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FRAGMENTATION OF AMINO ACIDS DUE TO INNER SHELL X RAY ABSORPTION IN SULFUR ATOMS

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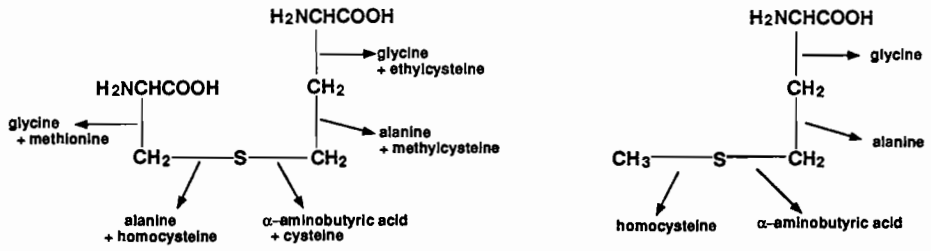
ABSTRACT

In order to obtain evidence for damage specific to the Auger processes in biological molecules, sulfur containing amino acids (cystathionine and methionine) were irradiated with monochromatic synchrotron soft X rays around the K shell absorption edge of sulfur and phosphorus, and fragmentation products were analyzed with high performance liquid chromatography (HPLC). It was demonstrated that the fragmentation patten of methionine depends upon the X ray energy. Alanine was not observed among the products following irradiation of methionine. This may indicate that alanine observed in the products following irradiation of cystathionine is a result of S-C bond cleavage, not C-C bond cleavage in the molecule. Experiments with a mixed sample of cystathionine and phosphate salts showed that Auger electrons emitted by phosphorus atoms do not affect the degradation pattern of the amino acid.

INTRODUCTION

The high lethality of Auger cascades has attracted much attention not only from the therapeutic point of view, but also from the basic biophysical point. The Auger effect induces radiological effects via two mechanisms. One is through the many low-energy Auger electrons ejected from the atom. These electrons produce many ionizations in close vicinity of the atom. The other is the multiply-ionized atom itself as a result of release of the electrons, which may lead to fragmentation of the molecule. Biological effects caused by the Auger effect have been studied using certain types of radionuclides, *i.e.* ^{125}I (1) or ^{77}Br (2). Recently, with the advance of synchrotron radiation technology, external irradiation with monochromatic X rays became available to efficiently induce inner shell ionization of specific atoms constituting the cell. One typical such atom is phosphorus which constitutes the backbone of DNA in the form of phosphate. On the basis of exposure, monochromatic soft X rays which are absorbed efficiently by K shell electrons of phosphorus showed higher lethality on yeast cells than soft X rays which can not be absorbed by the K shell electrons of phosphorus (3). This biological enhancement can not be interpreted by the increase of absorbed dose in the cell nucleus, indicating that the Auger effect may produce specific types of molecular damage. In order to get evidence for molecular damage specific to the Auger effect, we chose an amino acid having a sulfur atom in the backbone and surveyed the products when irradiated with monochromatic soft X rays at energies which correspond to the K shell absorption edge of sulfur. It was demonstrated that the degradation pattern of the amino acid depends upon the site of photoabsorption in the molecule (4). In this paper, we will describe further studies on the degradation mechanisms of the amino acid using methionine and a mixture of cystathionine and phosphate salt as samples. Methionine has a part of cystathionine including a sulfur atom covalently binding a methyl group (see Fig. 1), and hence the number of amino acids expected is less than in the case of cystathionine. The latter sample was irradiated with phosphorus K edge X rays in order to estimate the effects of Auger electrons generated outside of the molecule. These results indicate that bond breakage can occur efficiently at the site of photoabsorption but the damage is not caused by Auger electrons. Multiply ionized atoms are considered to be responsible for the molecular damage specific to the Auger effect in sulfur containing amino acids.



(a) Cystathionine (b) Methionine

FIG. 1. Molecular formula of the amino acids irradiated with monochromatic soft X rays. Arrows indicate the relation between the expected fragments (amino acids) and the cleaved bond.

