Estimation of the Extent of DNA Platination after Interaction of *cis*-DDP with DNA and Chromatin

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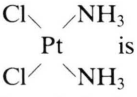
The aim of this work was to compare the kinetics of platinum binding to "naked" DNA and DNA remaining in a nucleoprotein complex. The incubation of chromatin and DNA from calf thymus was carried out with *cis*-DDP at different concentrations and incubation times. The coefficient r_b/μmolPt/μmolDNA/ of platinum binding to DNA was determined and the kinetics of *cis*-DDP binding to particular preparations was found and compared. The presence of chromosomal proteins decreases the extent of DNA platination. Hence it can be suggested that this drug induces the process of chromatin aggregation and condensation which in turn reduce the release of the total pool of DNA.

Introduction

Cis-diamminedichloroplatinum/II//  is a well known anticancer drug introduced to clinical research in 1972. Biological effects of this compound on the cell indicate its direct action with the genetic material. The DNA molecule is preferentially attacked by this compound and nitrogen bases are the main platination sites [1–3].

Among platinated derivatives mono- and bifunctional reaction products were stated to be present [4–7]. The possible formation of bifunctional compounds in case of the interaction of *cis*-DDP with DNA leads to intra- and interstrand crosslinks [8–11]. Structural and physicochemical changes of DNA formed following platination [12–18] modify the functional properties of DNA. It is well known that DNA in the eukaryotic cells occurs as a highly organized nucleoprotein complex and that this form of DNA is effected by *cis*-DDP.

Contrary to numerous experiments concerning the influence of *cis*-DDP on DNA and its components, the data on the interaction with chromatin are very limited. Some observations point out that the gross structural modifications of DNA brought about by its reaction with platinum compounds are the same for "naked" DNA and for its nucleoprotein complex [19]. Although it was confirmed that platinum can bind with DNA, nonhistone [20] and histone [21, 22] proteins, the role of proteins in this process has not been fully elucidated yet. It is not clear whether the chromosomal proteins are only a target or protection connected with their structural function. Preliminary comparative investigations of the platination of "naked" DNA and the DNA in a nucleoprotein complex may provide, though only to some extent, general data for the estimation of the protein participation in this process.

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Materials and Methods

Calf thymus obtained from slaughter house in Łódź directly after killing was used for experiments. Cell nuclei were isolated according to the method of Alffrey and Mirsky [23] and chromatin by Spelsberg and Hnilica's method [24]. DNA preparations were isolated by mild phenol method according to Gieorgiew and Struczkow [25].

The purity of DNA and chromatin preparations was determined

- spectrophotometrically by measuring spectra in the range of 230–300 nm
- chemically by calculating DNA content by Burton's method [26], protein by Lowry's *et al.* method [27] and RNA by Schneider's method [28].

Chromatin and DNA preparations of the concentration 1.5 mg/ml were incubated with chosen *cis*-DDP concentrations /0.05 mM, 0.25 mM, 0.5 mM/ for 0.5, 1, 2, 18 and 24 h. After incubation all the preparations were dialyzed to remove the excess of *cis*-DDP. In order to release DNA from chromatin previously incubated with *cis*-DDP, NaCl was added until the concentration 1 M, SDS up to 1% concen-

tration, proteinase K until 100 μg/ml and then 2 h incubation at 37 °C was carried out. Next the deproteinization was done according to Sevag. 5 mM *cis*-DDP solution in 5 mM $NaClO_4$ pH 6.4 was preincubated for 72 h at 37 °C. Platinum concentration was measured by the stannous chloride method determining absorption at 405 nm [29].

Results and Discussion

The data on the *cis*-DDP interaction with chromatin are very limited. Although the occurrence of the DNA-protein and protein-protein crosslinks has already been confirmed, the role of chromosomal proteins in the platination process of DNA remaining in the nucleoprotein complex still needs clarification.

In an attempt to approach the elucidation of this problem we compared the r_b values estimated for *cis*-DDP treated "naked" DNA and chromatin as well as DNA released from the second one. The results obtained at different *cis*-DDP concentrations are shown as dependence of r_b values and incubation time.

V_o values calculated for platination process of chromatin clearly pointed out that this reaction depends only on chromatin properties. Both "naked" and "released" DNA reacted with *cis*-DDP at similar rates. This reaction was effected not only by DNA properties but *cis*-DDP concentration as well, and the acceleration of platination process could be observed. The data on the platination of "naked" DNA indicate that the increase of the incubation time (0.5–24 h) and *cis*-DDP concentration (0.05–0.5 mM) enhances this process. None of the presented kinetics reaches the saturation point under the conditions used in this experiment. Taking into account the lowest *cis*-DDP concentration (0.05 mM) and the shortest (0.5 h) incubation time as well as the highest concentration (0.5 mM) and 24 h incubation a 10-fold increase of the r_b value can be observed. In case of "naked" DNA the effect of changing *cis*-DDP concentration is noteworthy at the shortest (0.5 h) incubation time. The change of *cis*-DDP concentration from 0.05 to 0.5 mM causes a 4-fold more intensive platination. The results obtained for the chromatin were very different both qualitatively and quantitatively. After 18 h all the curves tend to reach plateau.

