PS5.57 — TU

JOLANTA SWIATEK
KAZIMIERZ GASIOROWSKI
Medical Academy of Wrocław
Department of Basic Medical Sciences
14 Kochanowskiego str., 51-601 Wrocław
Poland
HENRYK KOZŁOWSKI
Institute of Chemistry
University of Wrocław
14 F. Joliot-Curie str., 50-383 Wrocław
Poland

POLAROGRAPHIC AND SPECTROSCOPIC STUDY ON Pb^{+2} ION INTERACTION WITH DNA

The toxic effects of lead polution seem to be already well established especially in the industrial countries [1]. The complexity of lead poisoning [2,3] was recently completed by the discovery of its carcinogenic actions in animals (for a review article, see ref. [4]). From the isotopic studies authors were not able, however, to establish whether metal ions are directly involved in DNA binding and if so which is the mode of lead binding to nucleic acid [5].
In this communication we present the polarographic and spectroscopic studies on the possible modes of interaction in the Pb^{+2} – DNA system.

RESULTS AND DISCUSSION

The lead-free DNA during a polarographic process in acetate buffer undergoes a reduction around −1.39 V. This process is usually assigned as a reduction of the adenine and cytosine residues of a double-stranded DNA molecule [6]. The relative height of a polarographic wave (h_{DNA}) is suggested to be a measure of a double-helical structure of nucleic acid [7,8].

Pb^{+2} – DNA solutions in 0.15 M acetate buffer

In the 0.15 M sodium acetate h_{DNA} reaches a value close to the maximum, which could indicate that most of the studied DNA is in a double-helical structure. The increase of Pb^{+2} to phosphate molar ratio (P) changes distinctly the reduction potential of DNA from −1.398 V (P = 0.05) to −1.443 V (P = 10). The fact that the presence of Pb^{+2} ions causes a slight increase of h_{DNA} with time of solution storage and variation of the reduction potential of DNA may indicate that lead ions compete with sodium ions in the interaction with DNA and that the main interaction site is the phosphate site.

Pb^{+2} – DNA solutions in 0.05 M acetate buffer

Addition of Pb^{+2} ions to 0.05 M acetate buffer causes a distinct increase of h_{DNA} which depends on the Pb^{+2} ion concentration as well as on the time of DNA exposition on metal ions (Fig. 1). These results could clearly indicate the direct involvement of lead ions in the stabilization of a double-helical structure of DNA (increase of h_{DNA}).

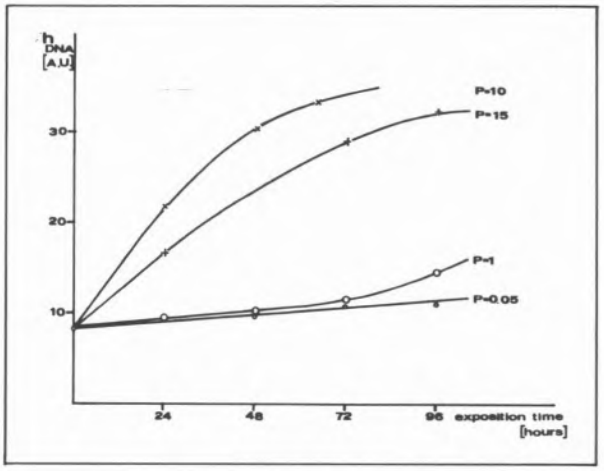

Fig. 1
Dependence of h_{DNA} on Pb^{+2} concentration and on exposition time (solution storage) (c_{DNA} = 25 μg/ml, 0.05 M acetate buffer)

The kinetics of this interaction is rather slow. The stabilizing effects of Pb^{+2} ions are supported by CD spectra. The results of CD spectra for P > 10 suggest the destabilizing effect of Pb^{+2} ions *i.e.* interaction with the base donors of DNA [9,10]. These results are supported also by the melting profiles for Pb^{+2} – DNA solutions (Table I). The data presented in Table I clearly show the stabilizing effects of lead ion of DNA for P < 10 and

for high excess of Pb^{+2}, decrease in T_m values, *e.g.* 69.2°C for P = 50 (Table I), generally indicates the interaction of metal ion with bases of DNA, which destabilizes a double-helical structure [9]. A new phase transition appearing around 40°C may indicate more specific interaction of Pb^{+2} ion with DNA.

