

## Non-thermal Effect of a Ceramics Radiation on a Yeast Glucose-6-phosphate dehydrogenase

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Non-thermal effect of a ceramics radiation on glucose-6-phosphate dehydrogenase has been investigated using the enzyme, glucose-6-phosphate and NADP<sup>+</sup> separately irradiated at 10°C by a ceracompo R plate and a ceramics un-sewed cloth (sheet). The  $K_m$  for glucose-6-phosphate was increased 20% after 6 h of irradiation by the plate, but the  $V_{max}/K_m$  was decreased 24%. After 3 h of irradiation by the sheet, the  $K_m$  was increased 17%, but after 6 h of irradiation it was decreased 11%. The 3 h of irradiation by the sheet slightly increased both enthalpy and entropy changes of the reaction, but the 6 h of irradiation significantly decreased them. Both thermodynamic parameters in the activated state were increased by the sheet irradiation. The promotion energy for both formations of the enzyme-substrate and their activated complex depended on enthalpy. The different effects of two ceramics radiators on G6PDH activity were discussed.

**Key words:** ceramics radiation; glucose-6-phosphate dehydrogenase; far-infrared; hydration; thermodynamics

A yeast glucose-6-phosphate dehydrogenase (G6PDH, EC 1.1.1.49; D-glucose-6-phosphate: NADP<sup>+</sup> 1-oxidoreductase) catalyzes the oxidation of  $\beta$ -D-glucose-6-phosphate in the presence of NADP<sup>+</sup> to D-glucono- $\delta$ -lactone-6-phosphate and NADPH + H<sup>+</sup>.<sup>1)</sup> The G6PDH activity can be changed, adapting to various environmental conditions.<sup>2)</sup> We have expected that the G6PDH activity may be affected by far-infrared (FIR) irradiation on the enzyme, since we have found that xanthine oxidase and lactic dehydrogenase (LDH) activities were significantly regulated by the enzymes that were modified with absorbing energy from FIR.<sup>3,4)</sup>

The two hydrogen atoms at the 4 position of NADPH are designated as *pro*-(R) and *pro*-(S). LDH selects the *pro*-(R) hydrogen of NADH and directly transfers the hydrogen of lactate to NAD<sup>+</sup> or the hydride ion of NADH + H<sup>+</sup> to pyruvate. G6PDH selects the *pro*-(R) hydrogen of NADPH.<sup>1)</sup> Therefore, we compared the effects of FIR irradiation on the chirality of the G6PDH molecule with the LDH one.

The electromagnetic waves in the FIR region that are radiated from a fine ceramics heater at a surface temperature below 300°C are resonantly absorbed into almost all organic molecules that have characteristic finger-

print regions of infrared waves.<sup>5)</sup> All these fingerprint regions are included in the FIR region of 5 to 1000  $\mu$ m. Water molecules also have several vibration levels in the FIR region that are caused by their stretching, deforming and rotational movement.<sup>5)</sup> Therefore, the FIR energy will affect thermally or non-thermally on the structure of biological molecules. Thermal effect is shown by the situation that the irradiation energy will be simply used as a heating energy for moving the molecules in fluids. Non-thermal effect is that the energy will be used for holding the structure of the molecules in fluids.

According to the Planck's and the Stefan-Boltzman's laws on black body, the subtle energy of about 36 to 52 mW/cm<sup>2</sup> is radiated from the FIR radiators including microorganisms and living cells at the surface temperature of 10 to 37°C, respectively.<sup>6,7)</sup> The Wien's displacement law about the maximum radiation wavelength ( $\lambda_{max}$ ) against the temperature of the radiator indicates that the FIR wavelength having  $\lambda_{max}$  of about 10 to 9  $\mu$ m was radiated from the radiator at 10 to 37°C, respectively.<sup>6,7)</sup> Since an enzyme reaction is done *in vitro* in a buffer system, the FIR energy or the vibration will be resonantly absorbed into the bulky aqueous solution and the buffer salts, and necessarily affect the structure of such a minor component of solute molecule as enzyme in the system.