MATERIALS AND METHODS

Methods of sample preparation, irradiation of monochromatic soft X rays and assay of degradation products, are the same as those described in our previous report (4). Briefly, sample molecules dissolved in double deionized ultrapure water were put and dried in an area of 4 mm(H) X 2 mm(V) on small aluminum plates. The sample weight was 50 μg. Mixed samples were prepared with Na_2HPO_4 and KH_2PO_4. Cystathionine was dissolved in the phosphate buffer instead of pure water and dried on the sample plates. The ratio of sulfur atoms to phosphorus in these samples was about one. These plates were set on a sample holder in a vacuum irradiation chamber. Synchrotron X rays, monochromatized with an InSb crystal, were used to irradiate the sample at the beamline 11B at the Photon Factory, National Laboratory for High Energy Physics, Tsukuba, Japan. The energies of monochromatic soft X rays used for irradiation were chosen after measuring the absorption spectra of the sample molecule. X ray energies for the irradiation of methionine were 2472 and 2466 eV, the former of which corresponds to the K shell resonance absorption of sulfur (4). Those for the mixed sample were 2153 and 2146 eV, which correspond to the resonance absorption of phosphorus and below the absorption edge of phosphorus, respectively (3). Exposure was determined by compensating the decrease of exposure in the sample due to the absorption of X ray by the sample

molecules. Irradiated samples were redissolved in water and injected into a high performance liquid chromatography (HPLC) system to analyze the products in combination with the OPA(o-phthal aldehyde) method to raise the detection sensitivity for amino acids (5). Quantities of the products were obtained from the peak areas on the HPLC chromatograms using the conversion factors previously determined. Production efficiencies were determined from the slope of the exposure-peak area relationship.

RESULTS

Products from Methionine

On the HPLC chromatograms, several peaks of the products were observed along with the remaining methionine, and the number of peaks was less than that in cystathionine (Fig. 2). The ratio of the peak height between α-aminobutyric acid and glycine was dependent upon the irradiation energy. Among the products, glycine and α-aminobutyric acid were identified. These same products were identified in the case of cystathionine. The peak areas were measured by an integrator. Peaks at the expected retention time of alanine could not be measured quantitatively at both irradiation energies, while the peak for alanine was observed in the irradiated cystathionine (4). Homocysteine can not be detected due to the HPLC condition adopted here. These results indicate that, in methionine, the C-C bond cleavage to produce alanine hardly occurs by the soft X ray irradiation irrespective of the photoabsorption site.

The production efficiencies of glycine and α-aminobutyric acid are obtained and listed in Table I. The production efficiency (41 pmol/MR) of α-aminobutyric acid per exposure at 2472 eV was 3.7 times the value (11 pmol/MR) at 2466 eV, while the production efficiency (1.9 pmol/MR) of glycine per exposure at 2472 eV was 2.5 times the value (0.77 pmol/MR) at 2466 eV. These efficiencies were close to the values obtained from cystathionine irradiated around the sulfur K edge (Table I). Energy dependence of the production efficiency of glycine from methionine was about twice that from cystathionine.

Products from Cystathionine-Phosphate Mixture

A mixed sample of cystathionine and phosphate was irradiated with phosphorus K edge X rays in order to estimate the effects of Auger electrons

generated outside of the molecule. Noticeable difference in the spectrum of the peak heights between the irradiation at 2153 eV and at 2146 eV was not observed (Fig. 3). The pattern of the degradation products on the HPLC chromatogram at 2146 eV were also very similar to that of cystathionine irradiated at the energy below the sulfur K edge (2466 eV). Production yields of the fragments were larger

