Summary of the doctoral thesis

Nano/micro-scale heating method using ferromagnetic particles and its application to bio-nano science and technology

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Chapter 1 Introduction

Magnetic nano/micro particles are widely used in the fields of nanotechnology, biological and biomedical sciences. For instance, nano/micro machines composed of magnetic particles for biomedical applications, such as drug delivery system, micro total analysis system, cancer therapy and so on have been developed. It is well known that ferromagnetic particles are heated up under a high frequency alternating magnetic field caused by magnetic hysteresis and eddy currents. Since magnetic particles can be manipulated utilizing their tumbling motion induced by a rotational magnetic field, nano/micro-scale objects can be selectively heated up using ferromagnetic particles as a heater. In this study, I develop a nano/micro-scale heating method utilizing the heat generation of ferromagnetic particles subjected to an ac magnetic field and demonstrate that enzyme reactions are encouraged by the present heating method.

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

I developed an experimental system for simultaneously performing manipulation and heating of ferromagnetic particles. The experimental system I developed was 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 iron particles were dispersed in dimethyl sulfoxide containing a thermosensitive dye of Eu-TTA. The particle solution was confined between two glass plates, where the gap between the plates was maintained by nonmagnetic spacer particles. The behavior of ferromagnetic particles under magnetic fields generated by the coils was observed by a fluorescent microscope. When a rotational magnetic field, the direction of the rotational axis of which was parallel to the grass plate, was applied, a cluster composed of ferromagnetic particles started to rotate following the field rotation and moved along the glass plate thanks to the friction force acting between the particles and the plate. The direction of the motion was controlled by changing the rotational direction of the magnetic field and the cluster was successfully moved to a target position. Next, a radio frequency alternating magnetic field was applied to heat up the particles. It was confirmed by fluorescent observation that a small region including the particles was selectively heated up under the ac magnetic field.

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

I immobilized α-amylase on the surface of ferromagnetic iron particles and analyzed the effect of an ac magnetic field on the activity of α-amylase on the particles. It was found that the activity of immobilized α-amylase was increased under an ac magnetic field caused by heat generation from the particles. I estimated the average surface temperature of the particles comparing the activity increase under an ac magnetic field and the temperature dependence of the activity in the absence of a magnetic field. I also calculated the amount of heat generated by a particle from the particle's surface temperature. I next analyzed the effect of heat dissipation from ferromagnetic particles on free, nonimmobilized enzyme molecules around the particles. I prepared a mixture solution, in which α-amylase/ferromagnetic particle hybrids and free, nonimmobilized chitinase were dispersed, and analyzed the enzymes' activities. When an ac magnetic field was applied to the solution, the activity of α-amylase immobilized on ferromagnetic particles increased whereas that of free chitinase hardly changed, which means that only α-amylase immobilized on the particles was selectively heated up and activated due to heat generation from the particles.

Chapter 4 Efficient DNA ligation by selective heating of DNA ligase

I immobilized T4 DNA ligase on ferromagnetic iron particles and performed the ligation of DNA fragments with cohesive ends using immobilized T4 DNA ligase. I analyzed the dependence of the ligation efficiency on the ambient temperature in the absence of a magnetic field and found that the ligation efficiency reaches maximum at a certain temperature. The activity of DNA ligase increases with an increase in the temperature up to its optimal temperature. However, DNA fragments joined by base pairing at their overhanging ends are dissociated by raising the temperature, which reduces the ligation efficiency. The temperature dependence of the ligation efficiency obtained in the present study can be explained qualitatively by the competition between the above two effects. I next carried out DNA ligation at a low temperature suitable for the annealing of DNA ends under an ac magnetic field. T4 DNA ligase immobilized on the ferromagnetic particles was selectively heated up and activated due to heat generation from the particles, as a result of which the ligation efficiency increased. The ligation efficiency increased with an increase in the amplitude of the magnetic field. I also carried out the ligation of DNA fragments with blunt ends and found that the ligation efficiency is increased by selective heating of DNA ligase with an ac magnetic field.

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

I investigated invert sugar production by hydrolysis of sucrose using invertase immobilized on iron and hematite particles. First of all, I analyzed invert sugar production by immobilized invertase after incubation at a high temperature and found that the thermostability of invertase is improved by immobilization on the particles. Invert sugar production was increased by applying an ac magnetic field since immobilized invertase was activated due to heat dissipation from the particles. In fact, invert sugar production by invertase immobilized on the particles at 4 °C under an ac magnetic field was higher than that by free invertase at 25 °C when the field amplitude was sufficiently high. This means that by using invertase immobilized on ferromagnetic particles, invert sugar can be produced efficiently even under low ambient temperature conditions inhibiting bacterial growth. The present enzymatic reaction also produced a fructo-oligosaccharide, that is, 1-kestose. 1-kestose production was also increased by applying an ac magnetic field.

Chapter 6 Conclusions

I developed a nano/micro-scale heating method utilizing the heat generation of ferromagnetic particles under an ac magnetic field. I successfully performed the manipulation and heating of ferromagnetic particles by applying an external magnetic field and confirmed that small regions surrounding the particles were selectively heated up under a radio frequency alternating magnetic field. Next I demonstrated that enzymes immobilized on the surface of ferromagnetic particles can be selectively activated by heating with an ac magnetic field. The present result suggests that the manipulation and reaction control of enzymes in micro reactors or micro total analysis systems (μ-TASs) can be carried out using ferromagnetic particles and therefore, I believe that the present heating method may make a great contribution to both bio-nano science and technology.

Publications

1. M. Suzuki, H. Hayashi, T. Mizuki, T. Maekawa and H. Morimoto, Efficient DNA ligation by selective heating of DNA ligase with a radio frequency alternating magnetic field. Biochem. Biophys. Rep. 8 (2016) 360-364.
2. M. Suzuki, A. Aki, T. Mizuki, T. Maekawa, R. Usami and H. Morimoto, Encouragement of enzyme reaction utilizing heat generation from ferromagnetic particles subjected to an ac magnetic field. PLoS One 10 (2015) e012763.