As the FIR energy is such a subtle one below 0.12 eV, it cannot cause an electron transfer in the irradiated molecule.<sup>5)</sup> However, our previous papers showed that the FIR irradiation accelerated the hydration of various biological molecules in aqueous solution and changed the basal energy state of the molecules, and that the hydration of enzyme and reaction components followed a large negative entropy change ( $-T\Delta S$ ) and then the enzymic reaction was effectively regulated.<sup>3,4,8,9)</sup>

In this paper using the thermodynamic analysis, we showed that the reaction kinetics of G6PDH irradiated by a ceramics plate differed from that by an un-sewed sheet tempering another ceramics, and that the molecular thermodynamics of the G6PDH reaction catalyzing a transfer of the *pro*-(S) hydrogen of NADPH using the ceramics sheet irradiation system was different from that of the previous LDH reaction catalyzing a transfer of the *pro*-(R) hydrogen of NADH using the ceramics heater irradiation system. The mechanism of regulation of

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Abbreviations: G6PDH, glucose-6-phosphate dehydrogenase; LDH, lactate dehydrogenase; FIR, far-infrared.

the G6PDH reaction by irradiation at 10°C using a ceramics un-sewed sheet is discussed.

### Materials and Methods

A bakers' yeast glucose-6-phosphate dehydrogenase (G6PDH, Type XV, crystallized, lyophilized, and sulfate-free, 200–400 units/mg protein) was obtained from Sigma. Distilled water for HPLC was purchased from Wako Pure Chemicals, Osaka.  $\beta$ -NADP<sup>+</sup> was from Oriental Yeast Co. Ltd. All other chemicals were of analytical grade.

The powdered G6PDH was dissolved in 1 ml of an ice-cold 5 mM trisodium citrate. The enzyme solution was distributed into Eppendorf tubes (10 units/20  $\mu$ l each) and stored at  $-80^{\circ}\text{C}$  before use. The frozen enzyme was melted in an ice cold water, and the solution was irradiated at 10°C for 3, 6, and 18 h in a Tabai Espec PL-1 GT constant humidity heater as shown in Fig. 1. The enzyme solution in an Eppendorf tube was irradiated by putting the tube on a 3 cm $\phi$  ceracompo R plate tempering a ceramics of silicon nitride/silicon carbon ( $\text{Si}_3\text{N}_4\cdot\text{SiC}$ ; emissivity at 2 to 25  $\mu\text{m}$  is about 0.90 against the black body, Nippon Keikinzoku Co. Ltd., Tokyo) or wrapping with an un-sewed cloth,  $T_4$ -sheet, tempering a ceramics of titanium oxide /silicon oxide ( $\text{TiO}_2\cdot\text{SiO}_2$ ; emissivity at 7 to 20  $\mu\text{m}$  is about 0.75 and that at 4  $\mu\text{m}$  is about 0.45, Nippon Vilene Co. Ltd.,

Tokyo). Each system containing the tube and the ceramics radiator was wrapped and sealed with a sheet of thick aluminum foil. A 10-ml polystyrene vial or tube was used for the irradiation of 20 mM glucose-6-phosphate and 6 mM NADP<sup>+</sup> solutions, respectively. A non-irradiated system was incubated at 10°C in the PL-1 GT heater for the same time by covering the tube or vial containing the samples with the aluminum foil or by wrapping with an un-sewed cloth,  $T_1$ -sheet, that had no tempering ceramics, and sealed with the aluminum foil. After an appropriate time of irradiation or incubation, the G6PDH solution was diluted 100-fold with ice-cold 5 mM trisodium citrate, and the glucose-6-phosphate solution was diluted 10-fold with 0.1 M Tris buffer, pH 8.0, and the diluted solutions were used for the reaction, but the NADP<sup>+</sup> solution was directly used without dilution.

