BNL-24809

CONF-780856--1

UV PHOTOBIOLOGY: DNA DAMAGE AND REPAIR*

Betsy M. Sutherland

Biology Department
Brookhaven National Laboratory
Upton, New York 11973

INTRODUCTION

Photobiology deals with the interaction of light and living systems. However, we will talk about only one kind of photobiology: the interaction of light in the wavelength range 200-300 nm (ultraviolet light) and its interaction with biological systems, in specific, living cells (1).

Why ultraviolet light? The first and most important reason is that this wavelength range is strongly absorbed by many biologically-important molecules.

Why biological molecules? Since we want to deal with photobiology, we must work in the range of doses in which the cell is still alive. This poses both a problem of measuring photoproducts in small numbers and provides a challenge to us to develop methods which enable us to do this.

When ultraviolet light interacts with a cell, what are the important targets which determine the fate of the cell? Cells are mostly water by weight. However, the important molecules in the cell which absorb in the ultraviolet range are a) proteins, be they enzymes or structural proteins - perhaps membrane components, and b) nucleic acids, the DNA which contains the genetic information and the RNA's which are structural molecules, mediators in

*Research supported by U. S. Department of Energy, Research Cancer Development Award CA00466, and the National Cancer Institute Grant CA23096.

DISCLAIMER

protein synthesis and also take part in the transmission of genetic information.

How could we decide which of these targets is a critical one for the cell? A popular method of doing this is action spectroscopy, measurement of the efficiency of different wavelengths in producing a given biological effect. If the action spectrum is straightforward, and many of them are, one can match the action spectrum for the given biological effect with the absorption spectrum of different biological macromolecules to get an idea of which molecule is doing the absorption, and thus which is the chromophore or "target" for the resulting biological damage. As an example, in the 1930's action spectroscopy provided important information that DNA was the target for the action ultraviolet light in the case of the killing of bacteria. Very recently Rothman and Setlow have shown that DNA is the important target for the killing of mammalian cells by ultraviolet light.

What are the biological effects of damage to DNA? In the first place, as we have said, a cell can die. The second possibility is: the cell does not die, but is affected by mutation, a change in the DNA leading to altered cell properties. The third possibility is: no effect on the cell. Now how can this happen? Let us consider the biological properties of DNA. It contains information in the genetic code which is read in groups of three called triplets. Each triplet specifies a tRNA intermediate, which in turn specifies an amino acid which is assembled into a growing protein chain. Since there are four bases and $4^3 = 64$, there are 64 possible triplets vs. about 20 amino acids. The cell's solution to this is degeneracy (more than one triplet specifying the same amino acid). So even if a triplet, for example UUU, is mutated to UUC, both triplets specify the amino acid serine and thus the protein coded for by that DNA segment will not be changed. Second, related triplets frequently code for amino acids with similar size and charge. So even if the DNA triplet GUA were mutated to GCA resulting in substitution of the amino acid alanine for valine, the protein would likely show little structural or functional differences since alanine and valine are closely related amino acids. The third possibility is an alteration of a nonessential part of the protein. Most proteins are present in excess anyway, so the cell can limp along even with a true change in its DNA and in the protein for which that DNA coded.

How does one measure a mutation? Bacteria are frequently used and we will talk about them here in some detail as an example. Many bacteria can be grown on defined chemical media consisting only of salts, minerals, and a carbon source. Upon mutation to auxotrophy, cells must be supplemented with an exogenous material,

for example, amino acids, vitamins, a nucleic acid base and so forth. In the case of bacterial viruses, mutations can be detected by their lack of growth on certain host, their slow growth or the appearance of the plaques which they make on a plate of bacteria. If the cell does not grow on a defined medium, the problem of finding mutations is somewhat harder. In mammalian cells, for example, in which growth on defined media is rather difficult to achieve, it is just in the past few years that systems have been developed for examining mutants. Here again one generally screens for the lack of some metabolic enzyme by specially developed selection procedures.

Chemical Damage to DNA

We have discussed the biological consequences of the detrimental effects of UV on cells. What is some of the chemistry leading to these biological changes? When ultraviolet light impinges upon DNA, although there are many photoproducts formed, one of these has been implicated in much of the resulting biological damage (2). Among the multiplicity of products formed in DNA, are pyrimidine dimers (formed between two adjacent pyrimidines on the same DNA strand), hydrates of pyrimidines, adducts within a DNA molecule (mainly pyrimidine to pyrimidine), DNA-DNA cross-links, and a product for mainly in dry and frozen DNA originally termed the "spore photoproduct." Of these, the dimer has been shown to be largely responsible for death and mutation in the bacteria and in simple eucaryotes, and now evidence is accumulating that the same is true in mammalian cells.

