ENDONUCLEASES SPECIFIC FOR APURINIC SITES IN DNA.

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ABSTRACT : Endonucleases specific for apurinic sites have been purified from bacteria, animals and plants. In eucaryotic cells, the enzyme is present in the non-histones of chromatin. The details of the *in vitro* repair of depurinated DNA by *E.coli* endonuclease VI, exonuclease III, DNA polymerase I and ligase, have been established experimentally.

In 1968, Strauss and Robbins (1) found, in a crude extract of *M.lysodeikticus*, an endonucleolytic activity on MMS-methylated DNA; the activity was not suppressed by heat-induced depurination and the authors suggested that separate enzymes might attack at apurinic sites and at sites of the methylated purines.

The work was resumed with *Escherichia coli* by two groups which used different substrates to purify the enzymes and came to what appeared as conflicting results : Goldthwait and his colleagues used MMS-alkylated DNA, whereas Verly and Paquette, who were more interested in apurinic sites, used alkylated-depurinated DNA. The two methods yielded different preparations : Verly *et al.* (2,3,4) purified an enzyme specific for apurinic sites with no action on alkylated sites, whereas Friedberg and Goldthwait (5) obtained a preparation active on alkylated bases which they named endonuclease II. This preparation was later found to be active on apurinic sites and Hadi and Goldthwait (6) thought mistakenly that the same enzyme, endonuclease II, was active on both substrates. This was the situation at the Squaw Valley meeting on the Molecular Mechanisms for Repair of DNA held in February 1974. Kirtikar and Goldthwait (7) then made the important discovery of a N-glycosidase activity in their endonuclease II preparation. In the mean-time, Verly and Rassart (8) had completely purified, from *E.coli*, the main endonuclease specific for apurinic sites. Kirtikar and Goldthwait (9) subsequently realized that their endonuclease II preparation was a mixture of enzymes; they were able to separate the N-glycosidase, for

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which they kept the name of endonuclease II because it still had some endonuclease activity, from an endonuclease specific for apurinic sites.

The confusion, arising from the work of Goldthwait's group, led many people to name endonuclease II the enzyme acting on apurinic sites. To stop this misnomer, we finally gave the name of endonuclease VI to the enzyme we had been studying since the late sixties (10).

Escherichia coli endonuclease VI is thus the first endonuclease specific for apurinic sites which has been described (2,3); it has no action on normal DNA strands or on alkylated sites (4). The purified enzyme was found to be a monomeric protein of 32,000 daltons (8) which needs Mg^{++} to be active and is rather thermolabile. The enzyme hydrolyzes a phosphoester bond on the 5' side of the apurinic site (which might be not immediate neighbour of the base-free deoxyribose) leaving a 3'-OH and a 5'-phosphate (10,11).

According to Ljungquist et al. (12), endonuclease VI is responsible for only 90 % of the activity on depurinated DNA in E.coli; the remaining 10 % are due to a second enzyme, endonuclease IV, which is stable at 45°C and does not need Mg^{++}.

Weiss (13) published that endonuclease II is exonuclease III; what he should have written is not endonuclease II but possibly endonuclease VI. We indeed found exonuclease III in a preparation of endonuclease VI which was devoid of endonuclease II activity (10,11). Ljungquist et al. (12) found bacterial mutants in which exonuclease III and endonuclease VI had disappeared simultaneously. However, Kirtikar et al. (14) have separated exonuclease III from an endonuclease specific for apurinic sites which they present as if it were our endonuclease VI (and to which they give the name of endonuclease VI), but which Ljungquist and Lindahl (15) think to be rather endonuclease IV. The question whether exonuclease III and endonuclease VI are the same enzyme is thus not yet settled and it is much better to keep separate names for the two activities. Should they finally be recognized by everyone as the same enzyme, the name of endonuclease VI should be retained preferentially to exonuclease III : exonuclease III has been waiting many years for a biological function whereas endonuclease VI is most important for the repair of apurinic and apyrimidinic sites in DNA and has been, moreover, the first enzyme of its class to be discovered. We shall see later that the biological action of exonuclease III is likely quite accessory and, indeed, not absolutely necessary.

Depurinated DNA has been repaired in vitro by incubation with endonuclease VI, DNA polymerase I and the 4 dNTP, ligase

and its coenzyme (16); the endonuclease VI preparation contained exonuclease III. The details of the repair have been worked out (10,12) : endonuclease VI hydrolyzes a phosphoester bond on the 5' side of the apurinic site leaving a 3'-OH and a 5'-phosphate; exonuclease III removes a few nucleotides in the 3'-5' direction leaving the apurinic site in the DNA molecule (which prevents ligase from closing immediately the nick); DNA polymerase I, starting from the 3'-OH, fills the gap due to exonuclease III, excises the apurinic site in a di- or trinucleotide, then catalyzes the translation of a nick in the 5'-3' direction; the nick is closed by ligase. The antiligase activity of exonuclease III is apparently not absolutely required since apurinic sites are repaired by endonuclease IV in absence of exonuclease III (12).

Lindahl and Nyberg's paper (17) on the spontaneous depurination of DNA led us to suggest (18) that an endonuclease specific for apurinic sites must be present in every cell. We have isolated an endonuclease for apurinic sites from Bacillus stearothermophilus (19); its properties are closer to those of E.coli endonuclease IV than endonuclease VI. An endonuclease specific for apurinic sites was also purified from rat liver (20,21) and plant embryos (22). An endonuclease for apurinic sites was detected in all the rat tissues that were tested, in human fibroblasts, HeLa cells, in roots and leaves of higher plants, in fungi and algae (23). Other authors have also purified endonucleases for apurinic sites from bacteria, plant or mammalian tissues.

In eukaryotic cells, the endonuclease specific for apurinic sites is mostly located in chromatin. It is a non-histone protein which, when integrated in the chromatin architecture, has little activity on a foreign DNA; this suggests an orientation to work on chromatin DNA. These observations, first made in plant tissues (22,23), have since been confirmed using rat liver.

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