrome b5 and their chemical formulas were determined with the programs. Then, indicated structures of the fragments generated from the surface of the immobilized-cytochrome b5 were compared to its three-dimensional structure registered in the protein data bank. Finally, the surface parts of the immobilized-cytochrome b5 representing its orientation are determined.

- 1P341 飛行時間型二次イオン質量分析法によるタンパク質測定時の表面状態の基礎的検討
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Evaluation of surface condition at protein measurement with time-of-flight mass spectrometry
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Time-of-flight secondary ion mass spectrometry (TOF-SIMS) is one of promising techniques for evaluation of biomolecules on sophisticated bio nano-devices. However, secondary ion generation phenomena of TOF-SIMS measurement for biomolecules such as protein have not been clarified yet. In this study, relationship between measurement time, representing an ion dose condition and depth of secondary ion generation, was evaluated based on study of TOF-SIMS spectra change depending on measurement time. A protein of 130 amino acid residues, lysozyme, was employed as a model protein. Different types of the lysozyme-immobilized samples were prepared by controlling the binding parts, i.e., amino groups or carboxyl groups, of the protein. The samples were measured with TOF-SIMS, and the TOF-SIMS spectra at varying measurement times, i.e., 1, 2, 3, 5, 10, 15, and 20 min, were compared. As a result, there are no specific secondary ions in TOF-SIMS spectra of less than 5 min measurement in case of the lysozyme immobilized at carboxyl groups. On the other hand, there are some specific secondary ions in the spectra of less than 5 min measurement in case of the lysozyme immobilized at amino groups. Thus differences depending on sample types and measurement times are found, which indicate a surface condition depending on measurement conditions. Therefore it is expected that depth profiles of immobilized protein molecules will be obtained with detailed analysis of TOF-SIMS spectra and appropriate measurement conditions.