Non-Thermal Effects of a Ceramics Irradiation on the Dissociation State of Lysine

Masahiro Kohashi,* Hiromi Naka, Ken-ichi Tanaka, and Tatsuo Watanabe

School of Food and Nutritional Sciences, University of Shizuoka, 52-1, Yada, Shizuoka 422, Japan

Received July 20, 1994

The pH of a lysine aqueous solution was quickly lowered by about one unit when it was irradiated with a ceramics radiator with cooling. The maximum point of difference in pH of the irradiated lysine from that of the non-irradiated one occurred near the first equivalent point of the ω-carboxyl group. The molar extinction of lysine also decreased with the irradiation time. Dissociation constants of ω-carboxyl, and ω- and ω-amino groups significantly decreased. The entropy dependency of dissociation of the ω-carboxyl group was changed to an enthalpy one by the irradiation, while the enthalpy dependency of the ω- and ω-amino groups tended to be changed to an entropy one by the irradiation. The mechanism of promotion of the dissociation by far-infrared waves irradiated from the ceramics has been estimated by a thermodynamic analysis as a hydration effect of the waves on the dissociable groups.

The electromagnetic waves in the far-infrared (FIR) region longer than 4 μm, which are irradiated from a ceramics heater at its surface temperature below 400°C, are resonantly absorbed into almost all organic molecules having a number of vibrations in the FIR region. The Wien's displacement law about a radiation wavelength as a function of absolute temperature of a black body indicates that almost all internal biochemical reactions are influenced by the FIR energy that is radiated under temperature from a great internal ceramics-like radiator such as bones. Water molecules also have specific numbers of vibration which are caused by their rotation, liberation, and translation in the FIR region. It is natural that water molecules overwhelmingly exceed in quantity the solute molecules in aqueous media such as biological systems. Especially, the water content is above 60% of human body weight. Therefore, the FIR energy or the vibration absorbed resonantly into water molecules would necessarily affect the structure of the solute molecules. The FIR energy, however, is at the most 0.1 eV, which cannot cause an electron transfer from water to solute molecules and vice versa. We previously proposed a weak radiation system that can simulate an internal environment in living bodies, and showed from enzyme kinetics and its molecular thermodynamic analyses that the structure of irradiated substrate and inhibitor, and the active center or a regulatory site of the irradiated xanthine oxidase were greatly affected by a stable formation of solvation shells oriented around their molecules.

A lysine molecule has a long hydrocarbon group of (CH₂)₆CH and three dissociable groups at both sides of the molecule. The ω-NH₂ group exists in an extremely small quantity in aqueous solution at a pH below 10. The group, however, is potentially reactive, and is easily acylated, and participates in many protein cross-linkage reactions. Lysine also lies in a cleft containing the active site of many enzymes such as ribonuclease and vitamin B₆ enzymes mediating amino acid metabolism. Therefore, a procedure that locally changes the dissociation state of lysine molecule will greatly affect the enzyme activities and the cross-linkage reactions.

The dissociation state of an amino acid generally depends on the pH of the solvent, the stability of its ionic form in the solvent, and largely on the solvent temperature. When amino acid has been titrated with alkali in the presence of formaldehyde, dissociation of the ω-NH₂ group is promoted, and then a new equilibrium is built up between the ω-NH₂ and its hydroxymethylated one. Therefore, more alkali will be consumed for titrating the liberated H⁺, then the pK value of the amino group goes down as it appears. We previously showed that the FIR irradiation varied the pK values of twenty natural amino acids in aqueous solution. The mechanism of the irradiation causing such a variety of the pK values has not been discovered.

In this paper we studied by a thermodynamic analysis the mechanism of a decrease in three pK values of the lysine molecules when the molecule was irradiated with the FIR waves in an aqueous solution cooled to 10°C.

Materials and Methods

Lysine in free base, distilled water for HPLC, and other chemicals were purchased from Wako Pure Chemicals Ltd., Osaka.

Two milliliters of 25 mM lysine aqueous solution per well of a Nunclon 6-well (30 mm i.d., Nunc Ltd., Denmark) was irradiated in a water bath cooled to 10°C with the ceramics heater as shown in our previous paper. After the irradiation, the weight of the sample was adjusted to the initial weight with distilled water, about pH 5.5, which was prepared in our laboratory by ion-exchange, active-carbon filtration, and distillation, and stored in a plastic bottle. The non-irradiated lysine solution was completely covered with aluminum foil, and incubated in the water bath.

