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ELECTROPHORETIC ANALYSIS OF DNA-ACTIN LINKS INDUCED BY CIS- AND TRANS-DIAMMINEDICHLOROPLATINUM (II)

The aim of this paper is to investigate the effects of the platinum compounds: cis- and trans-diamminedichloroplatinum (II) on the interaction of DNA and actin *in vitro*. DNA, isolated from the peripheral blood leucocytes and actin, obtained from Sigma Chemical Co., have been used in the experiment. The investigations have been carried out with electrophoretic analysis method. It has been found that electrophoretic mobility of DNA was reduced after the incubation with cis- and trans-diamminedichloroplatinum (II) with r_i equal 0.5, 1, 2. In the case of the incubation samples containing cis-diamminedichloroplatinum and actin, it has been found that the electrophoretic mobility of DNA was also reduced. The electrotransfer of actin has shown a lower electrophoretic mobility of protein in incubation samples containing cis-diamminedichloroplatinum. These observations prove that DNA-actin crosslinks have been produced by cis-diamminedichloroplatinum.

INTRODUCTION

Cis-diamminedichloroplatinum (II) (cis-DDP) is a commonly used anticancer drug, while trans-diamminedichloroplatinum (II) (trans-DDP) does not show any properities that could permit its use in clinical treatment. Probably, this different biological activity of DDP isomers is the effect of their different ways of reacting with DNA which, as a number of experiments have shown [1, 2, 4, 5], is the main receptor of DDP in a cell. Platinum compounds produce the links of DNA–DNA, DNA–protein, protein–protein [11]. The proteins participating in DNA–protein links belong to the proteins of nuclear matrix [6, 7, 12] – a structure of a cell nucleus, whose basic function is a spatial „arrangement" of DNA [14]. The experiments carried out on the Chinese hamster ovary cells (CHO) have shown that one of the proteins of nuclear matrix which takes part in the links induced by cis-DDP is actin (45 kDa, pI 5.4) [12].

This paper presents an attempt to produce crosslinks DNA-actin by cis-DDP and trans-DDP *in vitro* conditions. The assessment of the lowering of electrophoretic mobility of DNA linked with protein after the incubation with cis-DDP and trans-DDP („band shift" assays) has been used as the criterium of the identification of the produced links. Changes in the electrophoretic mobility of the proteins participating in the analysed links have been shown by a transfer to nitrocellulose.

MATERIAL AND METHODS

Cis-DDP, trans-DDP and actin were obtained from Sigma Chemical Co., DNA was isolated from the peripheral blood leucocytes have been used in the experiment. The blood has been collected on anticoagulant in a local slaughtery.

Stock water solutions of cis-DDP and trans-DDP of 0.2 mg/ml concentration have been used in the experiment. Actin was dissolved in buffer solution containing: 1 M Tris-HCl pH 7.5, 0.1 mM ATP, 0.1 mM $CaCl_2$ (c = 1 mg/ml). DNA was isolated from the peripheral blood leucocytes [8]. The obtained preparation was then dissolved in buffer TE (10 mM Tris-HCl, 1 mM EDTA, pH 7.8). The concentration of the obtained DNA preparation was spectrophotometrically determined on Spectromom 195 D. Purity of the DNA preparation was assessed by giving the value of the ratio A_{260}/A_{280} [15].

All the electrophoretic separation have been carried out in 1% agarose gel in TBE buffer (0.045 M Tris-borate, 0.001 M EDTA, pH 8.0). Etidium bromide was being added to TBE buffer till the final concentration of 0.5 μg/ml. The electrophoretic separations have been carried out at the voltage that gives the electric field intensity of approximately 5 V/cm. Gels have been analysed in light of 254 nm wave length.

Electrotransfer of protein to nitrocellulose (0.2 μm) was carried out in a buffer solution containing: 330 mM glycine and 25 mM Tris-HCl pH 8.3. The electrotransfer was carried out for about 15 h at the electric field intensity of 500 mA. Nitrocellulose was stained with 0.25% solution of amido black in 50% ethanol and 10% acetic acid. After the visualization of protein had been, nitrocellulose was destained with water : ethanol : acetic acid solution (5 : 4 : 1) [3, 12].

DNA has been incubated (37°C, 18 h) with different doses of cis-DDP and trans-DDP defined as r_i. Molar ratio DNA : actin in incubation samples was equal 1 : 1.