The standard reaction mixture (3.0 ml), which was modified from that in the literature,<sup>10</sup> contained: 0.1 M Tris-HCl, pH 8.0, 0.1 mM glucose-6-phosphate and 0.3 mM NADP<sup>+</sup>. The reaction mixture except enzyme was incubated in a turret cuvette holder at 30°C and the reaction was started by adding the enzyme (0.0033 units/0.1 ml). The reaction mixture was not irradiated during incubation. The initial velocity at 30°C was measured by monitoring the increase in absorbance of NADPH at 340 nm ( $\epsilon=6220/\text{mol}/\text{cm}$ ) for 2 min with a

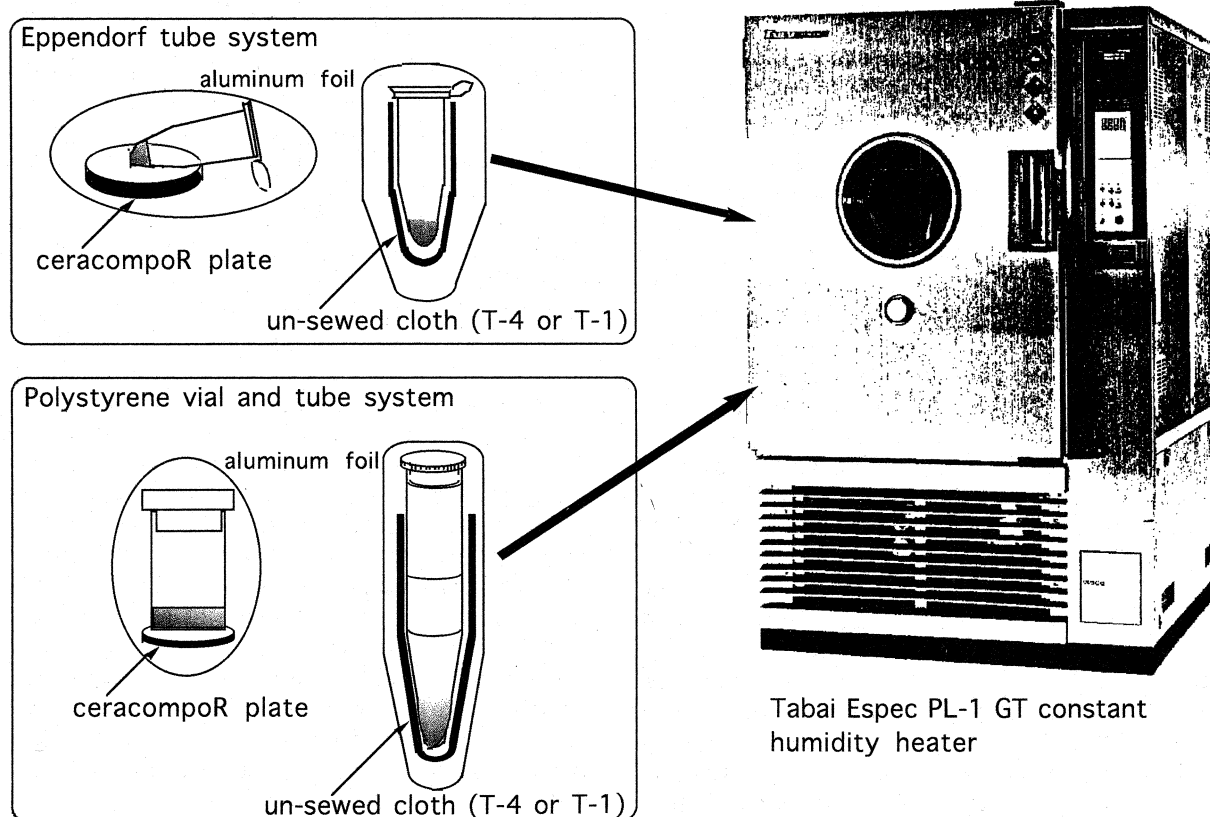


Fig. 1. Irradiation Equipment.

