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Chemical evolution of amino acid induced by soft X-ray with Synchrotron Radiation

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Abstract
Soft X-ray induced chemical evolution from glycine (Gly) to glycyl-glycine (glycine dimer or Gly-Gly) was studied for sublimated films of Gly. Values of quantum yield of Gly dimer formation were determined by HPLC technique to be , $(1.2\pm0.2)\times10^{-1}$ for 400 eV, $(4.6\pm0.5)\times10^{-2}$ for 407 eV and $(3.2\pm1.5)\times10^{-2}$ for 860 eV irradiation, respectively. We measured X-ray absorption spectra of irradiated Gly films at near edge region of nitrogen K-edge, as a function of X-ray dose. Formation of Gly-Gly was confirmed by the appearance and growth of 402 eV peak which is a fingerprint of the peptide bond. The important role of surface to chemical evolution is discussed.

Key words: Chemical evolution, Amino acid, Peptide synthesis, VUV and soft X-ray irradiation Quantum efficiency

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1. Introduction

Amino acids are known as key molecules in chemical evolution from small organic molecules to biomolecules. Recently amino acids were found from some meteorites [1]. This means that chemical evolution was able to occur in universe and achieved up to amino acids. It is of interest to study whether the next evolution from amino acid to peptide occurs in universe. Because amino acids mainly absorb electromagnetic radiation of which wavelength λ is shorter than 200 nm [2], vacuum ultraviolet light (VUV) or X-ray are possible candidate of energy sources responsible for chemical evolution in universe.

Pioneer works by Khoroshilova et al. [3] and Simakov et al. [4] reported that 146 nm VUV light induced peptide formation in solid amino acids. The reason why they used solid phase is that in universe amino acids are thought to solidify on the surface of meteorite, comet, and space dust. Tanaka et al. [5] studied the chemical evolution from alanine (Ala) to alanyl-alanine (Ala-Ala) induced by VUV light ($h\nu$ = 6, 6.8 and 14.2 eV). They reported the values of quantum efficiencies to be $(5.7\pm0.5)\times10^{-5}$, $(1.3\pm0.1)\times10^{-3}$, and $(2.4\pm1.0)\times10^{-4}$, for 6, 6.8 and 14.2 eV light.

Absorption spectra of amino acids are important basic data for the study of chemical evolution. Owing to a rapid advancement of synchrotron radiation, a wealth of absorption spectra of amino acids is available. Tanaka et al. [6] reported X-ray absorption near edge structure (XANES) of evaporated films of amino acids in the oxygen K edge region and examined obtained spectra with DV-Xα calculations. Boese at al. [7] studied XANES spectra of amino acid films near the carbon K edge energy region using a scanning transmission X-ray microscope (STXM). Solid films were carefully prepared from trifluoroethylene solution. Kaznacheyev et al. [8] also measured carbon K XANES spectra of all 20 amino acids which compose body of life-form and compared those with theoretical calculation. They suggested the concept of additivity, namely spectrum of an amino acid can be reproduced with superimpose spectral component of constituent molecular parts. Gordon et al. [9] reported results obtained from inner-shell electron energy loss spectroscopy (ISEELS) and XANES spectroscopy using STXM technique. They expanded energy regions to nitrogen K edge and oxygen K edge region. Recently Cooper et al. [10] studied comparison of XANES spectra of Gly and Gly-Gly.

Based on these spectral data, we studied chemical evolution from Gly to Gly-Gly in evaporated films with irradiation of soft X-ray photons emitted from an undulator. Produced Gly-Gly molecules were detected by XANES spectroscopy of irradiated samples.

2. Experimental procedure

Gly powder (purity > 99%, Wako Chemicals Co.) was used without further purification. Thin films of Gly were prepared with evaporation technique [11]. Evaporated glycine molecules at about 350 K were sublimated in vacuum on Si (111) (Nilaco. Co.) substrates covered with 30 nm Au. Thickness of Gly films was estimated to be about 1 to 2 μm by quartz oscillator. No thermal decomposition of sample during evaporation was confirmed by high performance liquid chromatography (HPLC) analysis of the sublimated films.