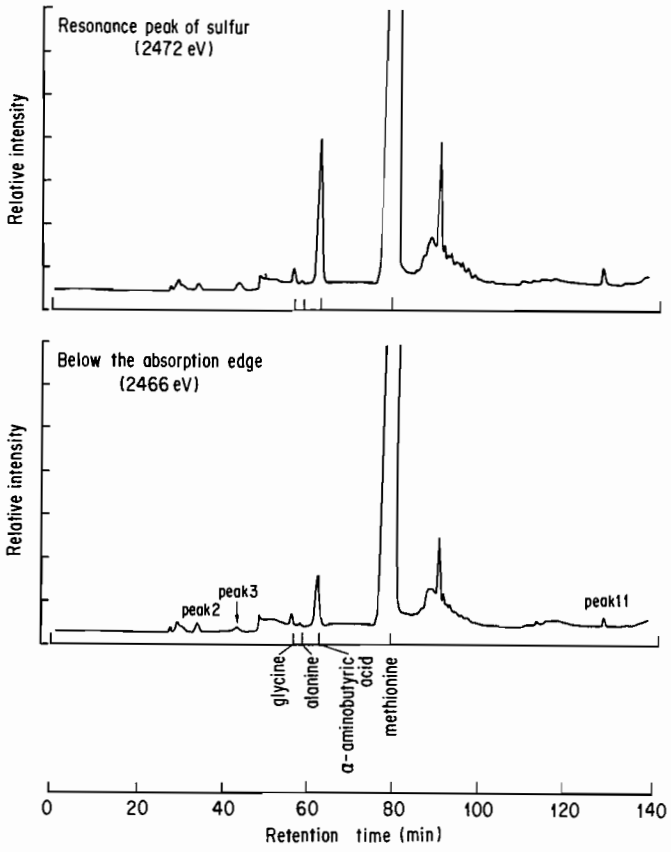


FIG. 2. HPLC chromatograms of irradiated methionine at 2472 eV and at 2466 eV. The names of the expected amino acids are inscribed on the abscissa. Exposures are 70 MR (1.8×10^4 C/kg) for the upper panel and 94 MR (2.4×10^4 C/kg) for the lower. Peaks for alanine were observed but were not large enough for quantitative analysis.

at 2153 eV than at 2146 eV due to the large absorption cross section of phosphorus at 2153 eV. The ratios of the production efficiencies of the products at the two energies were between 1.4 and 1.8, and did not vary much among the

products as seen in Table II. These results are clearly different from the case when irradiated with X rays at the sulfur K edge, where the ratios of the yield varied from 1.3 (glycine) to 4.1 (unidentified peak at the retention time of about 130 min), depending upon the products (4)).

TABLE I

Production Efficiencies† (pmol/MR) of α-Aminobutyric Acid and Glycine

Product	Methionine		Cystathionine*	
	2472 eV	2466 eV	2472 eV	2466 eV
α-Amino-butyric acid	41	11	38	13
Glycine	1.9	0.77	1.2	1.0
Alanine	n.d.	n.d.	75	28

†Efficiencies for alanine was calculated by assuming the peak of alanine and/or ethylcysteine contains alanine only.
*Data were taken from Yokoya et al. (4) for comparison
n.d.: not detected

DISCUSSION

Yield of C-C bond Cleavage Producing Alanine

Glycine and α-aminobutyric acid were identified on the chromatograms of irradiated methionine. However, the peak was not seen at the position of alanine on the HPLC chromatogram of the irradiated methionine at neither of the irradiation energies. In methionine, the C-C bond cleavage to produce alanine hardly occurred with the soft X ray irradiation. From these results, it might be suggested that alanine produced by the cleavage of the C-C bond in cystathionine did not contribute to the peak observed at the position of alanine on the HPLC chromatograms of the irradiated cystathionine; in other words, alanine was produced by the cleavage of S-C bond (see Fig. 1). Ethylcysteine, which might be produced from cystathionine as a counterpart fragment of

glycine, might be included in the alanine peak under the HPLC conditions used in this study (4). However, the amount of ethylcysteine can be considered very small upon estimation from the amount of glycine (about 1 pmol/MR at both irradiation energies). From these considerations, the large peak area observed at the position of alanine and/or ethylcysteine on the HPLC chromatograms of irradiated cystathionine could be considered as alanine produced by the S-C bond cleavage in cystathionine.

