Reversing DNA damage from the sun

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Researchers have synthesized an artificial enzyme that can break down thymine dimers, a type of DNA damage that is a major cause of skin cancer. The work is still at an early stage, but could offer a way of reversing sun-induced DNA changes before cancer develops.

Skin cancer is now the most common form of cancer in the US[1], with the incidence rising steadily for the past two decades. Further depletion of the ozone layer is likely to magnify this trend. UV radiation from the sun causes covalent linkage of adjacent thymine bases (T) in the DNA to form a *cis,syn* cyclobutane thymine dimer (T–T) via cycloaddition (Fig. 1).

Repairing DNA

T–T dimers can be broken down naturally by DNA photolyases, enzymes found widely in nature but not yet detected in humans[2]. These carry out a cycloreversion catalysed by the transfer of an electron donated by a 1,5-dihydroflavin adenine dinucleotide ($FADH^-$) molecule located in the active site. T–T dimers have been extensively studied and several groups are currently working in this area. Applied Genetics Inc Dermatics (Freeport, NY, USA) has completed Phase III trials on liposome-encapsulated bacteriophage T4 endonuclease V, a general DNA repair enzyme which acts, *inter alia*, on T–T dimers. This slowed the development of pre-cancerous skin lesions in young patients with xeroderma pigmentosum[3].

Because of the large MW of natural photolyases, delivery of these proteins is problematic. Olaf Wiest and Marco Jonas of the University of Notre Dame (Notre Dame, IN, USA) have therefore synthesized an artificial photolyase based on one found in the bacterium *Escherichia coli*[4]. This is only about 1% of the MW of the natural enzyme. It is also more stable than the natural enzyme and would be easier to make in the quantities required for preventing skin cancer on a large scale. To make the artificial enzyme, a flavin active unit (as found in the natural enzyme) was combined with a zinc–cyclen complex, known to selectively recognise pyrimidines in aqueous solution (Fig. 2). The synthesis is described by Wiest as 'fairly straightforward', starting with a commercially available cyclen, building up the flavin core using standard routes, coupling the two parts together and then deprotecting the final compound[4].

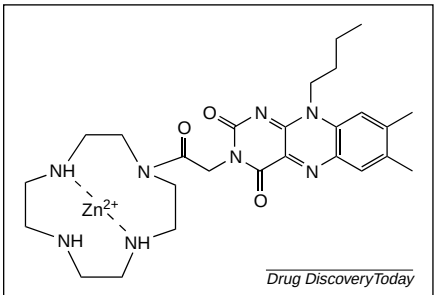

Figure 2. The artificial DNA photolyase synthesized by Wiest and Jonas, with assistance from Burkhardt Koenig (University of Regensburg, Regensburg, Germany).

In a paper recently presented to the American Chemical Society[4], Jonas and Wiest showed that the synthetic enzyme recognizes and breaks up T–T dimers outside of DNA. Thymine dimers were synthesized independently and then irradiated with visible light in the presence of the enzyme. Their cycloreversion to thymine was monitored by HPLC. Controls consisting of the dimer alone or dimer complexed with zinc–cyclen were also irradiated, but cycloreversion did not occur. Irradiating T–T with flavin alone, without the recognition portion of the enzyme, produced significantly slower reversion.

The Notre Dame Group will now test the enzyme on dimers within DNA molecules. They will first have to make a piece of DNA duplex, probably a 16-mer, containing T–T in a well-defined position. This is a difficult and time-consuming operation that will take several months. Testing the enzyme in DNA will involve essentially the same experiment as described above. DNA with the dimer

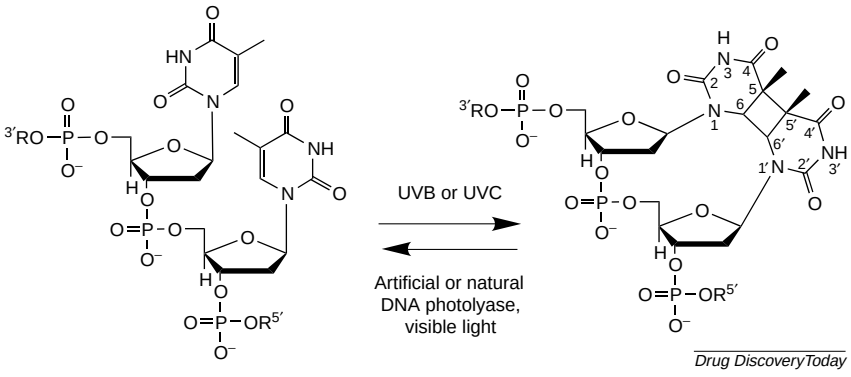

Figure 1. Formation and breakage of thymine dimers via cycloaddition/cycloreversion in the presence of ultraviolet radiation.

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can be distinguished from repaired DNA by HPLC.

Refining the enzyme

Wiest warns against premature optimism about developing a drug from the enzyme. 'I would be more than happy if we could get the experiment with DNA to run,' he says. 'This is fairly basic research and a proof-of-concept rather than a drug that will make it to market any time soon.' The enzyme version shown in Fig. 2 is not a likely drug candidate and will not be patented. If it is shown to function in DNA, a decision will have to be made on whether the fundamental structure can be developed for use *in vivo* or whether a different structure that works using the same principle would be required. 'My feeling is that we will have to move to a very different recognition unit in the end,' says Wiest. 'But this lays the foundation for a potential drug and I think it's promising.'

Any product that does derive from the work will be for prevention of skin cancer rather than for a cure, and will not reach the market for at least ten years. The Notre Dame group is hopeful that the long interval between DNA damage and cancer development offers a window for neutralizing the effects of past overexposure to the sun.

References

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