When we compared the results of measurements performed under extreme experimental conditions only a 3-fold increase of the r_b value was noted.

The results obtained for DNA released from chromatin treated with *cis*-DDP seem to be quite different. For the two lower doses (0.05 and 0.25 mM) the curves are similar to the ones for chromatin *i.e.* the extreme r_b values are alike and the

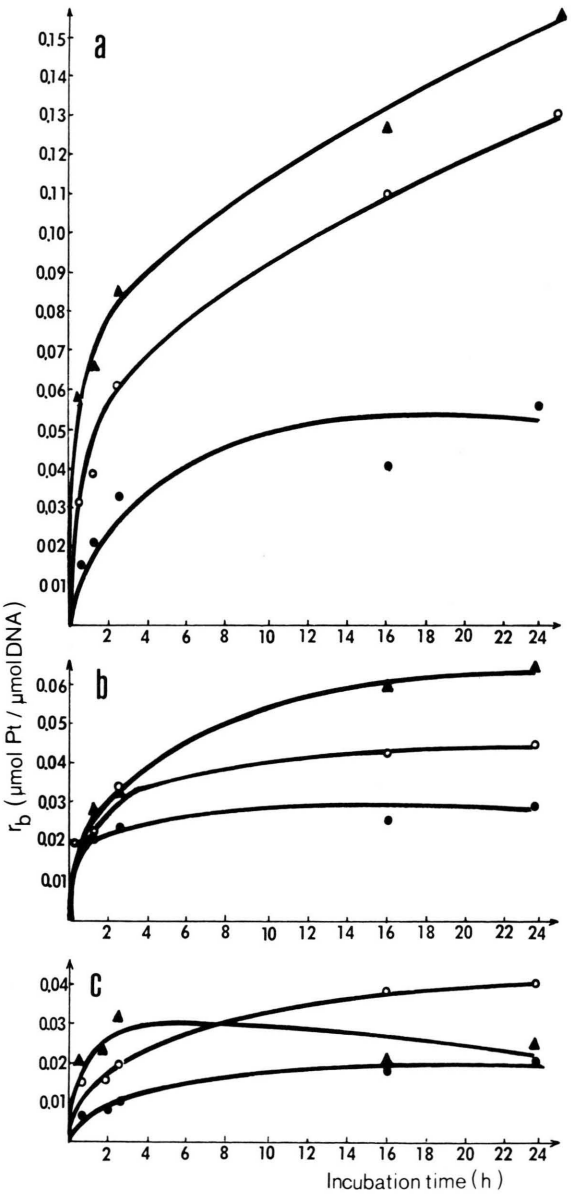

Fig. 1. Kinetics of the reaction between *cis*-DDP and a) "naked" DNA, b) chromatin, c) "released" DNA, as a function of *cis*-DDP concentration (0.05 mM ●—●—●, 0.25 mM ○—○—○, 0.5 mM ▲—▲—▲).

curves tend to reach saturation point. The highest r_b was observed at the concentration 0.25 mM and 24 h incubation. The highest r_b at the 0.5 mM *cis*-DDP was stated after 2 h incubation and the longer incubation the lower the coefficient. Comparing the r_b values for DNA released from chromatin and "naked" DNA some specific changes were found. The r_b values for the "released" DNA at all *cis*-DDP concentrations and short incubation time constitute half of the r_b values determined for "naked" DNA. Further increase of incubation time and *cis*-DDP concentration decreases the ratio of above mentioned r_b values to one third. Therefore the presence of proteins seems to decrease DNA platination 2–3-fold depending on the condition of experiment.

The results obtained for the "naked" DNA and chromatin indicate that are no drastic changes of r_b at low *cis*-DDP concentrations and short incubation times. The r_b values determined for chromatin and "naked" DNA at higher dose of drug and longer incubation times were 2.5-fold lower for chromatin than for "naked" DNA.

