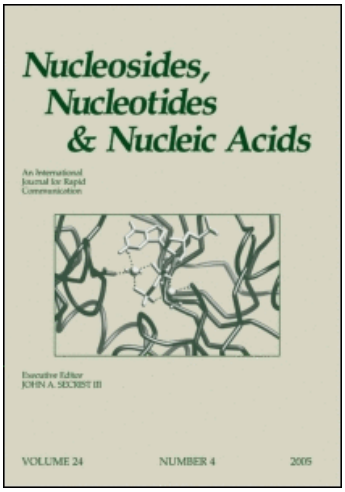

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DNA PHOSPHODIESTER BOND HYDROLYSIS MEDIATED BY Cu(II) AND Zn(II) COMPLEXES OF 1,3,5,-TRIAMINO-CYCLOHEXANE DERIVATIVES

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ABSTRACT. The hydrolytic activity of the 1,3,5-triaminocycloxexane derivatives TACH, TACI and TMCA complexed to Zn(II) and Cu(II) towards a model phosphoric ester and plasmid DNA has been evaluated by means of spectroscopic and gel-electrophoresis techniques. At conditions close to physiological, a prominent cleavage effect mediated by the nature of the ligand and metal ion was generally observed. TACI complexes are the most active in relaxing supercoiled DNA, the effect being explained by the affinity of the hydroxylated ligand for the nucleic acid. As indicated by the dependence of cleavage efficiency upon pH, Zn(II)-complexes act by a purely hydrolytic mechanism. In the case of Cu(II)-complexes, although hydrolysis should be prominent, involvement of an oxidative pathway cannot be completely ruled out.

INTRODUCTION

Hydrolysis of phosphoric esters is a reaction of major interest both in environmental and biological chemistry[1]. In the latter field, a number of enzymes act as DNA or RNA hydrolizing agents, cleaving phosphodiester bonds of the polynucleotide chain[2]. Clearly, it is very difficult to reproduce chemically the enzyme-driven processes. In fact, nucleic acids are quite resistant to nucleophilic attack, due to the strong electrostatic repulsions originating when a usually negatively charged nucleophile approaches the highly charged polyanionic backbone[3]. Indeed, most of the synthetic DNA cleaving agents so far reported act by an oxidative, rather than by a hydrolytic, mechanism. They are basically complexes of metal

ions having appropriate redox potentials, such as Fe(II) and Cu(II) or undergoing photochemical activation, such as Rh[4]. Notwithstanding their useful applications, these compounds are not suitable as artificial restriction enzymes.

Recent efforts to develop effective nucleic acid hydrolizing agents have produced new systems based on metal ions complexes able to polarize the P=O bond and activate a coordinated water molecule to allow nucleophilic attack. Among these, beside lanthanide ions based systems[5], the most efficient reported are generally Cu(II) or Co(III) complexes with polyamine ligands[6]. Among these the Cu(II) complex of 1,3,5-triaminocyclohexane has been shown to be the most active[7]. These findings stimulated our study of a number of 1,3,5-triaminocyclohexane derivatives (FIG. 1) complexed to Cu(II) or Zn(II). The present work is aimed at investigating their reactivity toward a model phosphate ester and DNA.

EXPERIMENTAL SECTION

All-*cis*-1,3,5-triamino-2,4,6-trihydroxycyclohexane (TACI)[8a], *cis-cis*-1,3,5-triamino-cyclohexane (TACH)[8b], all-*cis*-1,3,5-triamino-2,4,6-trimethoxycyclohexane (TMCA)[8c] and dinitrophenyldiethylphosphate (DNDEP)[8d] were synthesized as described. $Cu(NO_3)_2$ and $Zn(NO_3)_2$ were analytical grade products. Metal ion stock solutions were titrated against EDTA following standard procedures. Metal complexes solutions were prepared by mixing ligand and metal solutions in the appropriate amounts, adjusting the pH to about 7-8 with NaOH.

The reactions of the metal complexes with DNDEP were followed on a Perkin Elmer Lamda 16 spectrophotometer equipped with a thermostated cell holder ensuring a temperature of 25±1 °C. Reactions were started by addition of 20 μL of a solution of a $(1\text{-}2)\cdot10^{-3}$ M solution of DNEP in CH_3CN to a 2 ml solution of metal complex in the proper buffer and monitored by following the formation of 2,4-dinitrophenol. The initial concentration of substrate was $(1\text{-}2)\cdot10^{-5}$ M and the kinetics were in each case first order up to 90% of the reaction. The pseudo-first order rate constants (k_ψ) were obtained by non-linear regression analysis of the absorbance versus time data and the fit error on the rate constant was less than 1%. The second order rate constants were obtained by non-linear regression analysis of the k_ψ versus concentration of the complex.