All samples were prepared *ex situ*, and installed in an ultra high vacuum chamber (pressure was about 10^{-6} Pa) for XANES measurement and irradiation.

Measurement of XANES spectra and irradiation of soft X-ray photons were carried out at the soft X-ray undulator beamline BL23SU [12] of SPring-8 in Japan. Energy calibration was carried out by XPS measurement of Au. Energy resolution E/ΔE was about 10^4 at nitrogen K-edge energy region when we used the monochromator with the narrow exit slit width (about 0.5 mm).

Absorption spectra were obtained with drain current measurement, in which absorption A was determined from sample drain current I divided by incident photon flux I_0 monitored as drain current on a post focusing mirror ($A = I / I_0$). Absorption spectra were measured at near nitrogen K-edge and oxygen K-edge energy region.

When we irradiated samples, we opened the exit slit up to 2 mm. Photon flux was determined with a calibrated photodiode to be about 10^{11} photons s^{-1} for

the spot size of about 1.5 x 0.6 mm^2. In order to achieve the uniform irradiation for the sample (area was about 8 x 8 mm^2), sample was moved step by step during irradiation. Total area of irradiated part was estimated to be about 3 x 3 mm. Photon energy of irradiation was determined to tune at characteristic resonance in absorption spectra at nitrogen K-edge edge (400eV and 407eV). Off-resonant photon energy of 860 eV was also used for irradiation.

After irradiation, XANES spectra of the nitrogen K edge were measured and formation of Gly-Gly was confirmed by the appearance of 402 eV peak which is known to be the fingerprint transition peak associated with peptide bond [5, 8, 9]. Total number N_0 of irradiated photons in this experiment was determined to be about 10^{14} to 10^{15} photons for each sample. Irradiated samples were taken out from the vacuum chamber after irradiation and dissolved in distilled water. HPLC analysis was carried out to examine products. HPLC was already calibrated to determine absolute numbers of produced Gly-Gly molecules.

3. Result and Discussion

Fig. 1 shows XANES spectra of Gly films irradiated at 860eV with various irradiation times. Numbers near the each spectrum shows the irradiation time, respectively. As seen from the figure, a new peak at 402eV was found to grow as the increase with irradiation time. This peak is known to originate from peptide bond [5, 8, 9]. HPLC analysis for the same sample confirmed that Gly-Gly molecules were produced by 860eV irradiation. Glycyl-glycyl-glycine or higher glycine peptide were not detected. Based on this analysis, we conclude that the peak at 402eV was due to Gly-Gly. It should be noted that another peak was observed at 398 eV. However, careful examination showed that the area of this peak was found to be independent from irradiation. Thus we eliminate this peak from analysis of results.

In Fig.2, the area of the peak at 402 eV was plotted as a function of total number of absorbed photons ($h\nu$ = 860eV). Absorbed photon number was estimated on the basis of mass absorption coefficient of Gly at 860 eV [13] and the thickness of our Gly films (about 10^{-6} m).

As seen from the Fig. 1, 402 eV peak seems to have background baseline. To eliminate this effect, we drew a straight line between about 402 eV and about 399 eV, and integrate the area lower than experimental spectrum curve and above the straight line. Changing the straight line to reasonable spline function curves, we estimated the error bars shown in Fig. 2. In this procedure, vertical axis of each curves in Fig. 1 were translated into absolute values of mass absorption coefficient on the basis of summation of atomic absorption coefficient around at 395 eV and 420 eV[13].

Fig. 2 shows clearly that area of 402eV peak increased in proportional to irradiated photon number. Similar increase of 402 eV peak area was also observed for irradiation of 400eV and 407eV photons.

