ISSN 1070-3284, Russian Journal of Coordination Chemistry, 2015, Vol. 41, No. 9, pp. 599–606. © Pleiades Publishing, Ltd., 2015.

A Novel Mn(II) Complex with 3-(2,5-Dicarboxyl)-5-Carboxylpyridine: Synthesis, Crystal Structure, and Interaction with DNA[1]

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Received February 2, 2015

Abstract—A novel Mn coordination polymer, $\{[Mn_2(L)_6] \cdot 2H_2O\}_n$, has been synthesized by the reaction of $C_4H_6MnO_4 \cdot 4H_2O$ (manganese acetate) with 3-(2,5-dicarboxyl)-5-carboxylpyridine (L). Elemental analysis, IR spectra, thermal analyses, and X-ray single crystal diffraction (CIF file CCDC no. 1061752) were carried out to determine the composition and crystal structure of the complex. The polymer was characterized by UV spectrum, fluorescence spectrum showing that the complex has the ability of interaction with DNA, Gel electrophoresis assay demonstrated the ability of the complex to cleave the HL-60 DNA (HL-60 DNA, which was extracted by ourselves). Further more, the apoptotic test indicates that the complex has an apoptotic effect on JEKO.

DOI: 10.1134/S107032841509002X

INTRODUCTION

Recently, researchers have centered an increasing attention on synthesising metal-complexes, rather than individual theory development due to their potential applications as anticancer medications. During the past decades, synthetic coordination chemistry has developed rapidly [1]. Study in this field has provided lots of examples of rationally designed sundry coordination polymers possessing interesting structural motifs and significant properties in catalysis, gas adsorption, magnetism, DNA recognition, and so on [2–6]. There are a large amount of achievements for researchers in seeking drug treatment for cancer. We can hardly control the extended structures, and even the chemical composition of the final products which is one of the key points [7]. Therefore, it's still a long-term challenge to study design and construction of coordination polymers on the basis of the different metal ions and ligands.

There has been substantial interest in the design and investigation of transition-metal anticancer drugs [8–11]. Transition different metal complex of drugs plays an important role in the treatment of the disease. As is well-known, manganese metal is one of the indispensable part of the metal element, carboxylic acids are widely used as ligands as they can easily coordinate with metal by deprotonation. As an important transition-metal element, manganese can also form complexes and some manganese complexes exhibit excellent biological activities [12, 13].

In the present work, based on Mn(II), we synthesized $\{[Mn_2(L)_6] \cdot 2H_2O\}_n$ by 3-(2,5-dicarboxyl)-5-carboxylpyridine (L). The DNA-cleaving has been investigated by gel electrophoresis. The ability of complex to induce apoptosis is evaluated in JEKO cell line using Annexin V conjugated with FITC and propidium iodide (PI) counterstaining by flow cytometry.

EXPERIMENTAL

Materials and physical measurements. All chemicals purchased were used directly without further purification. IR spectra were recorded in KBr pellets on a Nicolet FT-IR 470 spectrometer in the range of 4000–400 cm^{-1}. UV spectra was recorded on a Shimadzu UV-240 instrument.

Fluorescence spectra were carried out on a PerkinElmer LS55 fluorescence spectrometer. For all fluorescence measurements, the entrance and exit slits were maintained at 10 and 10 nm, respectively. The samples were excited at 526 nm and its emission observed at 611 nm. The buffer used in the binding studies was 50.0 mM Tris–HCl, pH 6.8–7.3, containing 10.0 mM NaCl. The samples were incubated 4 h at room temperature (20°C) before spectral measurements. The measurement of EB binding to DNA–Mn complex was studied by increasing the concentrations of the complex and measuring the change in fluorescence intensity.

[1] The article is published in the original.