It can be stated that DNA is the primary target for *cis*-DDP action, especially at short incubation times at which no essential conformational changes of nucleoprotein complex are observed. Such results can neither confirm nor exclude the process of protein platination. However, the differences in the r_b values determined for chromatin and "released" DNA indicate some protein platination as the effect of *cis*-DDP interaction with nucleoprotein complex. The decrease of r_b for the "released" DNA at 0.5 mM *cis*-DDP and 18 and 24 h incubation can suggest that under the influence of this compound the processes of wide condensation of chromatin superhelical structure and aggregation take place thus restraining the release of the whole pool of DNA. This is confirmed by the decreased efficiency of "released" DNA from chromatin incubated with *cis*-DDP.

On the basis of the obtained results it can be concluded that chromosomal proteins are not only the competitive target for *cis*-DDP action; but their structural function in the occurrence of biologically active form of DNA plays an essential role.

[1] J. J. Roberts and A. J. Thompson, Mol. Biol. **22,** 71–133 (1978).
[2] B. Rosenberg, in: Inorgan. Chem. Biol. Med. (A. E. Martell, ed.), p. 143, 178th Meeting of Amer. Chem. Soc. (1979).
[3] T. Theophanides, P. K. Ganguli, and M. J. Bertrand, Anticanc. Res. **1,** 383–388 (1981).
[4] P. C. Kong and T. Theophanides, Inorg. Chem. **13,** 1167–1170 (1974).
[5] V. Kleinwachter and R. Zaludova, Chem. Biol. Interaction **16,** 207–222 (1977).
[6] A. T. M. Marcelis, G. G. van Kralingen, and J. Reedijk, J. Inorg. Biochem. **13,** 213–222 (1980).
[7] J. H. J. den Hartog, C. Altona, J. H. van Boorn, A. T. M. Marcelis, G. A. van der Marel, L. J. Rinkel, G. Wille-Hozeleger, and J. Reedijk, Eur. J. Biochem. **134,** 485–495 (1983).
[8] J. J. Roberts and J. M. Pascoe, Nature **235,** 282–284 (1972).
[9] H. C. Harder, Cisplatin Current Status and New Developments, Academic Press, New York 1980.
[10] J. J. Roberts and F. Friedlos, Biochim. Biophys. Acta **655,** 146, 151 (1981).
[11] A. Eastman, Biochem. Biophys. Res. Commun. **105,** 869–875 (1982).
[12] J. P. Macquet and J. L. Butour, Biochimie **60,** 901–914 (1978).
[13] R. C. Srivastava, J. Froehlich, and G. L. Eichhorn, Biochimie **60,** 879–890 (1978).
[14] J. L. Butour and J. P. Macquet, Biochem. Biophys. Acta **653,** 305–315 (1981).
[15] A. J. P. Alix, L. Bernard, M. Mantait, P. K. Ganguli, and T. Theophanides, Inorg. Chim. Acta **55,** 147–152 (1981).
[16] M. Arguilla, L. M. Thompson, L. F. Pearlman, and H. Simpkins, Canc. Res. **43,** 1211–1216 (1983).
[17] V. Brabec, O. Vrana, V. Kleinwachter, and F. Kiss, Studia Biophys. **101,** 135–139 (1984).
[18] H. Simpkins and L. T. Pearlman, FEBS Lett. **169,** 30–34 (1984).
[19] C. Houssier, M. C. Depauw-Gillet, R. Hacha, and E. Fredericq, Biochim. Biophys. Acta **739,** 317–325
[20] Z. M. Banjar, L. S. Hnilica, R. C. Briggs, J. Stein, and G. Stein, Biochemistry **23,** 1921–1926 (1984).
[21] S. J. Lippard and J. D. Hoeschele, Proc. Natl. Acad. Sci. USA **76,** 6091–6095 (1979).
[22] L. M. Thompson, M. Arquilla, and H. Simpkins, Biochem. Biophys. Acta **698,** 173–182 (1982).
[23] V. G. Allfrey and A. E. Mirsky, in: Method in Enzymology (S. P. Collowick and N. O. Kaplan, eds.), **vol. XII,** p. 418, Academic Press, New York 1967.
[24] T. C. Spelsberg and L. S. Hnilica, Biochem. Biophys. Acta **228,** 212–222 (1971).
[25] C. P. Georgiew and B. A. Struczkow, Biophysica V **6,** 745–748 (1960).
[26] K. Burton, J. Biochem. **62,** 315–322 (1956).
[27] J. O. H. Lowry, N. J. Rosenbrough, A. L. Farr, and R. J. Randall, J. Biol. Chem. **193,** 265–275 (1951).
[28] W. C. Schneider, in: Method in Enzymology (S. P. Colowick and N. O. Kaplan, eds.), **vol. III,** p. 680, Academic Press, New York 1957.
[29] Z. Marczenko, Spectrophotometric Determination of Elements, Ellis Horwood, Chichester 1976.