Using the HPLC analysis, we determined absolute number of produced Gly-Gly in irradiated samples. For each irradiated photon energy, number of produced Gly-Gly molecules was plotted as a function of total irradiation photon numbers.

Those plot were similar with Fig. 2. Namely, number of produced Gly-Gly molecules was found to be in proportional the number of total photon numbers.

From the viewpoint of radiation chemistry or photochemistry, we supposed the first order reaction actually in which reaction formula is; $h\nu$ + Gly +Gly $\rightarrow$ Gly* + Gly $\rightarrow$ Gly-Gly. This formula gives typically exponential decay in [Gly] as a function of irradiated photon number when the total photon number is comparable to the number of reactant (Gly in this case). In this case, numbers of Gly-Gly should increase at the first stage, and saturate, and finally decay according to the increase irradiation. However, even in the case of largest irradiation, the absorbed photons number 1.6×10^{15} was sufficiently smaller than Gly number 1.2×10^{17} in irradiated sample volume of $3 \times 3 \times 10^{-3}$ mm^3. Thus number of Gly in the system was reasonably assumed to be constant during irradiation. This means the [Gly-Gly] increase in proportional to the irradiated photon number. Based on this assumption, we determined the quantum efficiency ϕ of Gly-Gly formation to be (number of produced Gly-Gly molecules) / (number of absorbed photons). This assumption may be verified as seen from the Fig. 2.

According to this definition of ϕ, we determined values of ϕ from the HPLC analysis to be; $(1.2\pm0.2) \times 10^{-1}$ for 400eV, $(4.6\pm0.5) \times 10^{-2}$ for 407 eV, and $(3.2\pm1.5) \times 10^{-2}$ for 860eV photons. The origin of large error in the case of 860 eV is not clear now. Although this error, it is reasonable to roughly conclude that the magnitude of ϕ in soft X-ray region is around these values.

It is noteworthy that these values are larger than the value of ϕ in the case of 8eV VUV light irradiation, $(6.0\pm0.4) \times 10^{-3}$. We think that the reason for this larger value was due to the contribution of secondary electrons with relatively high energy.

Based on this result, we conclude that photons with the wide range of energies from VUV to soft X-ray can contribute to the chemical evolution of amino acids.

In Fig.3 we tried to reproduce the obtained XANES spectrum of irradiated Gly film by 860 eV photons. In the figure, the curve EXP is the spectrum after 240min irradiation shown in Fig.1. Vertical axis of Fig.3 was translated to the mass absorption coefficient estimated from the summation of the atomic absorption coefficient at about 420eV [13]. Assuming that the 402eV peak was due to only Gly-Gly, we determined the Gly-Gly component as the curve Gly-Gly in Fig.3. The peak height of Gly-Gly at 407 eV was about 31% of the curve Exp. The remaining 69% was attributed to Gly (curve Gly). Superposition of the curves Gly-Gly and Gly resulted in the curve CALC. As seen from the Fig.3, the curve EXP was well reproduced by the curve Gly + Gly-Gly, namely CALC.

Because XANES spectrum is surface sensitive (about 1nm depth), the result in Fig.3 shows that the surface concentration of Gly-Gly was 31% after the irradiation of 1.6×10^{15} photons at 860eV. This result suggests that the surface of solid amino acids may play an important role in chemical evolution in universe.

4. Conclusion

Because Gly-Gly was found to produce by irradiation of photons VUV light to soft X-ray, we conclude that a wide rage of photon energy can contribute to chemical evolution. We tentatively conclude that the surface of solid amino acids on space dust or meteorites can make an important role to the chemical evolution in space environment.