Table 1. Crystal data and details of the structure refinement for complex

Parameter	Value
Formula weight	716.32
Crystal system	Orthorhombic
Space group	*Pnna*
Unit cell dimensions:	
a, Å	14.5326(18)
b, Å	25.046(4)
c, Å	7.4669(8)
Z	4
Crystal size, mm	0.2 × 0.2 × 0.2
θ Range for data collection, deg	3.07–27.48
Limiting indices	$-18 \le h \le 18$, $-32 \le k \le 32$, $-9 \le l \le 9$
Reflections collected/unique (R_{int})	23699/3109 (0.0677)
Refinement method	Full-matrix least-squares on F^2
Data/restraints/parameters	3109/3/216
Goodness-of-fit on F^2	1.124
Final R indices ($I > 2\sigma(I)$)	$R_1 = 0.0408$, $wR_2 = 0.1191$
R indices (all data)	$R_1 = 0.0467$, $wR_2 = 0.1265$
Largest diff. peak and hole, e Å^{-3}	0.476 and –0.601

For the gel electrophoresis experiments, HL-60 DNA was treated with the complex in Tris-buffer (50.0 mM Tris–acetate, 18.0 mM NaCl buffer, pH 6.8–7.3), and the contents were incubated for 1.5 h at room temperature. The samples were electrophoresed for 3 h at 90 V on 0.8% agarose gel in Tris–acetate buffer. After electrophoresis, the gel was stained with 1.0 mg/mL EB and photographed under UV light.

Absorption measurements were performed on a Shimadzu UV-2550 double beam spectrophotometer in the range of 200–350 cm^{-1}, using 1 cm path length quartz cuvettes.

The ability of complex to induce apoptosis is evaluated in JEKO cell line using Annexin V conjugated with FITC and propidium iodide (PI) counterstaining by flow cytometry. The JEKO cell in a usable condition were seeded in a 6-well culture plate at 1 × 10^6 cells per well in a 3 mL culture medium. In 6 and 12 h later the medium including the Mn(II) complex was given. After 12 h (18 h) incubation, cells were gathered, wash cells twice with cold phosphate-buffered saline (PBS) and then resuspend cells in 1× binging buffer at a concentration of 1 × 10^6 cells/mL. Transfer 100 μL of the solution (1 × 10^5 cells) to a 5 mL culture tube. Add 5 μL of FITC Annexin V and 5 μL PI. Gently vortex the cells and incubate for 15 min at RT (25°C) in the dark. Add 400 μL of 1× binding buffer to each tube. Analyze by flow cytometry (Accuri C6, USA) within 1 h.

Synthesis of complex. The concentration of all reagents was 15 mmol/L. For the preparation of the title complex, an aqueous solution (10 mL) containing $C_4H_6MnO_4 \cdot 4H_2O$ was added to ligand L aqueous (10 mL). Then the pH of the mixture with KOH solution was adjusted to 5.8. The mixture was stirred for 3 h in air. The clear solution was obtained by filtration under atmospheric pressure. About a month later, the transparent crystals were formed by evaporating the solution at room temperature.

IR (RBr; ν, cm^{-1}): 3380 s, 1578 s, 1408 s, 1246 m, 1019 m, 821 s, 780 s.

For $C_{28}H_{18}Mn_2N_2O_{14}$

anal. calcd., %:	C, 46.99;	H, 2.55;	N, 3.97.
Found, %:	C, 46.95;	H, 2.53;	N, 3.91.

X-ray crystal determination. Structure measurements of the topical complex was performed on a XtaLAB mini X-ray single-crystal diffractometer with MoK_α radiation (λ = 0.71073 Å) at 293 K, and the intensity data were obtained in a range of 3.07° ≤ θ ≤ 27.48° at 293 K by using scan technique. A suitable single-crystal of dimensions 0.2 × 0.2 × 0.2 mm was mounted in a glass fiber capillary. A direct method using SHELXS-97 resolved the structure [14, 15]. All non-hydrogen atoms were refined anisotropically. Hy-