Titration was done in a 50-ml Pyrex bottle (40 x 75 mm), closed with a silicone rubber stopper, which was bored with four holes: one for the Toa GST 5312S glass electrode (Toa Electric, Ltd., Tokyo), one for a nitrogen-delivering capillary, one to admit the tip of a Pipetman P-1000 (Gilson Co., Ltd.) for alkali or acid, and one for an exhaust pit. Nitrogen gas was introduced in a slow stream during magnetic stirring. A Toa HM60S pH meter was used for the pH measurement. The irradiated and non-irradiated lysine solutions (25 mm each) were diluted 5 times with the distilled water, pH 5.5. The aqueous solution (45 ml) of 5 mm lysine was titrated at 25, 30, and 35°C with 0.1 N KOH or HCl. The pH was read

* To whom correspondence should be addressed.

Abbreviations: FIR, far-infrared.
between each addition of 250 μl of alkali or acid using a Pipetman. After titration with 0.1 n KOH or HCl, pK1, pK2, and pK3 values of α-carboxyl, ϵ- and ε- amino groups, respectively were calculated from a modified Henderson-Hasselbalch equation. The resulting pK values having a spread of more than ± 0.05 are not valid, and were discarded. An average of four or more points was taken.

In the experiment measuring a change in pH of lysine solution and the extinction, 5 nm lysine solution was prepared by dissolving in the distilled water prepared for HPLC, pH 6.7, purchased from Wako Pure Chemicals. After irradiation, the weight of the sample was adjusted with the distilled water to the initial weight. The pH of the solution was directly read at 25°C using a Toa flow analysis reference unit FAR-201A, which could stably measure pH of the distilled water even in a small quantity. Ultraviolet spectra of the solution was measured with a Jasco Ubest V-560 UV/VIS spectrophotometer (Japan Spectroscopic Co. Ltd., Tokyo).

Results and Discussion

Change in pH of the irradiated lysine

As shown in Fig. 1, the initial pH 9.752 ± 0.002 of the lysine solution that was dissolved in the distilled water prepared for HPLC quickly fell during 3 h of irradiation. A longer irradiation than 3 h slowly lowered the pH. After 24 h of irradiation, the pH was shifted to 8.426 ± 0.006. The pH of the non-irradiated lysine, however, changed little even after being incubated for 24 h at 10°C. This suggests that dissociation of the lysine molecule has been promoted by the FIR irradiation.

UV absorption spectra of the irradiated lysine

A change in pH of the solvent causes a shift in UV absorption of the chromophore in amino acids, and an increase or decrease in the molar extinction. Although lysine has no chromophores with an absorption maximum near the UV region, the molar extinction in a longer wave length region than 200 nm of the irradiated lysine decreased (Fig. 2). This indicates that the energy level in the excited equilibrium state of the irradiated molecule is significantly higher than the non-irradiated one, which situation is similar to a change in a n→π* transition. This proves that the FIR irradiation has caused a change in the molecular structure of a ground state of the lysine molecule in aqueous solution. Lysine has no λmax near the ultraviolet region, and the spectra show only a skirt. The UV absorption spectra of lysine may be blue-shifted with the irradiation time, because the FIR energy is not strong enough to destroy the structure of lysine. The molar extinctions at 200 nm were 802, 574, 490, 496, and 487 after 0, 3, 6, 16, and 24 h of irradiation, respectively. This indicates they decreased by about 50% of the non-irradiated lysine after irradiation for 6 to 24 h.

A blue-shifted spectrum of ionic aromatic amino acids is caused by hydration of the dissociable species. Therefore, blue-shifted spectra of the irradiated lysine as shown in Fig. 2 suggest that structure of the irradiated lysine molecule has been affected by some hydration forces in the aqueous medium.

The water molecule has potential abilities for hydrogen-bonding and Coulombic interactions with ionic solute molecules, which are caused by amphiprotic properties of the water molecule. Three groups of α-COO anion, α-NH2 cation, and ε-NH2 cation of the lysine molecule, which existed at pHs below 10, were solvated in aqueous solution by these forces. The water molecules also make a hydration shell by Lifshitz–van der Waals interactions around apolar molecules such as the pentyl moiety in this study. Moreover, these lysine molecules are completely surrounded by a hydration shell of the bulk water molecules forming a cluster through hydrogen bonding. The possibility of changes in effective dielectric constant and reflectance of the solvent adjacent the irradiated lysine

![Fig. 1. Effects of Irradiation Time on pH of Lysine Solution.](image1.png)

Five nm lysine dissolved in the distilled water prepared for HPLC was irradiated at 10°C. After the irradiation, weight of the solution was adjusted to the initial weight with distilled water. The pH of the solution was measured at 25°C by a flow analysis reference unit as shown in the text.

