Short Communication

Interaction of cis-diamminedichloroplatinum(II) with φX174 DNA

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The binding of cis platin (cis-diamminedichloroplatinum (II)) to cellular DNA is thought to be the major mechanism of its antitumour activity (Harder & Rosenberg, 1970; Pasco & Roberts, 1974a,b; Roberts & Thompson, 1979; Rosenberg et al., 1969). The drug produces DNA-DNA and DNA-protein cross-links (Pasco & Roberts, 1974a,b; Zwelling et al., 1978a,b). In bacteria cis platin has been shown to inhibit cell division (Rosenberg et al., 1965) and also reduces the viability of DNA repair mutants to a greater extent than in the wild-type organisms (Beck & Brubaker, 1973). Work with isolated DNA of lambda bacteriophage shows that cis platin damages the viral DNA by alteration of the helical structure (Kelman & Buchbinder, 1978; Mong et al. 1980a,b).

Covalently closed circular DNAs from bacteriophages and plasmids have been used as models to investigate the interaction of cis platin with DNA (Cohen et al., 1979; Mong et al., 1980a,b). These studies have in the main used agarose gel electrophoresis to characterise the various forms of DNA. The present study, rather than assessing the molecular topography of closed circular DNA, measures the biological activity of φX174 DNA as regards its ability to replicate and produce progeny phage and is a sensitive measurement of the direct consequences of DNA damage. The effect of cis platin on the biological activity of φX174 DNA in both its single-stranded and replicative form in wild-type and excision repair-deficient strains of E. coli is presented.

Bactotrypton and bactoagar were purchased from Difco (Detroit, Michigan, USA). Yeast extract was obtained from Oxoid (Basingstoke, UK). E. coli DNA, cis platin and other common laboratory reagents were purchased from Sigma (Dorset, England). The single-stranded form of φX174 was prepared according to Blok et al. (1967). The double-stranded (RF) form of φX174 DNA was isolated as described by Baas et al. (1981). The E. coli strains AB1157 (wild-type), AB1884 (uvrC−), AB1886 (uvrA−) were obtained from Dr J.A. Brandisma (Department of Biochemistry, State University of Leiden, The Netherlands) and maintained on nutrient agar plates.

DNA and cis platin were allowed to react at a specified drug-nucleotide ratio at room temperature. In most of the experiments the DNA was a mixture of E. coli and φX174 DNA. In such experiments the quoted D/N ratio was with respect to E. coli where the concentration was calculated from the relationship $E_{260}^m$ (nucleotides) = $6600 M^{-1} cm^{-1}$ (Waring & Henley, 1975). In these experiments the concentration of the φX174 DNA was between 0.4–1.0 µg cm−2 and is quoted for each case. In this range the surviving fraction measured for the phage is independent of the initial concentration but in the absence of E. coli the D/N ratio refers to the single-stranded or the replicative form (RF) of the φX174 DNA. The concentration of the φX174 DNA was determined spectrophotometrically using $E_{260}^m$ (nucleotides) = $6400 M^{-1} cm^{-1}$ (Sinsheimer, 1959).

Spheroplasts were prepared by removing part of the cell wall with lysozyme and EDTA according to Guthrie and Sinsheimer (1963). At frequent intervals 0.1 ml of sample was withdrawn from the cis platin-DNA reaction system and diluted 10-fold with ice-cold 0.25 M tris buffer before the determination of its biological activity according to Blok et al. (1967). Briefly, 0.1 ml of DNA samples was mixed with an equal volume of freshly prepared spheroplasts from E. coli AB1157 (wild type) or with the excision repair mutant strains AB1886 (uvrA−) and AB1884 (uvrC−). After 10 min at room temperature 0.8 ml of liquid broth prewarmed to 37°C was added. The mixture was shaken and incubated in a 37°C water bath for 1.5 h. For the replicative form of the DNA the incubation time was at least 2 h. Then the active viruses were released by osmotic shock and the phage titrated using E. coli C as the indicator organism and the plates scored for the plaques.
Table I  Cytotoxic effect of cis-platin at various concentrations on the survival of φX174 DNA at constant D/N ratio

| DNA conc. (mM) | DNA type | cis-pl conc. (mM) | D/N ratio | t₃₇ (min) |
|---------------|-----------|------------------|-----------|-----------|
| 0.30          | RF + E. coli | 0.10             | 0.33      | 0.71 (±0.05; 2) |
| 0.012         | RF        | 0.004            | 0.33      | 22.1 (±3.2; 2) |
| 0.006         | RF        | 0.002            | 0.33      | 52.4 (±7.5; 2) |
| 0.003         | RF        | 0.001            | 0.33      | 98.6 (±14.1; 2) |
| 0.006         | s.s.      | 0.002            | 0.33      | 43.2 (±7.1; 3) |
| 0.003         | s.s.      | 0.001            | 0.33      | 58.3 (±10.9; 2) |
| 0.0015        | s.s.      | 0.0005           | 0.33      | 78.3 (±9.6; 1) |

In parenthesis, s.e. of the mean and the number of experiments. RF is the double stranded (replicative form) of φX174 DNA, s.s. is the single stranded form of φX174 DNA.