Table 2. Bond lengths (Å) and angles (deg) for complex*

Bond	*d*, Å	Bond	*d*, Å
Mn(1)–O(1)	2.1542(16)	Mn(2)–O(2)	2.1389(17)
Mn(1)–O(6)	2.2065(16)	Mn(2)–O(6)	2.2334(16)
Mn(1)–N(1)	2.2748(19)	Mn(2)–O(7)	2.311(2)
Angle	ω, deg	Angle	ω, deg
O(1)Mn(1)O(1)$^{\#1}$	84.90(10)	O(2)$^{\#2}$Mn(2)O(2)	140.60(11)
O(1)Mn(1)O(6)	90.06(6)	O(2)$^{\#2}$Mn(2)O(6)	113.62(7)
O(1)$^{\#1}$Mn(1)O(6)	95.65(7)	O(2)Mn(2)O(6)	92.37(6)
O(6)Mn(1)O(6)$^{\#1}$	172.27(8)	O(6)$^{\#2}$Mn(2)O(6)	98.06(8)
O(1)Mn(1)N(1)	162.63(7)	O(2)Mn(2)O(7)	84.23(9)
O(1)$^{\#1}$Mn(1)N(1)	91.06(7)	O(6)Mn(2)O(7)	77.99(8)
O(6)Mn(1)N(1)	73.50(6)	O(2)Mn(2)O(7)$^{\#2}$	73.66(8)
O(6)$^{\#1}$Mn(1)N(1)	101.26(6)	O(6)$^{\#2}$Mn(2)O(7)$^{\#2}$	77.99(8)
N(1)$^{\#1}$Mn(1)N(1)	97.50(10)	O(6)Mn(2)O(7)$^{\#2}$	162.00(9)

* Symmetry codes: $^{\#1}$ $x, -y + 1/2, -z + 1/2$; $^{\#2}$ $x, -y + 1/2, -z - 1/2$.

drogen atoms were included in ideal geometrical positions. Crystal data and structure refinement parameters are listed in Table 1. Selected bond lengths and angles are given in Table 2.

Supplementary material for complex has been deposited with the Cambridge Crystallographic Data Centre (CCDC no. 1061752; deposit@ccdc.cam.ac.uk or http://www.ccdc.cam.ac.uk).

RESULTS AND DISSCUSSION

The crystal unit structure of complex $\{[Mn_2(L)_6] \cdot 2H_2O\}_n$ was determined by single-crystal X-ray diffraction. The molecular structure of the title complex is shown in Fig. 1. Interestingly, there are two coordinated types of Mn(II) in the complex. The first coordinated mode of Mn(1) is defined by three atoms, namely, four oxygen atoms (O(1), O(1)$^{\#1}$, O(6), O(6)$^{\#1}$) and two nitrogen atoms (N(1), N(1)$^{\#1}$), which reveals O(1) and O(1)$^{\#1}$ coming from 3-(2,5-dicarboxyl) , O(6), N(1) and O(6)$^{\#1}$, N(1)$^{\#1}$ coming from 5-carboxylpyridine. The second coordinated type of Mn(2) is ligated to six atoms with four oxygen atoms coming from the ligand (O(2), O(2)$^{\#2}$, O(6), O(6)$^{\#2}$), and the other oxygen atoms coming from the water molecules (O(7) and O(7)$^{\#2}$). The O(1)Mn(1)N(1) angle is 162.63(7)°, the O(6)Mn(1)O(6)$^{\#1}$ angle is 172.27(8)° and the O(1)$^{\#1}$Mn(1)N(1)$^{\#1}$ angle is 162.63(7)°. Also the O(2)$^{\#2}$Mn(2)O(2) angle is 140.60(11)°, the O(6)$^{\#2}$Mn(2)O(7) angle is 162.00(9)° and the O(6)Mn(2)O(7)$^{\#2}$ angle is 162.00(9)°. Therefore, the coordination geometry of the Mn(1) and Mn(2) atom exhibit a slightly distorted octahedral structure.

As is well-known that hydrogen bond is one of the key factors in the formation of coordination complex, consequently supramolecular polymers was formed. Two adjacent 1D chains (Fig. 2) interact with each other through hydrogen bonds to form two-dimensional layered structure, which is shown in Fig. 3.

The cleavage efficiency of extracted HL-60 DNA can be characterized by agarose gel electrophoresis. In the past research, PBR322 plasmid DNA was frequently used in the text, it is not originate from cell which studies [16, 17]. The influence of the complex on the structure of DNA is evaluated by their DNA