![Fig. 2. Effects of Irradiation on Spectra of Lysine.](image2.png)

A 5 nm lysine aqueous solution was irradiated at 10°C for 0 (--), 3 (--), 6 (--), 16 (--), and 24 h (--). The spectra of the solution were measured at 25°C.
molecule could not be discarded.\textsuperscript{1,2,15)}

\textbf{Titrations of the irradiated lysine with KOH and HCl}

The titration curve of lysine with KOH and HCl significantly shifted to a lower pHi after 3 h of irradiation as shown in Fig. 3. The difference in pHi of the irradiated lysine from that of the non-irradiated one was about 0.4. Difference in pHi of the species over the \( pK_1 \), \( pK_2 \), and \( pK_3 \) range between the irradiated and non-irradiated lysine was gradually increased. At the first equivalent point of the \( x \)-carboxyl group, proton bound 0.9 g ion per mol of the non-irradiated lysine. A steepening titration curve near the point changed to a broadening one after irradiation. The maximum difference in pHi between the irradiated and non-irradiated lysine was observed in the ionic form which bound 0.75 g ion of \( H^+ \) per mol of lysine. These results suggest that the FIR irradiation affects the molecular structure that exists near a neutral point.

\textbf{Dissociation constants of the irradiated lysine}

As shown in Table I, the dissociation constants, \( pK_1 \), \( pK_2 \), and \( pK_3 \) of the irradiated lysine significantly decreased from those of the non-irradiated one. The major ionic form of lysine at a pHi below 10 is shown as \( H_2^+ \), which bears two positive charges on the \( x \) - and \( \epsilon \) -amino groups and one negative charge on the \( x \)-carboxyl group. Protons removed from these groups of the irradiated lysine were 1.2, 3.2, and 3.2 times, respectively that of the non-irradiated one as it appears. This result suggests that the ionizable groups of lysine have been modified to a more dissociable form by irradiation.

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|}
\hline
 & Non-irradiated & Irradiated \\
\hline
\( pK_1 \) & 2.62 \( \pm \) 0.01 & 2.56 \( \pm \) 0.01 \\
\( pK_2 \) & 8.81 \( \pm \) 0.01 & 8.31 \( \pm \) 0.02 \\
\( pK_3 \) & 10.80 \( \pm \) 0.03 & 10.30 \( \pm \) 0.02 \\
\hline
\end{tabular}
\caption{Effects of Far-infrared Irradiation on Dissociation Constants of Lysine}
\end{table}

\textbf{Thermodynamics of dissociation constants of the irradiated lysine}

In the experiment for thermodynamics, 3 h of irradiation was selected because of its dynamic effect.\textsuperscript{20} The van't Hoff plots of dissociation constants of lysine against temperature are shown in Fig. 4. Three \( pK \) values decreased inversely proportional to increasing temperature from 25 to 35°C. The \( pK \) values of the irradiated lysine titrated over the temperature range decreased more than those of the non-irradiated one. This indicates that the dissociation states of \( x \)-carboxyl, \( \epsilon \) - and \( x \)-amino groups of the irradiated lysine have been promoted more than those of the non-irradiated one over the temperature range of this experiment.

Enthalpy change (\( \Delta H \)) of \( pK \) values calculated from the van't Hoff plots, the free energy change (\( \Delta G \)), and the entropy change (\( \Delta S \)) at 30°C are shown in Table II. \( \Delta G = -2.518 \, pK_1 \, kJ \cdot mol^{-1} \) at 303 K. Therefore, the difference in \( \Delta G \) corresponding to a change of one unit in \( pK_1 \) is \(-5.8 \, kJ \cdot mol^{-1}\), which is similar to the literature.\textsuperscript{18} The less negative \( \Delta G \), the weaker the binding, or the greater the dissociation. Since \( \Delta G \) is negative, the dissociation of lysine is an exergonic reaction. Although the negative \( \Delta G \) values for three dissociation constants of the irradiated lysine is exergonic reaction. Although the negative \( \Delta G \) values for three dissociation constants of the irradiated lysine tended to decrease slightly from those of the non-irradiated one, it is uncertain whether \( \Delta G \) for their dissociation depends on energetic or entropic terms.
Table II. Effects of Far-infrared Irradiation on Thermodynamic Constants Related to pK Values of Lysine

| pK, Control | 15.2 | -4.5 | 35 | 10.7 | 236 |
| pK, Control | 14.8 | -11.5 | 11 | 3.3 | 601 |
| pK, Control | 51.1 | -48.9 | 7 | 2.2 | 2553 |
| pK, Control | -48.2 | 26.2 | 73 | 22.0 | 1372 |
| pK, Control | -62.7 | -36.3 | 87 | 26.4 | 1899 |
| pK, Control | -59.8 | -19.5 | 133 | 40.3 | 1020 |