Phage survival curves were obtained by plotting the logarithm of the fraction of surviving virus against time. The curves were exponential and thus the t₃₇ value at which 37% of the viral population survives is a measure of the average of one lethal inactivation per DNA molecule.

Table I summarises some of the results obtained with cis platin under different experimental conditions, at a constant drug/nucleotide (D/N) ratio. The data are obtained from complete survival curves (similar to those shown in Figure 3) which were fitted by a least squares method and correspond to an exponential decay, implying a Poisson distribution of the lethal damage among the DNA molecules. It is clear from Table I that single- and double-stranded DNA differ in their dependence on the amount of DNA and cis platin present during the reaction. At the given D/N ratio, for the double-stranded form of the DNA, the drug toxicity is proportional and for the single-stranded DNA is only slightly dependent on the absolute concentration. Figures 1 and 2 show the relationship between the drug/nucleotide (D/N) ratio and the toxicity of cis platin in terms of its t₃₇ value. This was done at a constant DNA concentration by varying the cis platin concentration. For the double-stranded φX174 DNA this relationship is exponentially proportional to the drug concentration as shown by Figure 1 whereas for single-stranded DNA there is a direct relationship between the two parameters as depicted in Figure 2. For the double-stranded DNA the relationship is described by the equation:

\[ Y = a + bX \]

where \( X = \frac{D}{N} \) ratio, \( Y = \log(t_{37} \text{ in min}) \), \( a = 4.788 \) and \( b = -16.95 \).

The t₃₇ value for a D/N ratio of 0.5 in Figure 1 could not be measured accurately, because at this ratio the process is extremely fast (~6 sec) and hence was excluded from the least squares analysis carried out on the other data. Varying the DNA concentration did not change the relationship as mentioned above although the absolute values of the t₃₇ differ. Figure 3 shows the cytotoxic effect of cis platin on the RF-DNA as measured with spheroplasts from wild type and a repair-deficient
The results clearly demonstrate that the lesions produced by cis-platin are very efficiently repaired by a uvrA-dependent repair pathway. Identical results (within error) are obtained with a uvrC− mutant of the excision repair pathway (data not shown).

The results show that cis-platin is capable of inactivating both single-stranded and double-stranded ΦX174 DNA and corroborates previous results obtained with double-stranded lambda DNA (Filipski et al., 1979). However, the cytotoxic effect of the drug is quite different for single- and double-stranded DNA. Although the results produced (Figure 1 and 2) establish that the reaction between cis-platin and DNA leading to lethal damage is dependent on the drug concentration, it is proportional for single and exponential for double-stranded DNA.

Single-stranded ΦX174 DNA lesions cannot be repaired in its bacterial host and probably the reaction of the drug leads to the formation of adducts between DNA and cis-platin, but not intermolecular crosslinking. Furthermore, a high fraction or even every lesion will be lethal as is found for example with AAF adducts (Lutgerink et al., 1984) and apurinic sites (Lafleur et al., 1981). Therefore, the amount of drug will proportionally increase the number of inactivated DNA molecules, which is reflected by the reciprocal of the t37 value.

For double-stranded DNA a different picture emerges. Here several types of damage, including crosslinks, are formed (Zwelling et al., 1978a,b; Boutour & Macquet, 1978; Horacek & Drobnik, 1971) of which at least part can be repaired by one of the different repair mechanisms of the bacterial host.

The repair capability was investigated by the transfection of the RF-DNA to E. coli spheroplasts which are deficient in one or more genes of the DNA excision-repair system. From these experiments it can be concluded that the majority of the lesions which are induced by the drug are removed by a repair system which is dependent on the proteins encoded by the uvrA and uvrC genes as shown by Figure 3. This work agrees with that of Beck and Brubaker (1973) with repair deficient mutants of E. coli. However, in DNA with a relatively low modification of its bases (D/N ratio=0.01) almost all the damage can be repaired in the wild-type spheroplasts in contrast to the DNA with much higher modification (D/N=0.2). These results also indicate that for cis-platin the
majority, if not all, of the lesions in double-stranded DNA are due to alterations of the helix structure as has been shown earlier (Kelman & Buchbinder, 1978, Mong et al., 1980a), because the uvrA and uvrC gene products are required for the recognition of a variety of these lesions which are likely to cause a local distortion of the DNA helix (Hanawalt et al., 1979). However, only part of these lesions are lethal events, while most of them (depending on the number of the modified bases) can be repaired in the φX174 RF-DNA.

Furthermore, we have found that the cytotoxic effect of cis platin is not only determined by the D/N ratio as it is sometimes assumed, but also by the absolute amounts of DNA and cis platin available during the reaction (see Table I). These effects can probably best be explained by assuming 2nd order kinetics of the reaction for cis platin with DNA, i.e. rate \( \approx [\text{DNA}] \times [\text{cis platin}] \) which confirms with data obtained by other methods (Kelman & Buchbinder, 1978; Zwelling et al., 1978a).

In summary, cis platin is capable of inactivating both single- and double-stranded φX174 DNA. The results also indicate that the excision-repair system is efficient in repairing the lesions produced in double-stranded DNA but the unrepaired lesions which remain are probably responsible for the toxic effects of cis platin. We conclude further, that a suitable viral transfection assay is a powerful tool in analysing the biological consequences of drug-DNA interactions.

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