Short Communication

Biochemical Effects of Folate Radiated with the Mild Energy of Far-Infrared Waves

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Introduction

The electromagnetic waves radiated from a ceramic heater include wavelengths of 3 to 30 μm or more of far-infrared (FIR) regions. The heater has been used as health equipment and by food manufacturers, since the heater could stimulate the blood circulation and the maturation of fermented foods such as miso and soy sauce. These effects suggest that FIR from the heater might have some biochemical functions other than heating objects. However, there is no scientific proof about these effects.

The effect of FIR-radiation on folate was investigated using a kinetic analysis of xanthine oxidase (XO) reaction. XO catalyzes the formation of uric acid and O_2^- from xanthine, O_2 and H_2O , and is inhibited competitively by folate (1). To minimize the heating effect of the ceramics, the sample was cooled during radiation. From the kinetic analysis of the reaction, V_{max} , K_m of xanthine, and K_i of folate were determined. After radiating folate, the pK value was measured. To evaluate the exact effect of FIR radiation on folate, the inhibiting effect of the irradiated folate on the XO reaction was evaluated by a thermodynamic analysis.

Material and Methods

Sample solution put in Nunclon wells 25 cm far from the ceramic heater was cooled to 10 $^{\circ}\text{C}$ during radiating XO (255 mU/ml) and folate (30 μM). There was no evaporation from sample for at least 6 hours.

Enzyme: Xanthine oxidase, xanthine : oxygen oxidoreductase (EC 1.2.3.2)

The energy accepted by the sample was calculated as 0.14 watt/well. The activity of XO was measured by two methods: trapping O_2^- with NBT and measuring absorbance of formazan at 540 nm (NBT method) (2); measuring absorbance of uric acid at 291 nm (UV-291 method) (3). The reaction was initiated by adding 51 μU of XO. The initial velocity was measured by monitoring the absorbance for 5 min at 25 $^{\circ}\text{C}$. The final concentrations of the radiated enzyme and folate in the reaction were 1/500 and about 1/100 of the amount used during radiation, respectively. The system is not radiated directly with FIR during reaction.

Results and Discussion

Effect of radiation time

The activity of the reaction with the enzyme radiated for 3 to 24 hours was 81–85% of that with non-radiated enzyme.

Kinetic analysis of the reaction

Figure 1 shows simulated graphs of a $s/v-s$ and Lineweaver-Burk plottings (4). K_m of xanthine and V_{max} of the reaction with non-radiated enzyme were $3.8 \pm 0.51 \mu\text{M}$ and $11.9 \pm 1.7 \text{ nmol/min/mg}$ (NBT method); $11.5 \pm 1.9 \mu\text{M}$ and $10.3 \pm 0.9 \text{ nmol/min/mg}$ (UV-291 method), respectively. K_m and V_{max} with the radiated enzyme were $1.94 \pm 0.26 \mu\text{M}$ and $10.1 \pm 1.7 \text{ nmol/min/mg}$ (NBT method); $8.3 \pm 0.2 \mu\text{M}$ and $9.4 \pm 0.7 \text{ nmol/min/mg}$ (UV-291 method), respectively. The K_m and V_{max} with the radiated enzyme were 51% and 81% (NBT method), 72% and

Figure 1. Effect of FIR-radiation on xanthine oxidase reaction

91% (UV-291 method), respectively, of those with non-radiated enzyme. The activity remained during at least 2-day storage at 4 °C after stopping the radiation.

Effect of radiation on *pK* value of folate

The *pK*s of N (1) and amide of the radiated folate were 2.18 ± 0.01 and 8.03 ± 0.01 , respectively, whereas those of non-radiated folate were 2.23 ± 0.02 and 8.08 ± 0.02 , respectively. These results show that the *pK*s of folate were slightly changed by the FIR-radiation (significant difference: $p < 0.07$).

Kinetic analysis of the radiated folate

The *K_i* of folate was measured using UV-291 method after various radiating conditions: A, XO (–) and folate (–); B, XO (–) and folate (+); C, XO (+) and folate (–); D, XO (+) and folate (+), where (+) and (–) indicate the radiating and not radiating conditions, respectively. The *K_i* values of folate were: A, 0.99; B, 0.77; C, 0.21; D, 0.21 μM. The *K_i* value of the radiated folate became lower by radiating folate and/or XO compared with the A condition.

Thermodynamic analysis of the reaction from *K_m*

The thermodynamic parameters, changes of free energy, enthalpy and entropy of the reaction were cal-

culated from *K_m* of xanthine: ΔG , -35.0 (-30.5) kJ/mol; ΔH , -48.9 (-27.6) kJ/mol; ΔS , -55.6 (9.6) e. u.; $\Delta G^\ddagger$, 62.7 (62.7) kJ/mol; $\Delta H^\ddagger$, 54.8 (59.8) kJ/mol; $\Delta S^\ddagger$, -26.8 (-10.0) e. u., respectively. Parentheses indicate each value of non-radiated enzyme. The symbol ‡ shows the value of the activated state of the reaction (5). The increase of $-\Delta G$ by the radiating enzyme indicates that the affinity of xanthine to XO increased. The remarkable decrease of entropy by radiating XO suggests that FIR radiation might keep folding the structure of the enzyme and would promote the formation of a XO-xanthine complex rather than the product formation.

Thermodynamic analysis of the reaction from *K_i*

The thermodynamic parameters of the reaction competitively inhibited by folate were calculated from *K_i* of folate: at A, B, C and D conditions as described above, ΔG were -33.8 , -33.9 , -35.7 , -37.7 kJ/mol; ΔH were -165 , -292 , -54 , -152 kJ/mol; ΔS were -441 , -867 , -61 , -383 e. u., respectively. A large negative value of entropy in the radiated folate system indicates that a remarkable electrostriction of water surrounding folate might be generated. In the radiated enzyme system, however, a large increase of entropy was observed. This indicates that approaching of folate and xanthine to the radiated enzyme might loosen the structure of water surrounding enzyme. In the radiated both enzyme and folate system, the large negative values of ΔH and ΔS were obtained. This indicates that electrostriction of water surrounding both folate and XO might occur and formation of the complex of XO-xanthine-folate could become easy. This reflects the large value of $-\Delta G$ in this system.

References

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