Purine insertase: a new enzyme

from Gerard O'Donovan

It is now generally believed that a large number of environmental agents cause damage to DNA. Even in the absence of environmental stimuli DNA has to cope with yet another type of damage, namely the formation of DNA apurinic/apyrimidinic sites (AP sites). These lesions are produced spontaneously by the loss of purine or pyrimidine bases from the DNA and also by the action of a specific class of enzymes known collectively as DNA N-glycosylases (previously known as N-glycosidases). Three distinct enzyme activities have been discovered to date. These N-glycosylases remove uracil (Lindahl *Proc. natn. Acad. Sci. U.S.A.* **71,** 3649; 1974), 3-methyl adenine (Laval *Nature* **269,** 829; 1977; Riazuddin & Lindahl, *Biochemistry* **17,** 2110; 1978), or hypoxanthine (Karran & Lindahl, *J. biol. Chem.* **17,** 5877; 1978) from DNA without perturbing the sugar-phosphate backbone.

Until recently these enzymes were called DNA N-glycosidases because traditionally the base-sugar bonds in nucleic acids which they cleave, were termed glycosidic bonds (Lindahl, *Proc. natn. Acad. Sci. U.S.A.* **71,** 3,649; 1974). However, the current rules for carbohydrate nomenclature (*Biochemistry* **10,** 3,983; 1971) state that a glycosidic bond is a linkage through oxygen obtained by replacement of the hydrogen atom of the hemiacetal hydroxyl group, while removal of the entire hydroxyl group results in a glycosyl bond (Riazuddin & Lindahl *op. cit*).

Deoxyribonucleases that act on DNA containing apurinic sites have been characterised from various sources including bacteria (Gossard & Verly *Europ. J. Biochem.* **82,** 321; 1978), plants (Thibodeau & Verly *J. biol. Chem.* **252,** 3,304; 1977) and human cells (Kuhnlein *et al. Nucl. Acids Res.* **5,** 951; 1978). These enzymes are part of a duplex DNA repair pathway in which the apurinic endonuclease (AP endonuclease) makes an incision in the vicinity of the apurinic lesion, then the apurinic lesion is excised by an appropriate exonuclease and the resulting gap is filled in with a DNA polymerase before closure with DNA ligase. The apurinic lesion presumably arose spontaneously, by reaction with chemicals or as the product of a DNA N-glycosylase, one of the enzymes described above which removes abnormal bases from DNA by hydrolysing the glycosyl bond.

Since excision repair is a multienzyme process it seems reasonable that a simpler and more straightforward pathway might exist. With respect to modified DNA, N-glycosylytic removal of abnormal bases from DNA to produce an AP site, with the concomitant reinsertion of the correct base, has been speculated on. In a recent issue of *Proc. natn. Acad. Sci. U.S.A.* (**76,** 141; 1979) Deutsch and Linn report the discovery of an enzyme activity from cultured human fibroblasts which seems to reinsert purines, but not pyrimidines, into DNA containing apurinic sites. The activity, which is partially purified (molecular weight 120,000) to be free from apurinic endonucleases, was first identified by its ability to bind specifically to partially depurinated DNA. Using radioactively labelled purines, Deutsch and Linn show that the free base is best utilised, and that the incorporation like the binding, is specific for DNA apurinic sites. Moreover, using depurinated synthetic polymers, they show that the insertion of purine bases seems to be template-specific, with guanine but not adenine being inserted into depurinated poly (dG-dC), and adenine but not guanine being inserted into poly (dA-dT).

The formation of a glycosyl bond would seem to require energy although the free energy of formation within a DNA molecule has not been measured directly. In the present study the reaction occurs in the absence of any obvious energy source. One suggestion offered by Deutsch and Linn for the free energy source in the case of a free purine substrate is the reforming of base pairs and stacking at the site of the reaction. With respect to an energy source, it is significant that another reaction in which the guanine base is utilised also occurs in the absence of energy. Here, the tRNA guanylating enzyme replaces the Q base of certain tRNAs with guanine (Okado *et al. Nucl. Acids Res.* **3,** 2,593, 1978; Farkas & Singh, *J. biol. Chem.* **248,** 7,781; 1977).

Clearly the most likely role for the AP endonucleases is in DNA repair. Such enzymes (purine base insertases) might be extremely active in repair during normal DNA replication. But because they are present in nature in such large amounts, almost universally, it is tempting to ascribe additional roles to the AP endonucleases. As pointed out by Deutsch and Linn it could be that the utility of base insertion may not be for DNA repair but rather for situations in which the generation of genetic diversity is desirable. In addition, the eukaryotic genome contains large numbers of modified bases which could be replaced (or inserted) as a regulatory or developmental signal. Such hypotheses are simple to formulate but difficult to prove; it will be of interest to see if future investigations unmask more exotic DNA N-glycosylases and perhaps related insertases.

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A surplus of carbon dioxide

from G. Skirrow

Two recent independent contributions (Brewer *Geophys. Res. Lett.* **5,** 997; 1978; Chen & Millero *Nature* **277,** 205; 1979), both based on analysis of Geosecs data, have outlined direct experimental evidence that part of the present, continuing, atmospheric CO_2 increase consequent on fossil fuel combustion is being transferred to the sea and that this process has probably been taking place for some time. Although it has always seemed reasonable to suppose that this transfer did occur, evidence has hitherto been mainly indirect and estimates of the extent of the exchange have relied on, for example, observations of the distribution of ^{14}C produced by nuclear bomb explosions.

The air–sea transfer of CO_2 is merely one facet, albeit an important one, of the global carbon cycle. Briefly, CO_2 is gained by the atmosphere through volcanic exhalations, by evasion from the sea and by respiration and combustion. It is lost from the atmosphere by invasion into the sea, by photosynthesis into the terrestrial and marine biospheres and by weathering processes leading to dissolution of, particularly, carbonate rocks and the transfer to the sea of HCO_3^--rich solutions by continental run-off. Within the oceans biogenic precipitation of $CaCO_3$ from the supersaturated surface waters (together with precipitation of some organic debris) transfers some carbon to the sediments, although some of the $CaCO_3$ dissolves during its descent in the oceans. Over geological periods of time sea-floor spreading makes the sediments available for reworking. A more detailed examination shows that the carbon cycle is intimately linked with those for other elements, notably calcium, oxygen and nutrients.

Although life forms on Earth have changed over the past few hundred million years, the general and continuous persistence of life has been taken to imply that, over this time, the various elemental cycles have never been very violently perturbed from

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0028-0836/79/0278—0121$01.00

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