Table I
T_m values for Pb^{+2} – DNA solutions

P	T_m [°C]
0	72.4
1	75.7
10	74.6
15	74.3
	$T_{m_1}^{a)} = 37.0$
50	69.2
	$T_{m_1}^{a)} = 41.0$

a) T_{m_1} temperature of the first phase transition.

CONCLUSIONS

Pb^{+2} ions interact with DNA in two different ways, *i.e.* via phosphate chain leading to stabilization of DNA and via base donors destabilizing a double-stranded structure. These results are supported also by the recent X-ray studies on Pb^{+2} interaction with RNA which has proved the involvement of base donors in metal ion binding [11].

REFERENCES

[1] K.R. MAHAFFEY, *Federation Proc.*, **42**, 1730 (1983).
[2] J.A. VALCIUKAS, R. LILIS, M. PETROCCI, *Amer. J. Industrial Med.*, **2**, 261 (1981).
[3] J.A. VALCIUKAS, R. LILIS, *Int. Arch. Occup. Environ. Health*, **51**, 1 (1982).
[4] M.R. MOORE, P.A. MEREDITH, *Arch. Toxicol.*, **42**, 87 (1979).
[5] E. SABBIONI, E. MAFARANTE, *Chem.-Biol. Interactions*, **15**, 1 (1976).
[6] V. BRABEC, *Bioelectrochem. Bioenerg.*, **8**, 437 (1981).
[7] E. PALECEK in W.E. COHN (ed.), «Progress in Nucleic Acid Research and Molecular Biology», Academic Press, N.Y., vol. 18, 1976, p. 151.
[8] E. PALECEK, F. JELEN, *J. Electroanal. Chem., Interfacial Electrochem.*, **116**, 317 (1980).
[9] G.L. EICHHORN, Y. AE SHIN, *J. Am. Chem. Soc.*, **90**, 7323 (1968).
[10] Y.I. IVANOV, L.E. MINCHENKOVA, A.K. SCHYOLKINA, A.I. POLETAYEV, *Biopolymers*, **12**, 89 (1973).
[11] R.S. BROWN, B.E. HINGERTY, J.C. DEWAN, A. KLUG, *Nature*, **303**, 543 (1983).

PS5.58 — TH

THOMAS D. TULLIUS
BETH A. DOMBROSKI
Department of Chemistry
The Johns Hopkins University
Baltimore, Maryland 21218
U.S.A.

USING A TRANSITION METAL COMPLEX TO MAKE AN IMAGE OF THE HELICAL TWIST OF DNA

We have developed a new method for determining the helical twist along a molecule of DNA of particular sequence. In this technique, a transition metal complex produces an image of the helicity of DNA, by fragmenting a DNA molecule bound to a flat inorganic surface. This method offers a rapid way of surveying the structure of any DNA molecule of moderate size, up to a few hundred base pairs in length. Such studies can be used to determine common motifs in the three-dimensional conformation of DNA that are not apparent at the level of simple base sequence. It is possible that regions of DNA important in biological regulation (promoters, for example) exist in conformations that have different helical twists from those of neighboring sequences [1], and could thus be recognized by proteins involved in regulatory processes.

RESULTS AND DISCUSSION

Our experiment is based on the determination by Rhodes and Klug of the helicity of DNA in solution, in which the enzyme deoxyribonuclease I (DNase I) was used to cut random sequence nucleosomal DNA bound to an inorganic surface (crystalline calcium phosphate, for example) [2]. The idea behind this experiment is that only certain bonds in the phosphodiester backbone are accessible to the nuclease; the backbone bonds near the inorganic surface are shielded from the enzyme, and are thus cut with much lower frequency