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【論文審査】Review of the thesis

The development of nano/micro-scale heating methods is one of important subjects in the field of bio-nano science and technology. For instance, a technique of heating a specific spot on a substrate allows for the development of lab-on-a-chip devices that perform chemical processes with different optimal temperatures simultaneously. Precise local heating technique is also important to perform hyperthermia cancer therapy properly. Various heating methods using laser, microwave and electric field have been developed, but more powerful and versatile heating method is still needed. In the present doctoral thesis, a nano/micro-scale heating method utilizing heat generation from ferromagnetic particles subjected to an ac magnetic field is developed and the application of the present heating method to enzyme engineering, genetic engineering and food industry is demonstrated.

The thesis entitled “Nano/micro-scale heating method using ferromagnetic particles and its application to bio-nano science and technology” is composed of 6 chapters; that is, “Chapter 1: Introduction”, “Chapter 2: Nano/micro-scale heating method using ferromagnetic particles”, “Chapter 3: Encouragement of enzyme reaction utilizing heat

generation from ferromagnetic particles", "Chapter 4: Efficient DNA ligation by selective heating of DNA ligase", "Chapter 5: Production of invert sugar and fructo-oligosaccharides using invertase immobilized on ferromagnetic particles" and "Chapter 6: Conclusions".

Chapter 1 Introduction

Recently, magnetic particles have been widely used in the research fields of bio-nano science and technology. The chapter starts with some introductory explanation about the characteristics of magnetic particles and describes two mechanisms for the heat generation of ferromagnetic particles subjected to a radio frequency alternating magnetic field; that is, magnetic hysteresis and eddy currents. The chapter also outlines various methods of manipulating magnetic particles developed recently. Finally, the basic idea of the nano/micro-scale heating method developed in the present study, which consists of the manipulation and heating of magnetic particles using external magnetic fields, is described and the advantages of the present heating method are discussed.

Chapter 2 Nano/micro-scale heating method using ferromagnetic particles

In this chapter, the experimental system, which can generate simultaneously both a rotational magnetic field and a radio frequency alternating magnetic field, is developed and the manipulation and heating of ferromagnetic particles on a substrate are performed using this system. The present experimental system is composed of three pairs of coils generating a rotational magnetic field for manipulation of particles and a coil generating a radio frequency alternating magnetic field for heating of particles. Ferromagnetic particles are dispersed in dimethyl sulfoxide containing a thermosensitive dye of Eu-TTA to visualize the heat generation of the particles under an ac magnetic field and the particle solution is confined between two glass plates, the gap between which is maintained by nonmagnetic spacer particles. The behavior of ferromagnetic particles under magnetic fields generated by the coils is observed by a fluorescent microscope. When a rotational magnetic field, the direction of the rotational axis of which is parallel to the grass plate, is applied, a cluster composed of ferromagnetic particles starts to rotate following the field rotation and moves along the glass plate thanks to the friction force acting between the particles and the plate. The direction of the motion is controlled by changing the

rotational direction of the magnetic field and the cluster is successfully moved to a target position. Next, a radio frequency alternating magnetic field is applied to heat up the particles. It is confirmed by fluorescent observation that a small region including the cluster is selectively heated up under the ac magnetic field.

Chapter 3 Encouragement of enzyme reaction utilizing heat generation from ferromagnetic particles

Enzymes are proteins, which catalyze chemical reactions in living organisms, and are widely used in various areas such as biology, medicine, food industry, agriculture and so on. The activity of an enzyme varies depending on the temperature and some enzymes can be activated by appropriate heating. This chapter is devoted to the application of the present heating method to enzyme engineering.

α-amylase is immobilized on the surface of ferromagnetic iron particles and the effect of heat dissipation from the particles subjected to an ac magnetic field on the enzyme's activity is investigated. The activity of immobilized α-amylase is increased under an ac magnetic field caused by heat dissipation from the particles and it increases with an increase in the amplitude of the magnetic field. The average surface temperature of magnetic particles is estimated comparing the activity increase under an ac magnetic field and the temperature dependence of the activity in the absence of a magnetic field. The amount of heat generated by a particle is also calculated from the particle's surface temperature. Next, a solution containing α-amylase/ferromagnetic particle hybrids and free, nonimmobilized chitinase is prepared and the enzymatic reactions occurred in the solution are investigated. When an ac magnetic field is applied to the solution, the activity of α-amylase immobilized on the ferromagnetic particles increases whereas that of free chitinase hardly changes, which clearly shows that only α-amylase immobilized on the particles is selectively heated up and activated due to heat generation from the particles.

Chapter 4 Efficient DNA ligation by selective heating of DNA ligase

Genetic engineering modifies the genetic makeup of organisms through various processes of manipulating DNA. Thermal conditioning is very important to carry out those processes efficiently and properly. This chapter focuses on DNA ligation and demonstrates that it can be performed efficiently by selective heating of DNA ligase using ferromagnetic

particles.

T4 DNA ligase is immobilized on ferromagnetic iron particles and the ligation of DNA fragments with cohesive ends using immobilized T4 DNA ligase is performed. First of all, the dependence of the ligation efficiency in the absence of a magnetic field on the ambient temperature is analyzed. The ligation efficiency increases with an increase in the temperature since DNA ligase is activated by raising the temperature. However, once the temperature exceeds a certain value, the ligation efficiency starts to decrease since DNA fragments joined by base pairing at their overhanging ends are dissociated under such high temperature conditions. Next, DNA ligation is carried out at a low temperature suitable for the annealing of DNA ends under an ac magnetic field. T4 DNA ligase immobilized on ferromagnetic particles is selectively heated up and activated due to heat generation from the particles, as a result of which the ligation efficiency increases. The ligation efficiency increases with an increase in the amplitude of the magnetic field. The ligation of DNA fragments with blunt ends is also carried out and the ligation efficiency is successfully increased by selective heating of DNA ligase under an ac magnetic field.