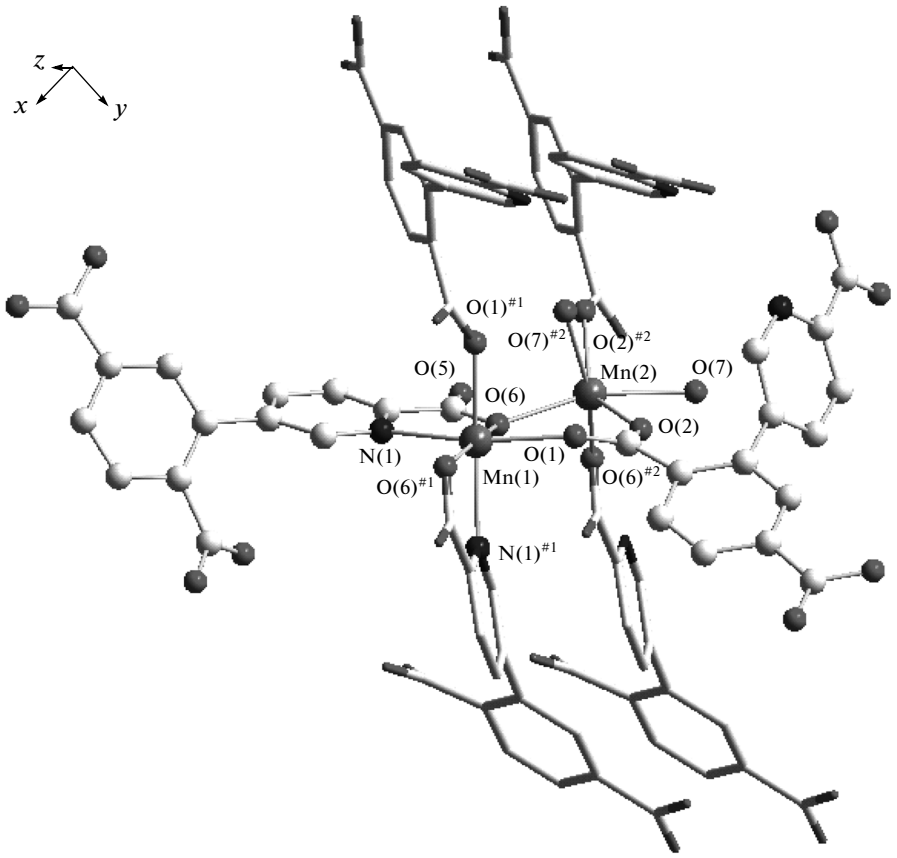

Fig. 1. The molecular structure of the ligand $[Mn_2(L)_6] \cdot 2H_2O$ (H atoms were omitted for clarity).

cleavage ability, which can be detected by agarose gel electrophoresis [18–20]. As seen from Fig. 4, the ligand and the complex induces obvious cleavage of the HL-60 DNA at 15, 7.5, and 3.75 μM. HL-60 DNA is cut into small molecular DNA strands, and each lane has obviously towed the marks. Lanes 2–4 show that the amount of supercoiled HL-60 DNA (Form I) gradually decreased and the linear form (Form III) appeared. It shows that the capability for cleavage is stronger at higher concentrations than it is at low concentrations.

Ethidium bromide (EtBr) is a conjugated planar molecule. Its fluorescence intensity is very weak, but it is greatly increased when EtBr is specifically intercalated into the base pairs of double-stranded DNA [21–23]. Figure 5 shows the emission spectra of EtBr bound to DNA in absence and presence of the complex, addition of the complex to DNA pretreated with EtBr causes appreciable reduction in the emission intensity, indicating replacement of EtBr by the complex [24], indicating the complex binds to DNA by intercalation.

One of the most important techniques to examine the binding mode of DNA with metal complexes is electronic absorption spectroscopy. A complex bound to DNA through intercalation usually results in hypochromism and red shift, due to the intercalation mode involving a strong stacking interaction between the chromophore and the base pairs of DNA. In Fig. 6, as the concentration of DNA was increased, the red shift and hypochromism were more obvious suggested that the rings of $\{[Mn_2(L)_6] \cdot 2H_2O\}_n$ in the planar complex interacted with DNA strongly.

Apoptosis is the programmed cell death that controlls the development and homeostasis of multicellular organisms by elimination of aged, damaged, or mutated cells, which has been shown to be the key cellular event responsible for the anticancer activity of the metal anticancer drugs. To determine whether the observed cell death induced by the complexes was due to apoptosis, the interaction of JEKO cells with the complex were further discussed using an Annexin V-FITC/PI assay (Fig. 7). As is known, phosphatidylserine (PS) exposure usually precedes loss of plasma membrane integrity in apoptosis, the presence of Annexin V^+/PI^- cells is deemed as an indicator of apoptosis. When treated with the complex after 6, 12, and 18 h, the population of Annexin V^+/PI^- cells (Q1-LR) were 9.9% (6 h), 20.1% (12 h), and 46.5% (18 h). Those datas