5. Acknowledgement

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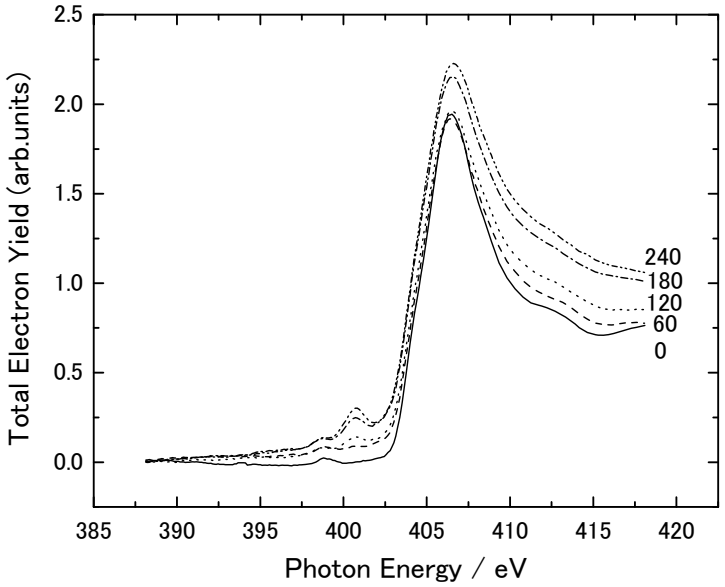

Fig.1. XANES spectra of glycine films irradiated at 860eV. The numbers 0 – 240 show irradiation time in unit of minute.

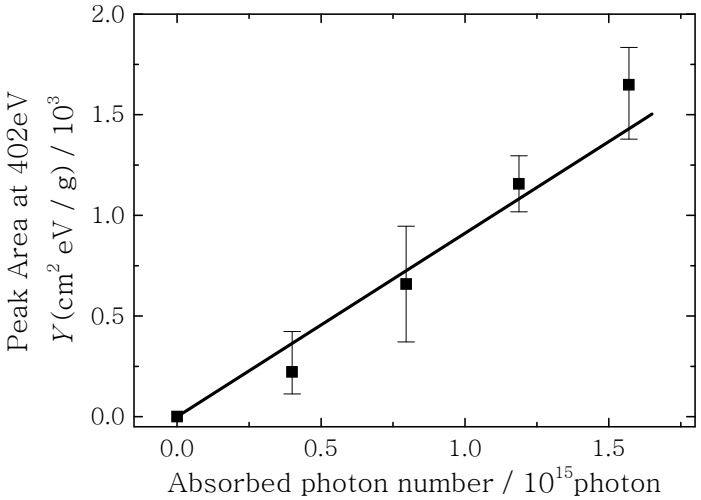

Fig.2 The area of the peak at 402eV as a function of absorbed photon number.

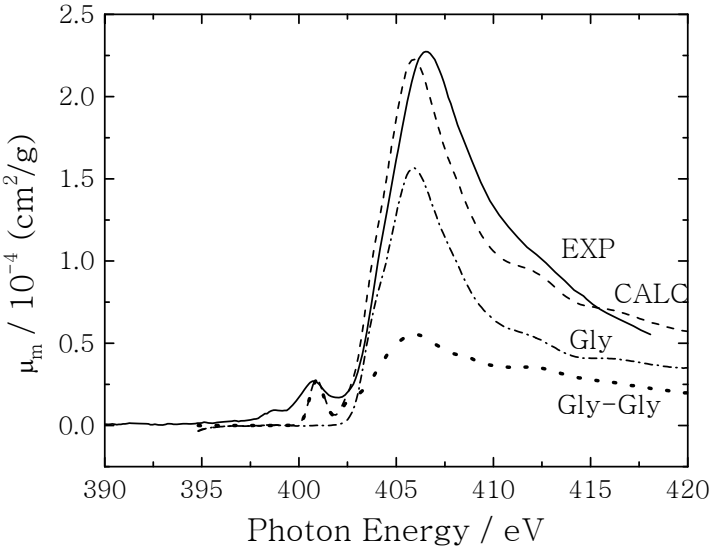

Fig.3 Reconstruction of the spectrum of glycine film irradiated 240 minutes ($1.6x10^{15}$ photons) at 860 eV. EXP: Observed spectrum. CALC: Reconstructed spectrum (see text in detail).