The left upper panel is for the enzyme irradiation system. The enzyme solution in an Eppendorf tube was put on a 3-cm $\phi$  ceracompo R plate or wrapped with an un-sewed cloth tempering a ceramics ( $T_4$ -sheet) or without ceramics ( $T_1$ -sheet). The lower panel is for the irradiation system of the buffer and NADP<sup>+</sup> solutions. Each system containing the tube and the ceramics radiator was wrapped and sealed with a sheet of thick aluminum foil. Materials for outer and inner shells are stainless steels SUS430CP and 304. Heat insulating materials used are a hard polyurethane foam, an expanded polystyrene, and a glass wool.

UVIDEC 220B spectrophotometer (Japan Spectroscopic Co. Ltd., Tokyo).

Kinetic constants ( $K_m$  and  $V_{max}$ ) were calculated from the x-axis intercept and the slope in  $[s]/v \sim [s]$  plots (Hanes-Woolf plots), where  $[s]$  is a substrate concentration, and  $v$  is velocity. Five concentrations between 20 to 100  $\mu\text{M}$  glucose-6-phosphate were used for the plots.

Thermodynamic parameters for the reaction at 25, 30, and 37°C were calculated from the  $K_m$  and  $k_{+2}$ , a reaction rate for the product formation in each system. They were calculated from the following equations at 303 K:  $\Delta G_s^0 = 2.303RT \log K_m$ ,  $\Delta H_s^0 = 2.303R \frac{d \log K_m}{d(1/T)}$ ,  $T\Delta S_s^0 = (\Delta H_s^0 - \Delta G_s^0)$ ,  $E = -2.303R \frac{d \log k_{+2}}{d(1/T)}$ ,  $\Delta H_s^* = E - RT$ ,  $\Delta G_s^* = 2.303RT [\log(kT/h) - \log k_{+2}]$ , and  $T\Delta S_s^* = (\Delta H_s^* - \Delta G_s^*)$ , where  $E$  is an activation energy,  $k$  is a Boltzmann constant ( $1.38 \times 10^{-23}$  J/K),  $h$  is a Planck constant ( $6.62 \times 10^{-34}$  Js),  $T$  is an absolute temperature (K) and  $R$  is a gas constant (8.31 J/K/mol).

Each set of experiments was repeated a minimum of five times. Data are the means of five different analyses from a representative experiment.

## Results

The irradiation system shown below indicates that the reaction components for G6PDH were separately irradiated by the plate or  $T_4$ -sheet, and the mixture was incubated under non-irradiated condition as shown in Materials and Methods.

### Effects of ceramics radiation on kinetic parameters for G6PDH reaction

The  $K_m$  for glucose-6-phosphate was increased by 20% after 6 h of irradiation by the plate, and after that it returned to an initial level (left panel in Fig. 2). After 3 h of irradiation by  $T_4$ -sheet, the  $K_m$  was increased by 17%, but after 6 h of irradiation it was decreased by 11%, and after that it returned to an initial level (right panel in Fig. 2). The  $V_{max}/K_m$  after 6 to 24 h-irradiation by the plate was decreased by 24%, and that after 6 h-irradiation by  $T_4$ -sheet was increased by 14%. After 24 h of irradiation by  $T_4$ -sheet, the value returned to the initial level, but that by the plate remained at the decreasing level.

The G6PDH under non-irradiated system using an un-sewed cloth without tempering any ceramics, a  $T_1$ -sheet, was stable for 24 h of incubation at 10°C.

### Effects of ceramics radiation with a $T_4$ -sheet on thermodynamic parameters for G6PDH reaction

Table I shows the thermodynamic parameters for the reaction of G6PDH irradiated by a  $T_4$ -sheet. There was little difference in the negative free energy change ( $\Delta G_s^0$ ) of  $-26.1$  kJ/mol between the irradiated and non-irradiated systems. Although the enthalpy change ( $\Delta H_s^0$ ) after 3 h of irradiation was slightly increased, that after 6 h of irradiation was significantly decreased to  $-24.4$  kJ/mol. The activated enthalpy changes ( $\Delta H_s^*$ ) after 3 to 6 h of irradiation were increased by 5.7 kJ/mol. The activated entropy change ( $T\Delta S_s^*$ ) was increased to  $-12.6$  kJ/mol by the irradiation.