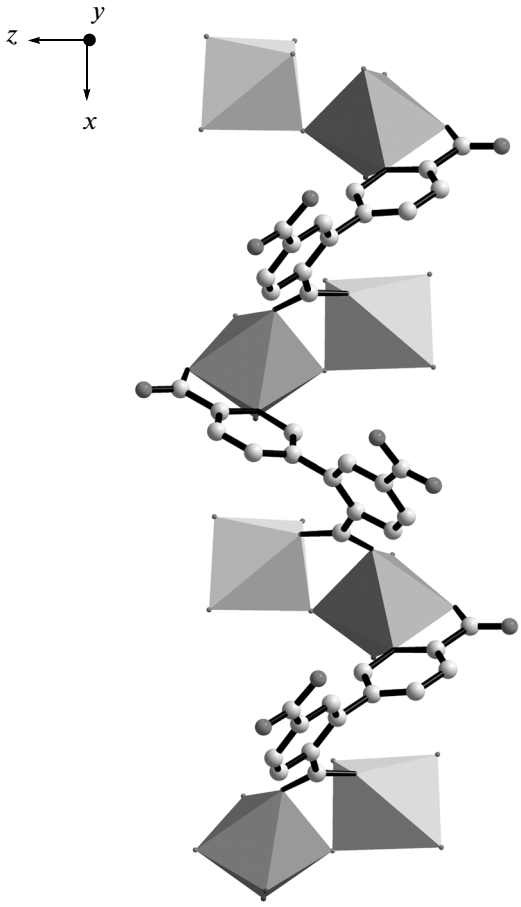

Fig. 2. 1D chain structure of the complex (H atoms were omitted for clarity).

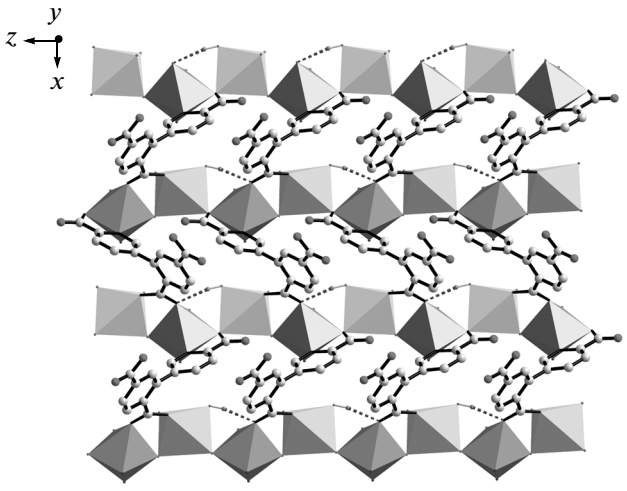

Fig. 3. 2D framework formed by hydrogen-bonding.

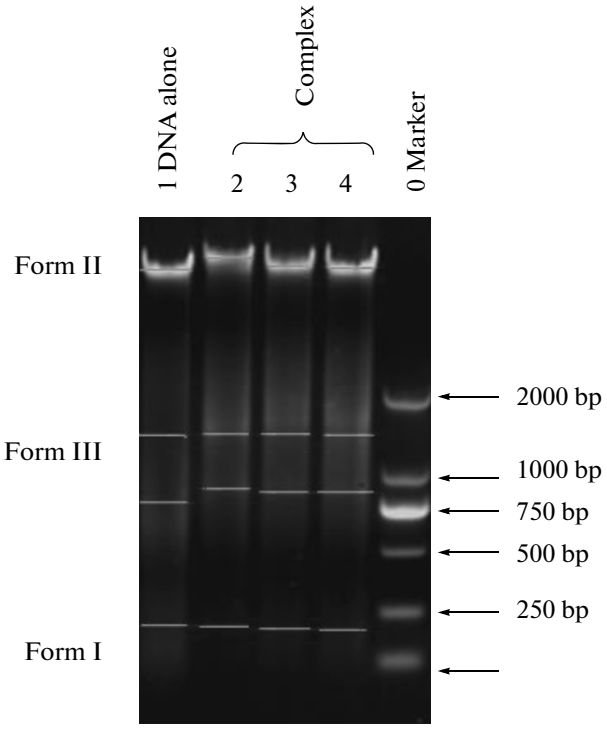

Fig. 4. Cleavage of HL-60 DNA in the present of the title complex: marker (lane 0), DNA (lane 1), DNA with different concentration of complex: 15 (lane 2), 7.5 (lane 3), 3.75 mM (lane 4).