What happens to a cell which receives damage to its DNA? Is the cell fated to death or mutation? Evidence now indicates this is not the case. Cells can repair damage to their DNA. There are at least three major modes of repair of DNA in biological systems. You might just imagine the possibilities. First is the reversal of the damage; the second mode is the cutting out of damage; and the third mode is one in which the cell procrastinates and tries to ignore the damage. We will talk about these mechanisms in more detail in this and in subsequent lectures. Much of what we know comes from E. coli. We will discuss not only what we know from bacterial cells, but also what we know and perhaps what we wish to extrapolate to higher cells.

What are the consequences of failure of repair? In bacterial cells, as we have said, cells can be mutated or die but what about people? Are there any implications that failure of DNA repair in people can lead to harmful effects? There are now a number of human diseases which have been implicated as resulting from lack

of DNA repair: one of these is xeroderma pigmentosum, which is the propensity to sunlight-induced skin cancer (3). Most of these individuals die before the age of about 20 from invasive tumors. Other such diseases are Fanconi's anemia and progeria.

PHOTOREACTIVATION

We have noted that there are three kinds of DNA repair. We will discuss the one which, at least in concept, is the simplest - simple reversion of the lesion called photoreactivation (4,5).

Photoreactivation was first found in 1949 by Dulbecco and by Kelner. They showed that UV-induced killing could be reduced by exposure to visible light after the ultraviolet. A decade later Rupert, in both the E. coli and in yeast, found that the active agent was a photoreactivating enzyme (PRE) and the substrate was ultraviolet-irradiated DNA. Rupert showed that the steps in this reaction were several: first, complex formation between PRE and UV'd DNA which did not require light, and then a repair step that was dependent on the presence of light. (Note that this reaction, unlike some which are known in photosynthesis, is not the activation of an enzyme by the light and in its subsequent action in an active state on a substrate. This enzyme must be complexed to the substrate when the light energy is supplied.) The Setlows showed that pyrimidine dimers in DNA were the photoreactivable lesion. They also provided evidence that the pyrimidine dimer was the only substrate. They showed that the product of the action of the photoreactivating enzyme on DNA were pyrimidine monomers. Photoreactivating enzymes are interesting not only because they catalyze a biological important repair step, but as unique proteins which require light for photolysis (6). Photoreactivating enzymes have been studied from a variety of procaryotic and eucaryotic sources. Most of photoreactivating enzymes have the following functions in common: first they have all been shown to be proteins, they require lignt for their function, they monomerize pyrimidine dimers, they have a molecular weight in the range of 35-40,000 and most are active in the wavelength range of 300-500 nm. It is the last of these properties which provides both interest and also a useful analytical tool.

The peaks of the action spectra of various enzymes range from about 360 nm to 380, 405 to even 435 nm, for the enzyme from Streptomyces griseus. The short-wavelength extent of the action spectrum is somewhat limited by the wavelengths at which one starts making dimers as well as photoreactivating them, that is about 300 nm. The long wavelength extent varies with the enzyme.

Many photoreactivating enzymes do not use light wavelength longer than about 500 nm. However, we have found that the human enzyme can use light which is between 500-600 nm and Giese has also found that the photoreactivating enzyme from Blepharisma can use light in this wavelength range.

One of the primary laws of photochemistry states that photochemical action occurs only when there is light absorption, so we would expect photoreactivating enzymes which act in the region 300 to say 500 or 600 nm to absorb in this region. Eker has found that the enzyme from Streptomyces griseus seems to contain an intrinsic chromophore which absorbs in the region where the Streptomyces enzyme is active. However, other photoreactivating enzymes, that from E. coli, that from the silverfish Thermobia domestica and perhaps the one from yeast, do not show any visible absorption in the region of the photochemical action. The solution to this dilemma was found by J. C. Sutherland and his postdoctoral fellow K. L. Wun (7). They found that the complex of E. coli with UV-irradiated DNA generated a new absorption which agreed in magnitude (molar extinction coefficient) and wavelength range with that predicted by the action spectrum of the E. coli enzyme acting in purified form in vitro or acting in the E. coli cell (in vivo). The new absorption was dependent on the presence of dimers in the DNA, and on complex formation, as it did not appear when PRE was added to irradiated DNA in the presence of so much salt that no complex was formed. The absorption also disappeared the same kinetics as dimers were monomerized.

