The Effect of Heat and of UV-Light on X-Ray Induced ESR-Centers in α-Alanine

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It has been shown that UV-light and X-rays produce the same type of electron spin resonance signals in certain compounds, whereas they give rise to qualitatively different spectra in some other compounds. Thus, Koch, Woenkhaus and Markau reported that UV-light and X-rays produced qualitatively equal signals in cysteine, whereas Koch, Fränz and Markau found that the two radiations gave rise to different signals in a number of other sulphur-containing compounds. Mönig and Koch have reported that on exposing bovine serum albumin, homocysteine, and methionine to UV-light following irradiation with X-rays, the ESR-spectra changed shape. Furthermore, the number of radicals decreased initially with time of UV-irradiation and increased during prolonged UV-exposure. Independent of this work we studied the qualitative and quantitative changes of the X-ray induced ESR signals in α-alanine when subjected to subsequent treatment either with UV-light (2537 Å) or with heat (150°C).

Gordy and Shields and Gordy have shown that α-alanine after X-irradiation at room temperature shows a nearly symmetrical five-line spectrum with an unresolved pattern superimposed. From work with single crystals of α-alanine Miyagawa and Gordy assumed this X-ray induced radical to be CH₃C–CHR, where the unpaired electron interacts with 4 protons, without any detectable coupling to the group R. Prydz and Henniksen found that the shape of the X-ray induced ESR-signal in α-alanine was independent of the radiation dose and of the time elapsed after the end of the exposure. They also found that the shape of the ESR-signal was unchanged even after heat treatment for several hours at about 110°C and concluded that X-rays probably produced only one single radical species in α-alanine.

In the present investigation X-rays from a "Stabilipan" unit with 0.2 mm Cu-filtration were used. The dose rate was about 5 × 10⁴ R per minute. The total dose given to each sample was about 4 × 10⁶ R. The ESR spectrometer used operates at 9200 Mc/sec with a modulation frequency of 110 kc/sec. The

![Graph](image)

*Fig. 1. The relative number of ESR-centers in X-rayed α-alanine as a function of the time the samples were subjected to subsequent treatment with:

□ Heat at 23°C; ○ UV-light; ● Heat at 150°C. X-Ray dose: 4 × 10⁶ R.*

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spectrometer and the procedure followed to obtain the number of ESR-centers in the samples were the same as those used by Prydz and Henriksen. The UV-light source used was a "sterile" liquid-pressure mercury resonance lamp in the form of a U-tube. The spectral line at 2537 Å completely dominates the emission from this lamp and there are no spectral lines between 2400 Å and 5400 Å other than those due to Hg. Based on inactivation of lysozyme in M/15 phosphate buffer, under experimental conditions identical to those used by Shugar, the light intensity at the position of the sample tubes during irradiation was estimated to be of the order of $1.5 \times 10^{18}$ quanta/cm²/min. Approximately 60 mg of α-alanine was added to each of a number of quartz tubes which were evacuated at about $10^{-4}$ mm Hg for 24 h. The tubes were sealed off and irradiated with X-rays. One group of irradiated samples was kept at room temperature (approximately 23°C) and the number of ESR centers followed as a function of the time the samples were kept at this temperature. Another group of samples was measured in the ESR spectrometer at room temperature immediately after the end of the X-ray exposure and also after storage at 150°C for various lengths of time. A third group of samples was measured in the ESR spectrometer at room temperature just after the X-irradiation and also after subsequent exposure to UV-light for various lengths of time.

Fig. 1 shows the relative number of ESR centers in the samples as a function of the three different treatments. In agreement with earlier investigations, it was found that the X-ray-induced signal was very stable at room temperature, with no significant qualitative or quantitative changes during the first week after the exposure.

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![ESR-spectra of α-alanine. A and A₁, immediately after the end of the X-ray exposure B, C, and D, after subsequent UV-exposure for 105 min, 2685 min, and 8385 min, respectively. B₁, C₁, and D₁, after subsequent heat-treatment at 150°C for 90 min, 2430 min, and 8010 min, respectively. Spectra D and D₁ are recorded with a gain 3 times higher than that of the other spectra shown. All other spectrometer settings are identical. The curves represent the first derivative of the absorption curves. The arrows show where the resonance of DPPH would be (G = 2.0036).](image-url)
On the other hand it was found that the number of ESR centers in those samples which after X-irradiation were subjected to UV-light and those subjected to heat-treatment at 150°C showed a very pronounced decrease during the first hours of the treatment, followed by a slighter decrease as the treatments continued.

The curves shown in Fig. 1 are drawn on the assumption that the decrease of the number of ESR centers can be described by two exponential decay processes: one with a half-life of the order of 3 hours under the present experimental conditions (a little more for the UV-treated samples, a little less for the heat-treated samples) and another component with a half-life of the order of 5 days, both for the heat-treated and for the UV-treated samples.

Fig. 2 shows some of the ESR spectra. Figs. A and A show the spectrum recorded about 10 min after the end of X-ray exposure. Figs. B, C, and D show the spectra after subsequent UV-treatment for 105 min, 2085 min, and 3385 min. B, C, and D, similarly, show the signals recorded after subsequent heat-treatment at 150°C for 90 min, 2430 min, and 8010 min, respectively. Under these conditions the percentage ESR centers remaining in D is approximately equal to that in D, the percentage in C approximates that in C, and so on. Fig. 1 shows that under the present experimental conditions the two treatments, i.e. exposure to UV-light and heat-treatment, result in approximately the same rate of change of the number of ESR centers, but from Fig. 2 it can be seen that the two treatments do give rise to somewhat different qualitative ESR signals. In the series of samples which was subjected to post-irradiation heat-treatment it cannot be excluded that the signal D, appears because of a preferential decay of one type of radicals which may dominate the ESR-signal before onset of the heat-treatment. If this is the case it would imply that at least two radical species are induced in α-alanine when exposed to X-rays at room temperature. UV-light, on the other hand, not only makes the X-ray-induced radicals unstable, but peaks appear in the central part of the resonance where no counterparts are seen in the original spectrum. This is particularly interesting since it was found that UV-exposure alone produced no detectable ESR signal in α-alanine even when the UV-exposure lasted for 10 days. Studies of the effect of UV-light during as well as after exposure to X-rays are being continued with enzymes as test materials in order to attempt to cast more light over the relative contribution to the radiation damage caused by excitations and by ionizations and to elucidate mechanisms of importance in radiobiological “after-effects”.

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