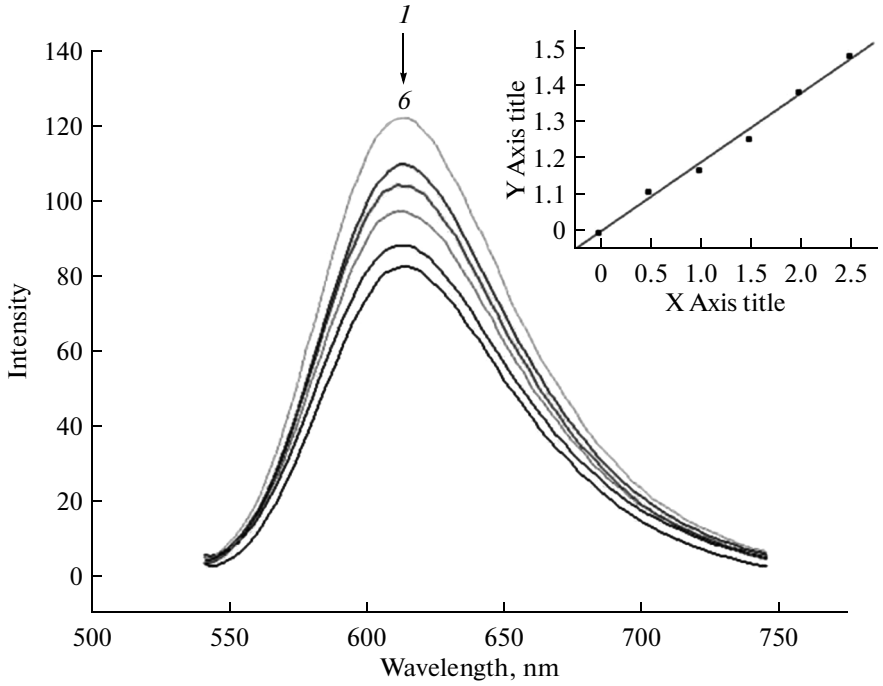

Fig. 5. Emission spectrum of EB bound to DNA in the presence of the title complex (c_{EB} = 1.0 μM, c_{DNA} = 5.0 μM, $c_{complex}$ = 0–37.5 μM, λ_{ex} = 611 nm). The arrow shows the intensity change on increasing the complex concentration.

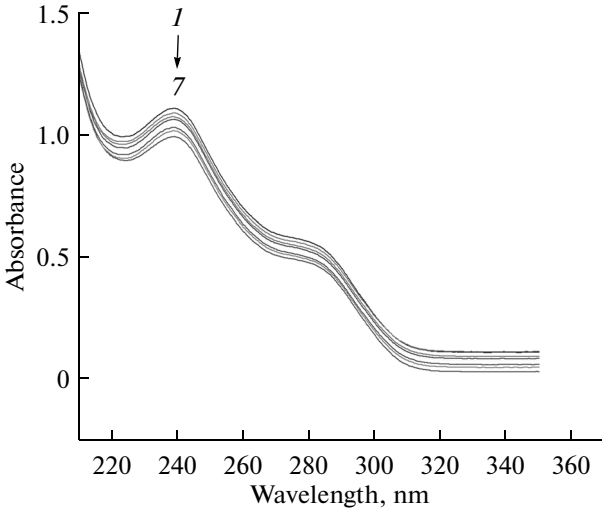

Fig. 6. Absorption spectra of the title complex in the presence of increasing amounts of HL-60 $c_{complex} = 20.0$ μM, $c_{DNA} = 0–48.0$ μM).

suggested that apoptotic death was induced in JEKO cells, which indicates that the ability of cell apoptosis enhances gradually as the length of aliphatic chain becomes longer.