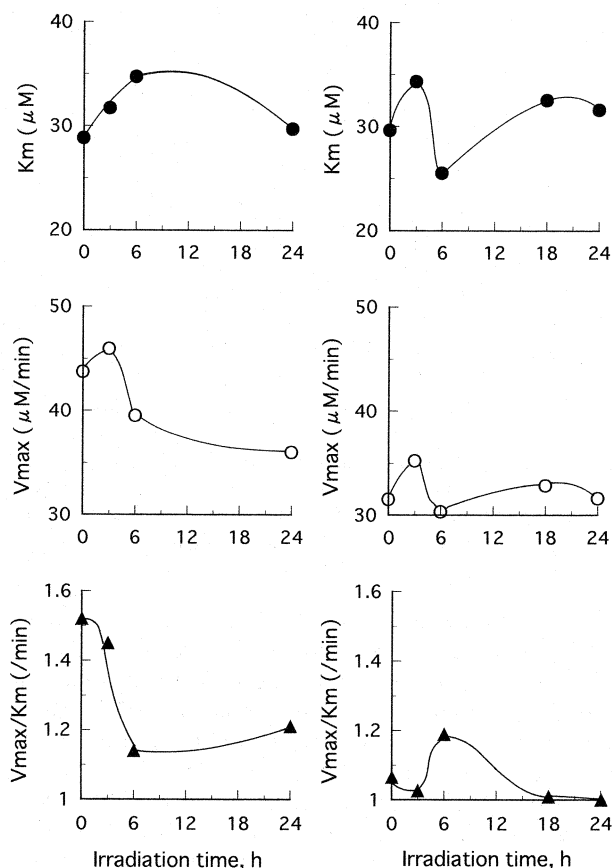


Fig. 2. Effects of Irradiation Time on Kinetic Parameters for G6PDH Reaction.

Five different concentrations of glucose-6-phosphate (20, 40, 60, 80 and 100  $\mu\text{M}$ ) and 0.3 mM  $\text{NADP}^+$  were incubated at 30°C with 0.033 units/ml of the non-irradiated and irradiated by a ceracompo R plate (left panels) and  $T_4$ -sheet (right panels). The activities were measured by monitoring the absorbance at 340 nm as shown in Materials and Methods. Kinetic parameters ( $K_m$  and  $V_{max}$ ) were calculated from the x-axis intercept and the slope in  $[s]/v \sim [s]$  plots. Values are means for five different experiments. G6PDH (10 units/20  $\mu\text{l}$ ), glucose-6-phosphate (20 mM) and  $\text{NADP}^+$  (6 mM) were irradiated for 3 to 18 h, and used for assay after 100, 10-time, and no time dilution, respectively.

Table I. Effects of Radiation Time on Thermodynamic Parameters for Glucose-6-phosphate Dehydrogenase

Five different concentrations of glucose-6-phosphate (20, 40, 60, 80, and 100  $\mu\text{M}$ ) and 0.3 mM  $\text{NADP}^+$  were incubated with 0.033 units/ml of the enzyme. The irradiation was done by a  $T_4$ -ceramics radiator and non-irradiation was by  $T_1$  non-tempering ceramics. The activity was measured by monitoring the absorbance at 340 nm as shown in Materials and Methods. The changes in the free energy ( $\Delta G_s^0$ ), the enthalpy ( $\Delta H_s^0$ ), and the entropy ( $\Delta S_s^0$ ) at 30°C were calculated from Arrhenius plots of  $K_m$ . Their values in activation state at 30°C were from Arrhenius plots of a reaction rate,  $k_{+2}$ , for the product formation.