RESULTS AND DISCUSSION

The obtained DNA preparation had a high degree of purity. Ratio A_{260}/A_{280} equalled 1.75 (A_{260}/A_{280} for a standard preparation is 1.8). DNA was incubated with cis-DDP and trans-DDP in three doses r_i: 0.5, 1, 2 (r_i – the ratio of the quantity of platinum molecules to the number of molecules of DNA nucleotides in incubation mixture). Incubation of DNA with cis-DDP and trans-DDP causes a reduction of electrophoretic mobility of DNA in agarose gel (Fig. 1). The reduction of the mobility of DNA is proportional to the used doses of platinum compounds (the larger dose of a platinum compound, the more visible is the reduction of DNA mobility). As the earlier experiments have shown, the reduction of electrophoretic mobility of DNA is caused by disorders in II-row DNA structure which occur because of the addition of platinum compounds. The investigations carried out on oligonucleotides have shown that both, cis-DDP and trans-DDP cause the unwinding and shortening of DNA helix of about 50% of the length of an undamaged molecule [16]. In addition to the unwinding of the helix and the disorders in II-row structure, coordinate binding of platinum may cause bending or twisting of the helix axis. The method of measuring electrophoretic mobility has been recently in the examination of structural changes of DNA according to the number of produced adducts of platinum. In double-strand oligonucleotides containing 1,2-intrastrand links cis-DDP d(GpG) and d(ApG) and 1,3-intrastrand d(GpXpG), the presence of bends in DNA chain at the angles of 32°, 34°, 35°C has been found [1]. The disorders which occur after the addition of trans-DDP are different. The occurrence of bends in DNA helix in such cases is most probably caused by the presence of labile sites where bends with angles which are hard to define are formed [16].

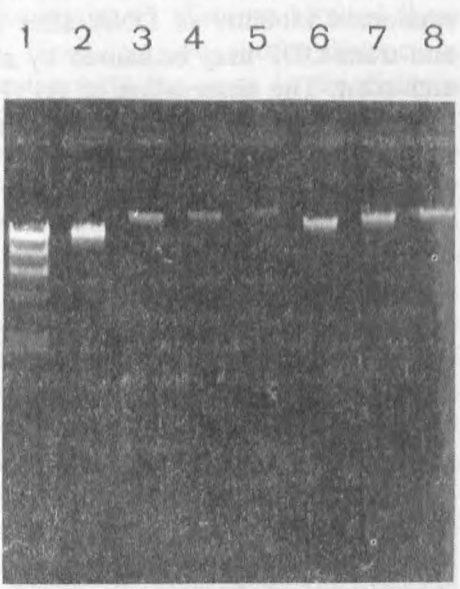

Fig. 1. Agarose gel fluorescence patterns of DNA treated (37°C, 18 h) with cis-DDP or trans-DDP

1 – λ phage Hind III standards, 2 – DNA, 3 – DNA + cis-DDP $r_i = 0.5$, 4 – DNA + + cis-DDP $r_i = 1$, 5 – DNA + cis-DDP $r_i = 2$, 6 – DNA + trans-DDP $r_i = 0.5$, 7 – DNA + trans-DDP $r_i = 1$, 8 – DNA + + trans-DDP $r_i = 2$

Electrophoretic analysis of incubation samples of DNA and actin with cis-DDP or trans-DDP have shown changes in the mobility of both, the protein and DNA. Changes in electrophoretic mobility of DNA are visible only for the samples containing cis-DDP (Fig. 2, lanes 3, 4, 5). The incubation of samples which contain trans-DDP show electrophoretic mobility approximating the control sample (DNA + actin). The reduction of the electrophoretic mobility of DNA after the incubation (37°C, 18 h) with actin and cis-DDP may reflect the formation of DNA-protein complexes. Such complexes, because of their greater mass, migrate more slowly than DNA and DNA incubated with actin. The lack of changes in the electrophoretic mobility of DNA after the incubation (37°C, 18 h) with actin and trans-DDP may be caused by non-formation of the link between DNA and actin. The observation of gel (Fig. 2) also shows that in these samples (lanes 6, 7, 8) the addition of trans-DDP to DNA has not occurred.

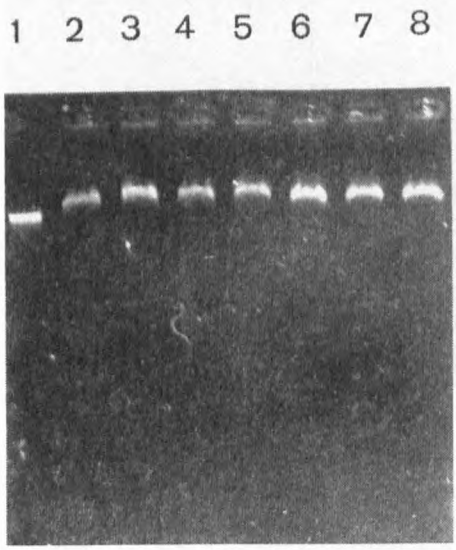

Fig. 2. Agarose gel fluorescence patterns of DNA treated (37°C, 18 h) with actin cis-DDP or trans-DDP

