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PULSED UV LASER GENERATED SHORT-LIVED FREE RADICALS FROM BIOLOGICAL SAMPLES

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EPR characterization of the short-lived free radicals generated by pulsed UV laser ablation of biological samples has been investigated using a spin trap method. The obtained EPR spectra suggest that the trapped short-lived free radicals generated by excimer laser ablation of collagen and myocardium are identical. The obtained results are discussed in association with the production scheme of free radicals and an empirical mechanism of laser generated short-lived free radicals.

KEY WORDS: Free radicals, laser ablation, collagen, myocardium, EPR.

INTRODUCTION

Laser photoablation of biological tissue samples has been of great interest. There is no doubt that various kinds of short-lived free radicals are formed by laser interaction with biological tissue samples.[1-4] By adjusting laser output power and laser modes, you may control the types of free radicals generated, and through the recognition of free radicals, you can prevent injuries induced by the reactive free radical intermediates. Their short lifetimes and complexity of the tissue samples make their nature difficult to understand. The short-lived free radicals can be converted to long-lived free radicals by reacting with a spin trap reagent. The long-lived free radicals are detected using a conventional cw EPR method at ambient temperature.

The laser generated free radicals have been measured indirectly using the spin trap method.[1,3,5,6] The indirect spin trap method has provided significant information about the free radicals produced by photoablation of the tissue sample. It has been found that different laser modes: cw and pulse, produce different radical species.[1,6] However, the detail mechanisms of laser-biological tissue interaction and free radical generation processes have not been established.

To compare effects produced when ablating biological tissue with a pulsed laser, a laser which has been reported by several groups to produce injury-free tissue removal,[7,8,9] with the typical thermal injury on cw laser irradiation,[1,3,4,6] we have examined the effects of laser ablation on the analogous series. We have postulated the free radicals and their production scheme depending on the laser irradiation mode.

In this research commuication, we report the pulsed UV laser generated free radicals from biological samples at ambient temperature. The formation of free radicals and the possible species are discussed. Furthermore, an empirical mechanism for the photoablation of the biological samples is considered.

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EXPERIMENTAL PROCEDURE

Post-mortem tissue sample preparation was described elsewhere.[1] The tissue samples were irradiated through silica fused optical fiber by pulsed UV laser in cyclohexane. Questic series 2240 pulsed excimer lasers were used to generate short-lived free radicals. The short-lived free radicals reacted with the organic spin trap compound, N-tert-butyl-α-phenylnitrone (PBN). PBN was purchased from Aldrich Chemical Co. and used as received. PBN was dissolved in cyclohexane (A.C.S. spectrophotometric grade, 99%) to a concentration of 1 M. Cyclohexane was purged with Argon gas for a few minutes prior to being poured into the EPR sample tube. Collagen (Sigma Chemical Co.) was ablated by excimer laser in the same manner as the tissue sample. Laser output power from the optical fiber was kept at 0.1 ~ 0.3 watts (e.g., 40 Hz, 7.5 millijoules/pulse, 20 second exposure; total energy delivered = 6 joules). Once photoablation of the tissue sample commenced, the solution in the EPR tube started bubbling. This cavitation observed for the pulsed laser ablation was less significant than for cw laser irradiation.

EPR measurements were made with a varian E104 X-band spectrometer. All EPR spectra were recorded at ambient temperature. Typical EPR conditions were the following: microwave power, 20 mW; microwave frequency, 9.10 GHz; modulation aplitude, 1.0 G; time constant, 1.0 s; scan rate, 20 G per minute. The resonance fields were measured relative to the standard free radical 2,2-diphenyl-1-picylhydrazyl (DPPH, g = 2.003).

RESULTS AND DISCUSSION

As a simple model compound for the photoablation of myocardium samples which are somewhat related to atherosclerosis, collagen is chosen. The basic structural unit of collagen consists of three polypeptide cross-linked chains. Pulsed UV laser ablation of the collagen produces short-lived free radicals. The short-lived free radicals are trapped by the spin trap reagent PBN and are converted to stable free radicals. These are called spin adducts. PBN is chosen because it is relatively stable to laser irradiation and has no EPR background signal. The drawback of PBN is that it normally does not provide complementary nuclear hyperfine information concerning the spin adducts.

Spin trapped short-lived free radicals produced by excimer laser ablation of collagen and myocardium samples are shown in Figure 1 (A) and (B), respectively. Strikingly both EPR spectra are similar. Both cases produce strong EPR spectra with a dominant three nitrogen hyperfine splittings. Even though there are subtle differences in signal to noise ratio (because of dispersion of the laser beam for collagen experiments), the trapped free radicals produced by excimer laser ablation of the collagen are identical to those from the tissue sample. Moreover, no thermal damage in the sample target site is observed in two different experiments.

To further interpret the EPR spectra for both samples, an EPR simulation is performed. This EPR spectral simulation of the laser ablated tissue sample is shown in Figure 1 (C). Parameters for the calculated EPR spectrum are a $^{N}_{NO} = 13.8$ G and a $^{H}_{\beta} = 1.4$ G. The beta hydrogen hyperfine value is too small to split each peak. This EPR simulation suggests that laser ablation of collagen and of the tissue sample results in very similar adducts.

