



Title	E.S.R. SPECTROSCOPY OF RADIATION-INDUCED FREE RADICALS IN IRRADIATED DNA-RO-07-0582 COMPLEXES
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of 629 rad. Thus, the oxygen enhancement ratio (o. e. r.) was calculated to be factor 5.

An electron affinic radiosensitizer, Ro-07-0582, revealed a pronounced sensitizing effect under an extremely hypoxic condition by an enhancement ratio of 3.9 at a concentration of 10 mM. The sensitizer reduced the D_0 value with an increasing concentration of the reagent. This evidence suggests that Ro-07-0582 acts as an oxygen-mimic, although the reagent shows no radiosensitizing effect at low concentrations and at low irradiation doses.

A supplemental study was undertaken to show the effect of a superoxide anion radical, O_2^- , which was known as a component of the oxygen effect on cell killing. No remarkable change of survival was observed in the X-irradiated aerobic cells, although O_2^- was inactivated by superoxide dismutase. This result suggests that O_2^- is not a component of the oxygen effect on mouse L cells.

E. S. R. SPECTROSCOPY OF RADIATION-INDUCED FREE RADICALS IN IRRADIATED DNA-RO-07-0582 COMPLEXES

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The present study was carried out to elucidate the effect of an electron affinic radiosensitizer, Ro-07-0582, on the radical formation in irradiated DNA.

DNA-Ro-07-0582 complex was γ -irradiated in dry state at 77°K, and free radicals produced in the complex were recorded on an electron spin resonance (E. S. R.) spectrometer at 77°K. The main component of the E. S. R. spectrum was derived from the Ro-07-0582 anion radical. The result seems to indicate that electrons liberated in the complex by ionizing radiation were localized preferentially on Ro-07-0582.

To obtain further information, the binary complexes between each of the four DNA nucleotides and Ro-07-0582 were employed as samples. The TMP- and dAMP-Ro-07-0582 complexes showed that the spectrum due to the Ro-07-0582 anion radical could be observed, and that the radical yield was higher than that of pure TMP or dAMP. The mechanism of radical formation in TMP and dAMP involves an electron transfer process from sugar moiety to base moiety. The above phenomenon was explained by assuming that electrons liberated from sugar by ionization were transferred to the Ro-07-0582. The considerable increase

in radical yields of the two complexes suggested that Ro-07-0582 scavenged electrons which may recombine with the cationic holes of sugar moiety.

The effect of Ro-07-0582 on dCMP- and dGMP-Ro-07-0582 complexes was almost indiscernible. The result shows that electrons do not transfer from the nucleotides to the sensitizer. This interpretation is supported by the observation that the mechanism of radical formation in dCMP and dGMP did not involve the electron transfer process.

It may be concluded that the TMP and dAMP moieties can be taken as possible sites in which Ro-07-0582 shows a remarkable effect on the DNA radicals. Damages in the sugar moieties of TMP and dAMP appear to result in an increase of double strand breaks of DNA. Thus, the increase of double strand breaks induced by Ro-07-0582 in the TMP-dAMP pair of DNA helix may be responsible for the radiosensitization mechanism.

DISTRIBUTION AND LOCALIZATION OF ADENYLATE KINASE ISOZYMES IN PORCINE TISSUES

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Porcine heart adenylate kinase can be separated into acidic and basic isozymes by isoelectrofocusing. The former is similar to the bovine liver mitochondrial adenylate kinase, and the latter is identical to the skeletal muscle and erythrocyte adenylate kinases (KUBO, S. and NODA, L. 1974; ITAKURA, T. et al. 1977; MIWA, N. et al. 1978).

The acidic enzyme prepared from the heart reacted only to rabbit anti-acidic enzymes but not to rabbit anti-basic enzymes in the double diffusion and antibody inhibition tests, whereas the basic enzyme prepared from the skeletal muscle reacted only to rabbit anti-basic enzymes and not to rabbit anti-acidic enzymes.

Adenylate kinase activities in porcine tissue extracts were almost completely inhibited by both anti-isozymes. Organ specificity was observed in isoelectrofocusing profiles of adenylate kinase from several tissues, but each enzyme fraction separated by the isoelectrofocusing was completely inhibited either by anti-acidic or anti-basic enzymes. These results indicate that there are two main kinds of adenylate kinase isozymes—acidic and basic adenylate kinases—in porcine tissues.