1 – DNA, 2 – DNA + actin, 3 – DNA + + actin + cis-DDP $r_i = 0.5$, 4 – DNA + + actin + cis-DDP $r_i = 1$, 5 – DNA + actin + + cis-DDP $r_i = 2$, 6 – DNA + actin + + trans-DDP $r_i = 0.5$, 7 – DNA + actin + + trans-DDP $r_i = 1$, 8 – DNA + actin + + trans-DDP $r_i = 2$

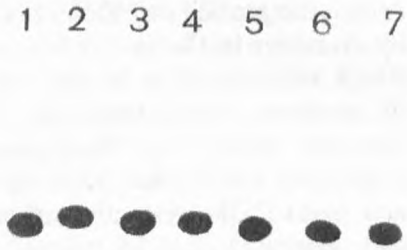

Fig. 3. Electrotransfer of actin after incubation (37°C, 18 h) with DNA and cis-DDP or trans-DDP (schema)

1 – DNA + actin, 2 – DNA + actin + + cis-DDP $r_i = 0.5$, 3 – DNA + actin + + cis-DDP $r_i = 1$, 4 – DNA + actin + + cis-DDP $r_i = 2$, 5 – DNA + actin + + trans-DDP $r_i = 0.5$, 6 – DNA + actin + + trans-DDP $r_i = 1$, 7 – DNA + actin + + trans-DDP $r_i = 2$

Electrotransfer to nitrocellulose of actin present in incubation samples has revealed changes in the rate of protein migration. These changes are clearly visible in the samples in which cis-DDP has been used (Fig. 3, lanes 2, 3, 4).

Cis-DDP is a commonly used now drug in treatment of many cancer: ovarian, testicular, bladder and among others [2]. It is not clear which of the ways of cis-DDP with DNA interaction is responsible for anticancer activity of this compound. There are reasons to believe that therapeutic qualities of cis-DDP are connected with the formation of 1,2-intrastrand links d(GpG) and d(GpA) [9]. These links make respectively 65% and 25% of cis-DDP adducts induced *in vitro* [1, 2]. Trans-DDP does not produce such a type of links because of the distribution of the reactive groups Cl (distance between these groups in a trans-DDP molecule 4,5 Å, if we compare, similar distance in cis-DDP molecule is 3,3 Å and is almost exactly the same as the distance between two adjoining bases in a double DNA helix) [13].

The links with DNA and proteins are responsible for antitumour activity of platinum compounds. The role of crosslinks DNA-protein, induced by platinum compounds, is a still little known problem when compared with biological activity of platinum complexes. This problem for years has been left out by research workers on account of practical difficulties which appeared when this type of addition had been attempted. In the recent years there has appeared a number of papers relating to the participation of DNA-protein crosslinks in the biological activity of platinum compounds. These experiments also provide a lot of new information on the issue of protein structure of cell nucleus. Proteins which participate in crosslinks induced by platinum compounds are separated and identified with great care [6, 7, 11, 12].

Our earlier experiments have shown that after the incubation of cell nuclei with cis-DDP and trans-DDP different proteins attach themselves to DNA. It has been found that after the incubation of cell nuclei with cis-DDP, protein of molecular weight 19 kDa gets attached to DNA, but it does not attach itself after incubation with trans-DDP [10]. This research requires further investigation. It is certain that it will bring a lot of interesting information on the issue of biological activity of platinum compounds, especially those ones which show anticancer activity, and it will also extend our knowledge about the structure and functions of cell nucleus.

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ANALIZA ELEKTROFORETYCZNA WIĄZAŃ DNA-AKTYNA INDUKOWANYCH PRZEZ CIS- I TRANS-DIAMMINODICHLOROPLATYNĘ (II)

Celem pracy jest zbadanie wpływu związków platyny: cis- i trans-diamminodichloroplatyny (II) na interakcję DNA i aktyny *in vitro*. W doświadczeniu wykorzystano DNA wyizolowany z leukocytów wieprzowej krwi obwodowej oraz aktynę firmy SIGMA. Badania były prowadzone metodą analizy elektroforetycznej. Stwierdzono zmniejszenie ruchliwości elektroforetycznej DNA po inkubacji z cis- i trans-diamminodichloroplatyną (II) przy r_i równym 0,5, 1, 2. W przypadku prób inkubacyjnych zawierających cis-diamminodichloroplatynę i aktynę stwierdzono zmniejszenie ruchliwości elektroforetycznej DNA. Elektrotransfer aktyny uwidocznił zmniejszenie ruchliwości elektroforetycznej białka w próbach inkubacyjnych zawierających cis-diamminodichloroplatynę. Obserwacje te świadczą o wytworzeniu wiązań krzyżowych DNA-aktyna przez cis-diamminodichloroplatynę.