For many years it was thought that photoreactivating enzyme was absent in mammalian cells, even though there were a few reports of biological photoreactivation of tumor formation in mice and of human erythema. In 1974 a photoreactivation enzyme was first isolated from human leukocytes (6,8,9). Since then Dr. Helga Harm has shown photoreactivating activity from rabbit, cow, cat and human cells. Photoreactivating enzyme from human cells has been characterized as a protein of a molecular weight of about 40,000 with action spectrum extending from about 300 to at least 600 nm.

If photoreactivating enzyme is present in these cells, do they use the enzyme to photoreactivate dimer lesions in their own DNA, or perhaps does the enzyme not have access to the DNA? A case of particular interest is that of human skin. Sunlight forms pyrimidine dimers in the DNA of human skin cells. If we wish to measure photoreactivation of dimers in skin, we would be faced with a problem: most measurements of repair, dimer excision, photoreactivation or whatever, depend on the use of

radioactive DNA. In order to have enough radioactivity to test, one would have to have a very radioactive human! A solution to this problem has been worked out in the laboratory of R. B. Setlow, using the technique developed in the laboratory of F. W. Studier at Brookhaven. The technique is electrophoresis in alkaline agarose. DNA extracted from cells is treated with an enzyme which specifically makes a nick beside every pyrimidine dimer. The DNA is then denatured and electrophoresed on the alkaline gels. DNA which contains dimers and therefore is nicked by the "UV-specific endonuclease" will be smaller and therefore migrate further into the gel. The absent of dimers, however, will be seen by the lack of nicking by the enzyme and by the presence of larger molecular weight DNA. Using such a technique we have been able to show that leukocytes (white blood cells) taken directly from humans do have dimers formed in their DNA and they do photoreactivate these dimers in a reasonably short exposure (about 20 to 40 minutes) to visible light.

What is the role of pyrimidine dimers in the induction of skin cancer by UV? We have developed a model system in our laboratory which we believe will allow us to examine the role of photoreactivation and of DNA repair in the prevention of the induction of skin cancer by ultraviolet light. This technique involves a conversion of normal human cells by ultraviolet light to cells which are "transformed" that is, with many properties of cancerous cells. We find that ultraviolet light, at rather small doses, produces this conversion of normal cells to transformed cells. Furthermore, an exposure of the cells to photoreactivating light immediately after the UV decreases the rate of conversion of the cells from normal to transformed (10).

Although much progress has been made in understanding the photobiology and photobiochemistry of photoreactivation, many outstanding problems remain. The first is the structural similarities and differences in photoreactivating enzymes. Do they reflect evolutionary similarities, divergence, or do they reflect perhaps true differences in function? For example, does the enzyme from Streptomyces, which seems to have its own intrinsic chromophore, have a different method of function from the enzymes from E. coli and yeast, which do not contain intrinsic chromophores?

Finally, we can ask some questions about photoreactivation in vivo. Since the reactivation seems to be specific for pyrimidine dimers, we can use its action as an analytical tool; that is, if a reaction is reversible by a true photoenzymatic reaction, the chemical lesion which lead to the biological damage

is likely to have been a pyrimidine dimer. Finally, we can explore the role of the photoreactivating enzyme in repair in human cells, determine the biological consequences of the failure of the enzyme to repair DNA.

REFERENCES

1. Jagger, J., Introduction to Research in Ultraviolet Photobiology, Prentice-Hall, Englewood Cliffs, New Jersey, 1967
2. Setlow, R. B., Science 153, 379-386 (1966).
3. Robbins, J. H., Karemer, K. H., Lutyner, M. A., Festoff, B. W. and Coon, H. G., Ann. Int. Med. 80, 221-248 (1974).
4. Symposium on Molecular Mechanisms in Photoreactivation, Photochem. and Photobiol. 25 (1975).
5. Setlow, J. K., Curr. Top. Radiat. Res. 2, 195-248 (1966).
6. Sutherland, B. M., Int. J. Cytol., in press (1978).
7. Wun, K. L., Gih, A., and Sutherland, J. C., Biochemistry 16, 921-924 (1977).
8. Sutherland, B. M., Nature 248, 109-112.
9. Sutherland, B. M., Runge, P. and Sutherland, J. C., Biochem. 13, 4710-4715 (1974).
10. Sutherland, B. M., Proceedings of the International Conference on Solar Carcinogenesis, Airlie House, in press (1978).