Chapter 5 Production of invert sugar and fructo-oligosaccharides using invertase immobilized on ferromagnetic particles

Food production using enzyme is sometimes carried out at a high temperature to prevent bacterial contamination. Under such high temperature conditions, enzyme immediately becomes denatured and therefore a large amount of enzyme is required. If the production is carried out at low temperature to suppress bacterial growth, the enzyme's activity is remarkably reduced. This chapter demonstrates that the present heating method overcomes the above difficulties.

In the present study, the production of invert sugar and 1-kestose from sucrose using invertase immobilized on iron or hematite particles is investigated. First of all, the production of invert sugar and 1-kestose using immobilized invertase after incubation at high temperature is analyzed, and the results obtained show that the thermostability of invertase is improved by immobilization on the particles. Invert sugar production is increased by applying an ac magnetic field since immobilized invertase is activated due to heat dissipation from the particles. Invert sugar production by invertase immobilized on the particles at refrigerator temperature (4 °C) is increased by applying an ac magnetic field to the same level as that by free invertase at room temperature (25 °C). 1-kestose

production by immobilized invertase at refrigerator temperature under an ac magnetic field is much higher than that by free invertase at room temperature when the field amplitude is sufficiently high.

Chapter 6　Conclusions

In the present doctoral thesis, a nano/micro-scale heating method utilizing heat generation from ferromagnetic particles subjected to an ac magnetic field is developed and its application to bio-nano science and technology is demonstrated. This final chapter summarizes the results obtained in the present study.

The present heating method can selectively heat nano/micro-scale target objects placed at any position and furthermore, the target position can be moved dynamically since the motion of heat sauces, that is, ferromagnetic particles, can be controlled using a rotational magnetic field. Therefore, hyperthermia cancer therapy could be performed more efficiently and accurately using the present heating method. If ferromagnetic particles are introduced into the microchannels of a micro total analysis system (μ-TAS), a particular spot in the system can be heated utilizing heat generation from the particles.

Enzymes immobilized on ferromagnetic particles can be activated utilizing heat generation from the particles under an ac magnetic field without any effect on the other molecules around the particles. The present activation method is quite simple and therefore it may be widely utilized for various enzymatic processes to increase their efficiencies. Since magnetic particles can be manipulated using an external magnetic field without any difficulty, the manipulation and reaction control of enzymes in μ-TASs can be carried out utilizing enzyme/ferromagnetic particle hybrids, which may make a great contribution to both bio-nano science and technology.

【審査結果】Summary and decision

The thesis entitled “Nano/micro-scale heating method using ferromagnetic particles and its application to bio-nano science and technology” focuses on the development of a nano/micro-scale heating method utilizing heat generation from ferromagnetic particles subjected to an ac magnetic field and its application to the encouragement of enzyme reaction, the promotion of DNA ligation and the efficient production of invert sugar and fructo-oligosaccharides. The results shown in the thesis are outstanding from an international point of view and the significant points in the present study are summarized

below;

(1) Nano/micro-scale objects can be selectively heated up by the present heating method. Furthermore, the heating region can be moved dynamically since the motion of heat sauces, that is, ferromagnetic particles, can be controlled using a rotational magnetic field.

(2) Enzymes immobilized on ferromagnetic particles can be activated utilizing heat generation from the particles under an ac magnetic field without any effect on the other molecules around the particles. The present activation method is quite simple and therefore it may be widely utilized for various enzymatic processes to increase their efficiencies.

(3) Since a small region surrounding a ferromagnetic particle is locally heated by an ac magnetic field, enzymatic reactions using enzyme/ferromagnetic particle hybrids can be carried out with a high reaction rate even under low ambient temperature conditions, which minimizes undesirable chemical reactions that can occur at high temperatures. This feature is clearly useful for enzyme reactions, which need to be carried out at lower temperatures compared to the optimal temperatures of enzymes for several reasons.

(4) DNA ligation can be promoted by selective heating of DNA ligase immobilized on ferromagnetic particles with a radio frequency alternating magnetic field.

(5) Invert sugar and fructo-oligosaccharides can be produced efficiently using invertase immobilized on ferromagnetic particles under an ac magnetic field at a refrigerator temperature. The present method could be expanded to other food manufacturing processes using enzymes.

(6) The surface temperature of ferromagnetic particles under an ac magnetic field can be estimated through measuring the activity of an enzyme immobilized on the particles. The present temperature measurement technique may contribute to the research of nano/micro-scale heat transfer.

(7) Since ferromagnetic particles can be manipulated using an external magnetic field without any difficulty, the manipulation and reaction control of enzymes in micro total analysis systems (μ-TASs) can be carried out utilizing enzyme/ferromagnetic particle hybrids, which may make a great contribution to both bio-nano science and technology.

The results obtained by the present doctoral study have been highly appreciated by

some academic societies; two first-authoring papers have been published by international journals (Public Library of Science and Elsevier).

Judging by the results shown in the thesis and the number of international papers published so far, the level of the present research results is definitely high by international standards and the present results may well make a great contribution to the improvement of hyperthermia therapy as well as the development of lab-on-a-chip devices. The present results may also contribute to the development of efficient methodologies for enzymatic processes. In conclusion, the thesis is considered to be a high quality, high standard one by international standards.