Irradiation time h	$\Delta G_s^0$ kJ/mol	$\Delta H_s^0$ kJ/mol	$T\Delta S_s^0$ kJ/mol	$\Delta G_s^*$ kJ/mol	$\Delta H_s^*$ kJ/mol	$T\Delta S_s^*$ kJ/mol
0	-26.1	-3.1	23	58.1	39.8	-18.3
3	-26.1	-0.9	25	58.1	45.5	-12.6
6	-26.2	-24.4	1.8	58.2	45.5	-12.6

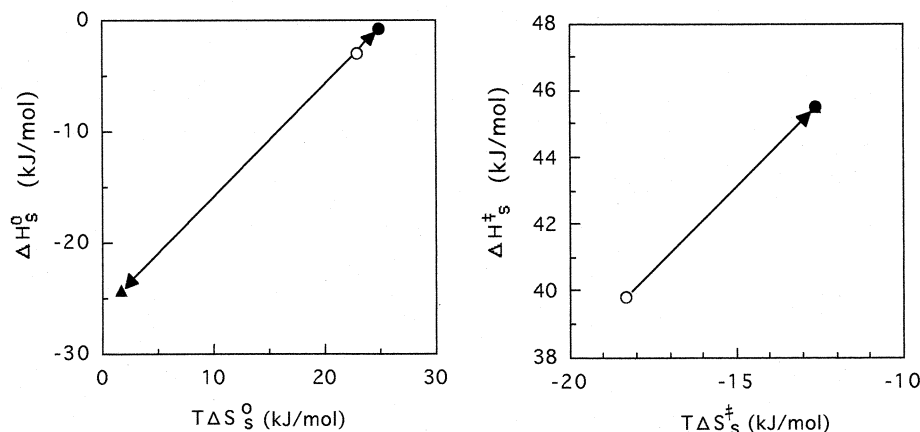


Fig. 3. Enthalpy/Entropy Compensation in the Reaction Using the Irradiated and Non-irradiated G6PDH.

Enthalpy changes were plotted against the entropy changes at 30°C in the reaction using the enzyme irradiated for 3 h (●) and 6 h (▲), and non-irradiated for 3 h (○) and 6 h (△) (left panel). Right panel is the results under the activated or transient state of the reaction. Arrows indicate directions of the changes obtained by the irradiation by  $T_4$ -sheet.

#### Compensation effect on the free energy change of G6PDH reaction by enthalpy and entropy changes

As shown in the left panel of Fig. 3, both enthalpy and entropy changes of the reaction of G6PDH irradiated for 3 h by a  $T_4$ -sheet were slightly increased, but those were decreased by 23 kJ/mol by 6 h of irradiation. Using the 6-h irradiated enzyme, the entropy dependency of free energy change for the G6PDH reaction was changed to an enthalpy one. Both of the activated enthalpy and entropy changes after 3 to 6 h of irradiation were increased by 5 kJ/mol (right panel in Fig. 3). The free energy change for the activated system continued to an enthalpy dependency by the irradiation.

#### Discussion

As shown previously, the promotion energy for the enzyme-substrate (E-S) complex formation by non-irradiated lactate dehydrogenase (LDH), *i.e.*, LDH-lactate and LDH-pyruvate, which select the *pro*-(*R*) hydrogen of NADH, depended on enthalpy. On the other hand, as shown in Table I, the energy for the formation by the non-irradiated G6PDH system depended on entropy. The enthalpy dependency of the LDH-S complex formation persisted using LDH that was irradiated by a ceramics heater, although the enthalpy dependency of the activated or transient state of the LDH reaction was changed to an entropy dependency by the 18 h-irradiation.<sup>4)</sup> The entropy changes ( $T\Delta S_s^0$ ) for LDH-lactate formation, its activated state and the activated state of the LDH-pyruvate system were decreased. On the other hand as shown in Fig. 3, the entropy dependency of the G6PDH-S complex formation persisted for 3 h of irradiation by  $T_4$ -sheet, and changed to an enthalpy dependency after 6 h of irradiation, although the enthalpy dependency of the activated state for G6PDH reaction was hold for 3 to 6 h of irradiation by a  $T_4$ -sheet. These differences in the dependency of promotion energy for the G6PDH and LDH reactions after irradiation suggest that various changes in the steric structure of both enzymes in relation to a steric discrepancy of NAD(P)H chirality as cofactor have been occurred. The discrepan-

cy of the radiation properties of the ceramics used may also affect the enzymic reaction in which the basal energy state of the enzyme, substrate and cofactor will be variously changed by hydration as in the case of xanthine oxidase.<sup>3,4,8,9)</sup>

