NOTES

PATHWAYS OF DNA REPAIR OPERATING IN YEAST TREATED WITH THE PYRROLO(1,4)-BENZODIAZEPINE ANTITUMOR ANTIBIOTICS

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Anthramycin, tomaymycin and sibiromycin are members of a group of structurally related antitumor agents which have been named the pyrrolo(1,4)benzodiazepine antitumor antibiotics.[1] The potent biological effects of these antibiotics are due to their ability to react covalently with DNA thereby inhibiting nucleic acid synthesis.[1,2] These antibiotics appear to have some unique properties with respect to their reaction with DNA and, thus, constitute an interesting group of chemicals for genetic and DNA repair studies. Recently we have demonstrated that one of these antibiotics namely anthramycin has strong recombinogenic effects in *Saccharomyces cerevisiae* while its mutagenic effects are highly specific and often absent in both *Salmonella typhimurium* and *S. cerevisiae*.[3] While studies are in progress to characterize the specific type(s) of adducts formed by these antibiotics in DNA, we attempted to identify the possible repair pathway(s) dealing with the potential inactivating lesions produced by them in the yeast *S. cerevisiae*.

Microbial strains defective in different pathways or steps of DNA repair have been successfully employed in earlier studies to gain an insight into the types of lesions in DNA and the pathways of repair occurring in cells after exposure to chemical mutagens.[4,5] In *S. cerevisiae*, both repair-proficient and repair-deficient strains are

Table 1. Relative sensitivity of different wild type and radiation sensitive mutants of *Saccharomyces cerevisiae* to anthramycin, sibiromycin and tomaymycin.

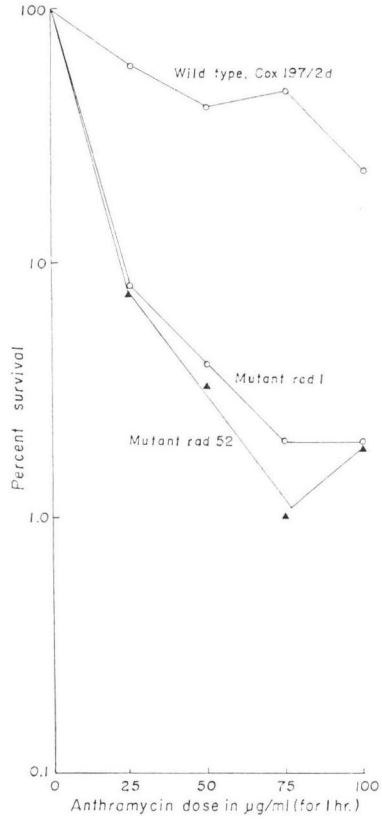
Designation of yeast strains	Characteristics of the strain (radiation sensitivity and repair deficiency)	Relative sensitivity to		
		Anthramycin	Sibiromycin	Tomaymycin
Cox 197/2*d*	Repair-proficient, normal sensitivity to UV and ionizing radiations	+	+	+
XV185-14*C*	"	+	+	+
C16-11*C*	"	+	+	+
rad 1	Deficient in excision of UV-induced dimers, very sensitive to UV and normal sensitivity to ionizing radiations	++++	+++	++++
rad 2	"	++++	+++	++++
rad 9	Deficient in recombination moderately sensitive to both UV and ionizing radiations	+++	+++	+++
rad 52	Deficient in recombination and in the repair of DNA double strand breaks, very sensitive to ionizing radiations, only slightly sensitive to UV	++++	++++	++++
rad 53	Repair deficiency not characterized, sensitive to ionizing radiation	+	+	+
rad 54	"	++++	++++	++++
rad 55	"	++++	++++	++++
rad 56	"	+	+	+

available. Repair-deficient mutants have been genetically analyzed and at least three functional repair pathways have been identified in this organism.[6,7] Mutants blocked in specific repair pathways have been used in the present study to determine whether or not, all, or a class of the mutants were hypersensitive to the antibiotics compared to the repair-proficient strains. Enhanced sensitivity of a repair-deficient strain to the antibiotics is considered to be due to its inability to repair the antibiotics-induced DNA lesions responsible for cell death. The repair-proficient strains designated *Cox* 197/2*d*, *C*16-11*C* and *XV*185-14*C* and the repair-deficient strains designated *rad* 1, *rad* 2, *rad* 6, *rad* 9, *rad* 51, *rad* 52, *rad* 53, *rad* 54, *rad* 55 and *rad* 56 were obtained from Dr. A. NASIM, NRC of Canada, Ottawa.

The degree of relative sensitivity of various strains to anthramycin, tomaymycin and sibiromycin is shown in Table 1. For a more precise comparison of sensitivities of the representatives of repair-proficient and repair-deficient strains, dose-effect survival curves for the selected strains are shown in Fig. 1. The results demonstrated that the mutants which are defective in the removal of UV-induced pyrimidine dimers (*rad* 1 and *rad* 2)[7] as well as those which have been characterized as being defective in recombination and, thus, presumably unable to carry out recombinational repair (*rad* 9 and *rad* 52)[8,9] were extremely sensitive to all three antibiotics tested. Among the strains tested, *rad* 50, *rad* 51, *rad* 52, *rad* 53, *rad* 54, *rad* 55 and *rad* 56 are known for their being exceedingly sensitive to ionizing radiation while their sensitivity to UV light is much less pronounced. In this class of ionizing radiation-sensitive mutants, all except *rad* 53 and *rad* 56 were highly sensitive to anthramycin, tomaymycin and sibiromycin. Of all the mutants sensitive to ionizing radiation, only *rad* 52 has been biochemically characterized as being defective in the repair of DNA double strand breaks as well as in recombination.[9] Some of these mutants may be defective in the repair of single strand breaks as well.

The present results showed that two principal repair pathways were involved in handling the DNA lesions produced by anthramycin, tomaymycin and sibiromycin. These pathways utilize *RAD* 1 gene product concerned with excision of UV-induced dimers and the *RAD* 52 gene product concerned with the repair of ionizing radiation-induced DNA strand breaks.

Fig. 1. Enhanced sensitivity of *rad* 1 and *rad* 52 mutants (representing the strains deficient in excision of UV-induced dimers and repair of double strand breaks respectively) to anthramycin.
Note: Similar results were obtained with sibiromycin and tomaymycin.



The involvement of both UV-excision repair and mechanism(s) for X-ray repair in the case of pyrrolo(1,4)benzodiazepine antibiotics may be interpreted to mean that these antibiotics produce two types of DNA lesions, one being base-damage type recognizable by UV-specific endonuclease while the other being strand breakage repairable through a recombinational process controlled by *RAD* 52-like gene product. Alternatively, one type of initial lesions produced by these antibiotics are channelled equally through two different pathways, *i.e.* UV-type excision repair and recombinational repair/strand break repair.

The involvement of recombinational pathway suggests that treatments with anthramycin-like

antibiotics do lead to DNA strand breakage. We have found that anthramycin is recombinogenic in repair proficient diploid cells of *S. cerevisiae* which support the above conclusion. Furthermore, involvement of the same repair pathways almost to the same extent in the repair of DNA lesions induced by the three structurally similar antibiotics reflect a similar mode of action of these agents with DNA.

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