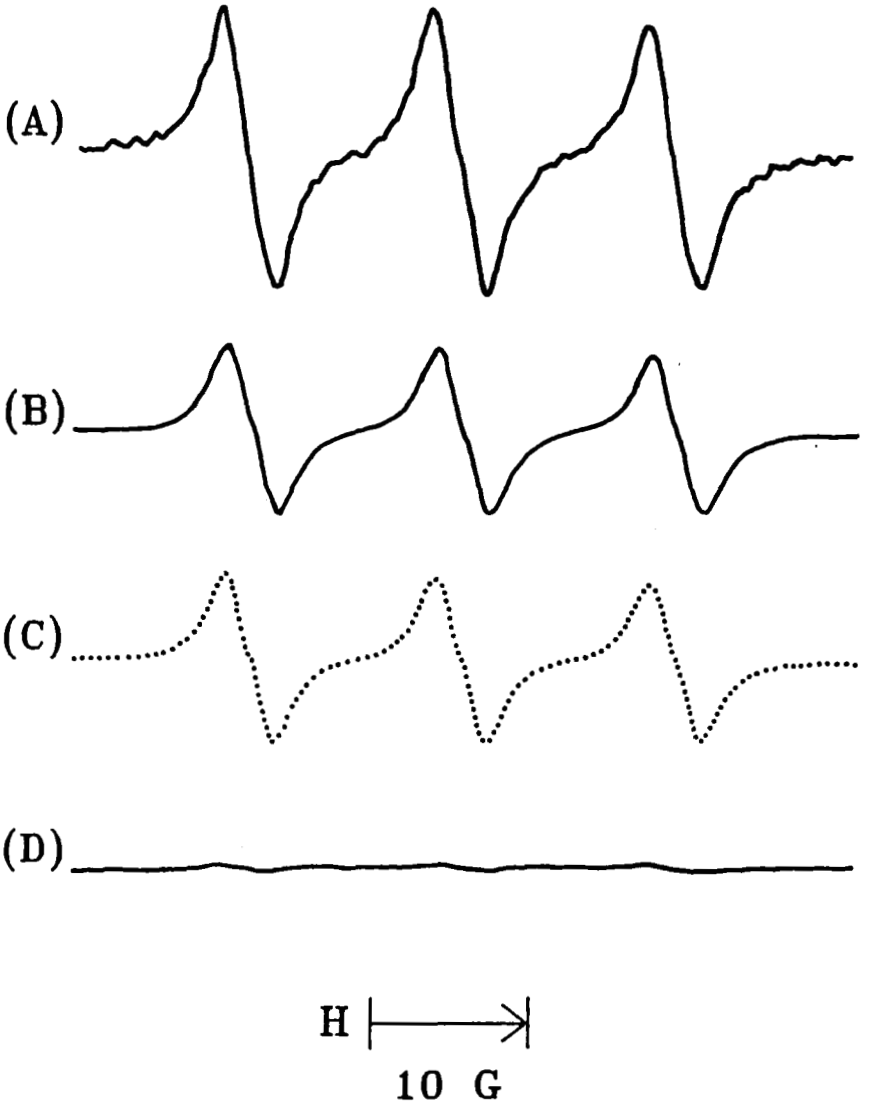

Figure 1. EPR spectra of the spin adducts from pulsed 308 nm excimer ablation of (A) collagen, (B) myocardium in the presence of spin trap reagent PBN. In both cases the same energy 7 [mJ/pulse] is delivered to the samples. Computer simulation of the EPR spectrum obtained by excimer laser ablation of the biological samples is shown in (C). Parameters for the EPR computer simulation are in the text. (D) is the background EPR spectrum observed for excimer laser irradiation of PBN solution in the absence of the sample.

In order to examine the decomposition of PBN reagent by laser irradiation, the PBN solution is irradiated in the same manner as the samples. It is found that the decomposition of spin trap reagent does not contribute to the resultant EPR signals. Therefore, the mechanism of the free radical production should be the same in both cases.

To specify the short-lived free radicals produced when ablating biological samples with the pulsed laser, we have examined the effects of excimer laser ablation on the polypeptide chain crosslinked collagen and cardiovascular tissue. The results observed are the same in two respects as those of the pulsed excimer laser ablation. First, a qualitatively same hyperfine pattern is observed on both biological samples; the EPR patterns are displayed in Figure 1 (A) and (B). The further splitting of the nitrogen triplet is clearly absent in the results with excimer laser ablation; the same results are observed with both 351 nm and 308 nm excimer laser wavelengths. A second similarity on the photoablation with pulsed excimer laser is in the individual nitrogen transition intensity ($m_I = 1,0,-1$) of the observed EPR signal. Therefore, the free radicals generated by excimer laser ablation are similar in the both systems.

The present results indicate that pulsed UV laser action on the biological samples could employ the same mechanism for the formation of free radicals. Excimer laser ablation of the biological samples shows nonthermal removal of the sample surfaces at the target sites. The pulsed laser beam emits a large number of photons within a 20 nanosecond laser pulse. This pulsed laser excitation could have enough energy to fragment the protein chains without thermal damage at the target site. The free radical molecules are initiated by photodisruption of the sample[5] and are ejected releasing excess energy from the target site. Then, they react with the spin trap reagent.

We postulate the identification of free radicals and their generation scheme for the pulsed laser ablation based on the present achievements. The appearance of free radicals accompanying laser ablation implies specific bond breakage and subsequent release of distinct molecular fragments, such as different sizes of carbon centered free radicals. We are also able to provide concrete evidence for the similarity of free radicals through examination of the EPR spectra of their nitroxide spin adducts in solution. The origin of short-lived free radicals produced by pulsed lasers is essentially identical with the two different types of biological samples.

Their origin at molecular levels may lead to understanding the detailed events of photoablation. The present experiments cannot distinguish the subtle EPR differences in both collagen and myocardium samples. However, further understanding of the nature of laser generated free radicals from biological samples leads us to postulate their production scheme along with the empirical mechanism.

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Abbreviations

EPR, electron paramagnetic resonance; cw, continuous wave; G, Gauss.

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