Thermodynamic constants used are calculated from the following equations at 303 K: \( \Delta G = -2.303 RT \ln K_a \), \( \Delta H = -2.303 R \ln K_a / (1/T) \), \( R = 8.31 \text{ J mol}^{-1} \text{ K}^{-1} \), and \( \Delta S = (\Delta H - \Delta G) / T \), where \( K_a \) is the dissociation constant of lysine. FIR indicates the lysine solution irradiated with far-infrared waves.

![Graph](image)

Fig. 5. Enthalpy/Entropy Compensation in the Dissociation Constants of the Irradiated and Non-irradiated Lysine.

Enthalpy of dissociation groups (\( \Delta H \)) were plotted against the entropy (\( \Delta S \)) at 303K. Key: (○) non-irradiated lysine; (●) irradiated lysine. Arrows indicate directions of the changes obtained by irradiation.

Since the enthalpy change is negative, the dissociation of lysine has been energetically promoted. The negative enthalpy for \( pK_1 \) of the irradiated lysine significantly increased, while the negative enthalpy for the \( pK_2 \) and \( pK_3 \) decreased more than those of the non-irradiated one. As shown in Table II, \( \Delta S \) was positive. The entropy change (\( \Delta S \)) for \( pK_1 \) of the irradiated lysine decreased, while the entropy change for the \( pK_2 \) and \( pK_3 \) increased more significantly than those of the non-irradiated one.

By inspecting compensation of entropy and enthalpy for the situation of small difference in \( \Delta G \) for dissociable groups of the irradiated and non-irradiated lysine molecules, it will be seen whether \( \Delta G \) of the dissociation equilibrium depends on the enthalpy or entropy terms. Compensation of enthalpy and entropy for \( pK \) values of the 3-h irradiated lysine is shown in Fig. 5. \( \Delta G \) for the \( \alpha \)-carboxyl group of lysine depended on the entropy term, but those for \( \epsilon \) and \( \alpha \)-amino groups depended on the enthalpy term. The entropy dependency for \( pK_1 \) of lysine was changed to an enthalpy one by the irradiation. However, the enthalpy dependency of \( pK_2 \) was significantly decreased by the irradiation, while the enthalpy dependency of \( pK_3 \) was changed to an entropy one by the irradiation. Since compensation effects are also a consequence of some unique property of solvation, these results suggest that the dissociation state of the irradiated lysine molecule has been affected by solvation.

The enthalpy term will govern dissociation of the \( \epsilon \)-NH\(_3\) ion in consequence of the strong repulsion of the positive charges. Promotion of dissociation of these ionic forms by the FIR irradiation and the formation of a hydrogen bonding around them have caused to increase in entropy dependency.

The implication of these studies on the non-thermal effects of FIR is that the hydration shells or hydrogen bondings oriented around the irradiated lysine molecule were stabilized when the lysine aqueous solution absorbed resonantly with a number of vibrations shorter than 120 cm\(^{-1}\) or a longer wavelength above 8.5 \( \mu \)m of the FIR region, and especially that the FIR irradiation lowered the pH of the lysine aqueous solution by promoting the dissociation of the molecule. Such a manifestation of hydration caused by the FIR irradiation is apparently operating in the irradiated lysine as shown by changes in the free energy, the enthalpy, and the entropy for three dissociation groups of lysine, and compensation of their entropy and enthalpy. Although the dissociation near \( pK_1 \) of the irradiation lysine molecule accompanied a decrease in entropy, those near \( pK_2 \) and \( pK_3 \) made an increase in entropy.

It is noticeable that not much energy of the FIR waves significantly affected the structure of lysine and its dissociation state in aqueous medium. The situation may occur in internal biochemical reactions, because the living body is constantly being irradiated with the FIR waves at normal temperatures, and the waves are similar to those irradiated from ceramics used in this study. Subsequent studies must be required for whether such a local change in dissociation of the irradiated lysine affects the activity of any enzymes having a lysine group in the cleft containing the active site.

References