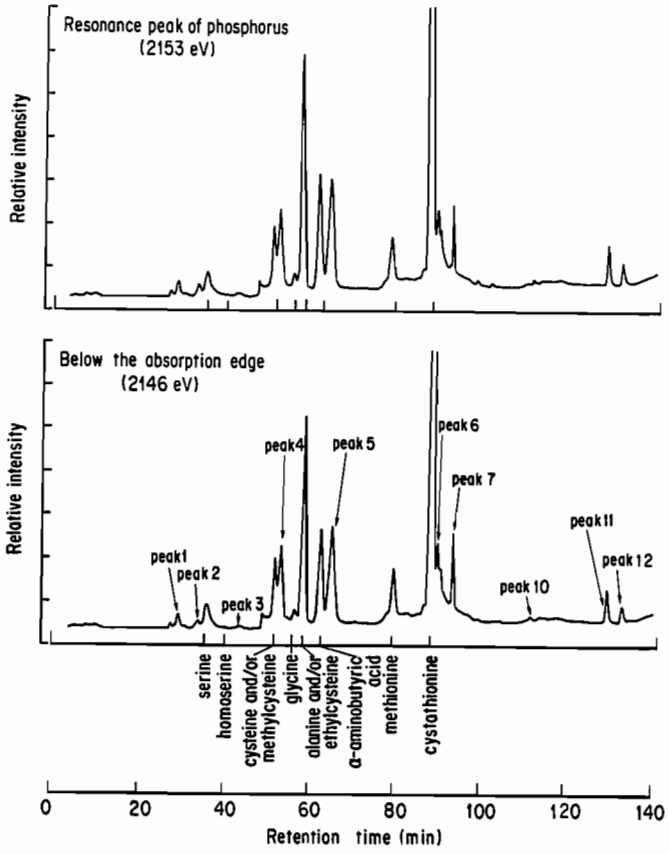


FIG. 3. HPLC chromatograms of the cystathionine-phosphate mixed sample irradiated with 2153 eV and at 2146 eV monochromatic soft X rays. Exposures are 57 MR (1.5×10^4 C/kg) for the upper panel and 82 MR (2.1×10^4 C/kg) for the lower. The names of the expected amino acids are inscribed on the abscissa. Peaks with numbers are unidentified ones.

The yield of alanine from cystathionine irradiated at the energy around the sulfur K edge was then calculated by assuming that the peak contains only alanine. The obtained yields are shown in Table I with the values of α-aminobutyric acid and glycine. Similar dependence of the yields of alanine and α-aminobutyric acid on the photoabsorption site may indicate that the production mechanisms have some part in common. Some role of the sulfur atom as the photoabsorption site can be suspected.

TABLE II

Production Efficiencies* of Irradiation Products Following Irradiation of a Mixed Sample of Cystathionine and Phosphate Salts

Product	Efficiency per unit exposure (pmol/MR)	
	2153 eV	2146 eV
α-Aminobutyric acid	47	26
Glycine	2.3	1.6
Alanine	108	63

*Irradiated at the resonance absorption peak of phosphorus (2153 eV) and at the energy below the peak (2146 eV)

Contribution of Auger electrons in the Degradation of the Amino Acids

The production efficiencies of α-aminobutyric acid and glycine at 2153 eV were larger than those at 2146 eV as shown in Table II, reflecting the large absorption cross section of phosphorus. From these results, it was confirmed that the Auger electrons from phosphorus did actually contribute to the degradation of cystathionine.

The ratio of the yields of three products; namely, α-aminobutyric acid, glycine and alanine, at the energy of 2153 eV was about 20:1:47. This ratio was

not so different from that at 2146 eV (16:1:39). At the energies around the sulfur K edge, on the other hand, the ratio clearly changed with the photoabsorption site from 13:1:28 to 32:1:62 (Table I). These results also indicate that the Auger electrons from phosphorus were not involved in the change of the degradation reactions of the amino acid. Inner shell excited atoms are multiply ionized after the Auger cascade. A multiply ionized sulfur atom in the amino acid, rather than the Auger electrons from the sulfur atom, is considered to play an important role in determining the degradation reaction of the molecule. These results may support the idea that the changes of the biological effects at the inner-shell absorption edges are due to the action of the multiple ionization of the atom after the Auger cascade rather than the action of the ejected secondary electrons. The Auger electrons are supposed to affect the chemical bonds non-specifically in the molecules in the sample.

CONCLUSION

Degradation pattern of amino acids depends much upon the photoabsorption site and the multiply ionized atom plays an important role in the degradation. These molecular changes (damage) could explain the enhancement of biological effect observed with irradiation of monochromatic soft X rays at the inner-shell absorption edge.

ACKNOWLEDGMENTS

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DISCUSSION

Harapanhalli, R. S. 1) What reasons do you ascribe to the fact that S-C bond cleavage predominates at the absorption edge of the spectrum (2472 eV)? 2) What are the consequences on the sulfur containing residue after the S-C cleavage?

Kobayashi, K. 1) The reason for the increase of S-C bond breakage with 2472 eV irradiation is multiple ionization of sulfur atoms which absorbed an X ray photon and ejected several Auger electrons. Molecules having multiply charged atoms in it are known to break up in to small fragments. 2) Since the peak in HLPC for sulfur containing residues are observed as a mixture with other fragments, we cannot say decisively. However, we suggest that other S-C bonds are also cleaved and quantities of sulfur-containing residues become very small, mainly due to the same reason above when Auger effects occur.