Irradiation of G6PDH and glucose-6-phosphate by  $T_4$ -sheet for 6 h at 10°C significantly decreased  $\Delta H_s^0$  of the E-S complex formation, although the irradiation increased the entropy change ( $T\Delta S_s^\ddagger$ ) of the activated complex formation. The  $K_m$  was decreased by the irradiation and the  $V_{max}/K_m$  was increased as shown in the right panel of Fig. 2. These results suggest that 6 h of irradiation by a  $T_4$ -sheet affected the structure of G6PDH molecule, accelerated the formation of the E-S complex, and promoted the overall G6PDH reaction. The decrease in  $T\Delta S_s^0$  of the E-S complex formation by 6 h of irradiation with a  $T_4$ -sheet indicates that the enzyme reaction was regulated by hydration of G6PDH and glucose-6-phosphate molecules as shown in the case of xanthine oxidase<sup>3)</sup> and LDH.<sup>4)</sup> As shown in Table I,  $\Delta G_s^0$  for the E-S complex formation and  $\Delta G_s^\ddagger$  for the activated complex one were not affected by the irradiation. Figure 3 shows the compensation effects of enthalpy and entropy changes on  $\Delta G_s^0$  at 30°C by the irradiation. Since a compensation effect is a consequence of some unique property of solvation,<sup>12)</sup> this result also suggest that the G6PDH reaction has been affected by solvation with water of the enzyme and the reaction components caused by the  $T_4$ -sheet irradiation.

As shown in left panel of Fig. 2, the  $K_m$  was increased after 6 h of irradiation by the plate, and the  $V_{max}/K_m$  was decreased. This situation similarly occurred only after 3 h-irradiation by  $T_4$ -sheet (right panel of Fig. 2), but after 6 h-irradiation, the kinetics parameters were changed. Upon longer irradiation than 18 h, they returned to the initial level. On the plate irradiation system for 24 h, however, the  $K_m$  returned to the initial level, but the  $V_{max}/K_m$  was holded in a repressed value (left panel of Fig. 2). This is the first case of different effects caused by a discrepancy of radiators that change the activity of one enzyme.

A subtle energy of FIR has been constantly radiated from and absorbed by living cells including yeast at its surface temperature near 37°C, because living cells are effective radiators. The G6PDH reaction is a major supplier of NADPH for reductive biosynthesis in cells, and the large free energy decrease for the overall reaction ensures that the ratio  $[NADPH]/[NADP^+]$  is kept high within cells.<sup>1)</sup> Therefore, such a regulation by the FIR radiation from ceramics as shown *in vitro* in this paper, *i.e.*, hydration and its stabilization of the enzyme G6PDH, will be apparently operating in cellular systems *in vivo* or *in situ*.

A hydrogen atom at the 1 position of hemiacetal in the D-glucose-6-phosphate molecule is directly transferred to  $NADP^+$  with no collaboration of water molecules, and then NADPH with a *pro-(S)* chirality is formed,<sup>1)</sup> although enzyme, substrate, and  $NADP^+$  in the cytoplasm are originally surrounded by bulk water, and then all these molecules are hydrated. Our previous paper on LDH that was irradiated by a ceramics heater showed that hydration of the enzyme molecule occurred by resonantly absorbing the FIR energy at 10°C and affected the reaction kinetics.<sup>4)</sup> Recently it was indicated that the existence of water molecules around enzyme and substrate promotes the enzymic reaction by van der Waals attraction.<sup>13)</sup> We had found that the second structure of serum albumin was affected by the FIR irradiation.<sup>11)</sup> Therefore, FIR irradiation may regulate the structure of G6PDH molecules through the hydration of the bulk water.

Detailed experiments will be necessary to show

whether the different effects on the chirality of the G6PDH and LDH may depend on emissivity of the ceramics radiator at a radiation wavelength or on the region of radiation wavelength. These experiments will be very difficult now because of the difficulty in production of a radiator that can effectively emit the energy of a restricted wavelength in the FIR region.

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