Thus, the mononuclear Mn(II) complex was strong DNA intercalator. This study on the synthesis and crystal structure of the complex provides important information which could help to understand the mechanism of activity of the manganese complex interacting with DNA. Thus the newly synthesized

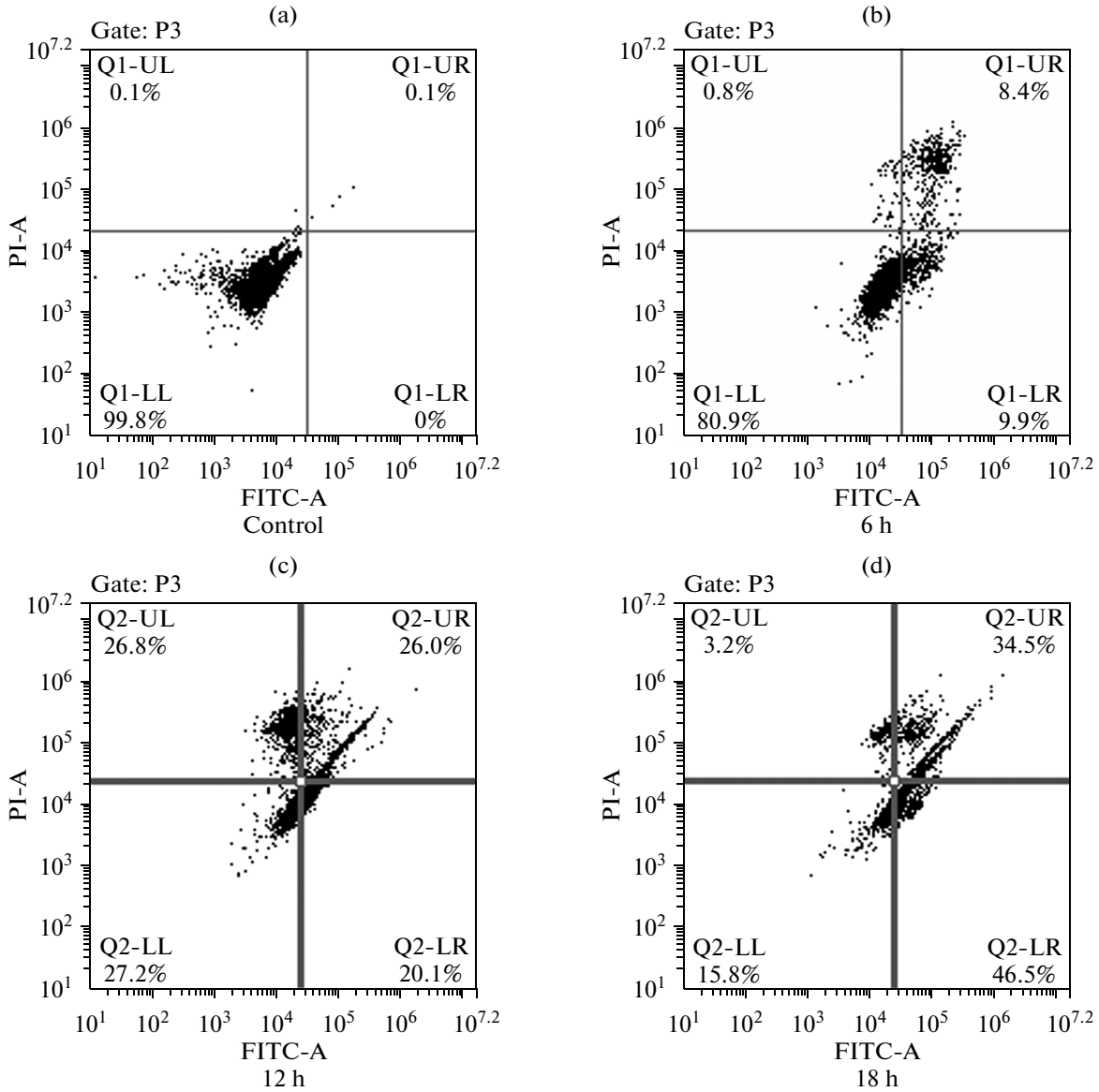

Fig. 7. The JEKO cells cultured with or without 1000 μg/mL of the complex for 6, 12, and 18 h. On quadrants, dot plots show percentages representing the population of cells: non-apoptotic (c), early apoptotic (d) or late apoptotic/necrotic (b). Quadrants were established using controls (a).

Mn(II) complex may be potential antitumor agent due to its unique interaction mode with DNA.

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