DNA cleavage experiment [9] were performed using pBR 322 (Gibco BRL) in 10 mM HEPES, pH 8.1 or 20 mM HEPES, pH 7.0. Reactions were performed incubating DNA (12 μM base pairs) at 35°C in the presence/absence of increasing amount of metal complex for the indicated time. Reaction products were resolved on a 1% agarose gel in TBE buffer (44.5 mM TRIS base, 44.5 mM, boric acid, 1 mM EDTA), visualized by ethidium bromide

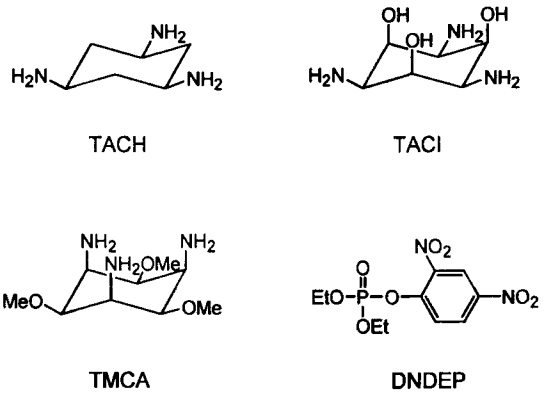


FIG 1 : Chemical structure of test compounds and of phosphate ester used as substrate

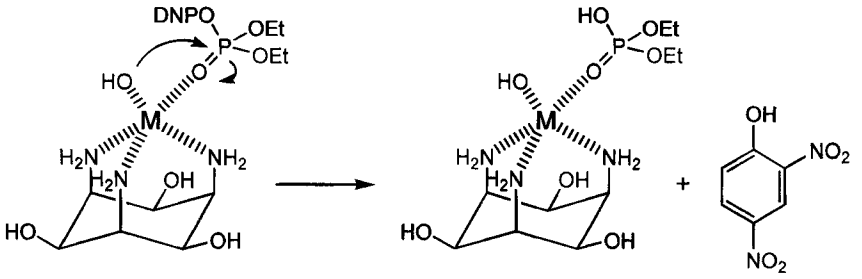
staining and photographed. The relative amounts of different plasmid structures were quantified using a BioRad Gel doc 1000 apparatus interfaced to a PC workstation.

RESULTS AND DISCUSSION

Cleavage of the model substrate dinitrophenyldiethylphosphate (DNDEP)

The model phosphoric ester DNDEP was used to evaluate the efficiency of the test complexes to induce phosphate ester hydrolysis. Kinetic measurements were performed to estimate the second order rate constants according to SCHEME 1. In the case of the Cu(II) complexes of TACI and TMCA, the formation of unreactive bimetallic dimers was observed and taken in account for the rate constants estimation. The results obtained are summarized in TABLE 1.

As expected[6a], Cu(II) ions are remarkably more efficient in hydrolyzing the model substrate than Zn(II) ions. On the other hand the structure of the ligand play only a minor role in determining the hydrolytic efficiency. The difference in the rate constants is within a factor of 2 in the case of the Zn(II) complexes and of 1.5 in that of the Cu(II) complexes. This may be easily explained by considering that the substitution on the cyclohexane ring strongly influences the formation constant of the complexes but not the acidity of the metal ion coordinated water molecule. In fact the $\log\beta_1$ values increase from 7.1 to 10.8 on going from TACH to TMCA in the case of Zn(II) complexes, and from 10.3 to 14.6 in the case of Cu(II) complexes[8c] while the pK_a of the metal coordinated water molecule are in the 8-9 range in the case of Zn(II) and all close to 8 in the case of Cu(II) complexes[11]. Therefore, the



Scheme 1

TABLE 1. Second order rate constants for the hydrolysis of DNDEP at 25°C in water. In parenthesis literature values [10].

Ligand	k_2 for the Zn(II) complex ($M^{-1}s^{-1}$)	k_2 for the Cu(II) complex ($M^{-1}s^{-1}$)
TACH	**0.15 (0.2)**	**1.1 (1.2)**
TACI	**0.26**	**1.5**
TMCA	**0.34**	**1.1**

nucleophilicity of the coordinated hydroxide anion is little influenced by the ligand structure at least in the series here considered.

DNA cleavage experiments

Incubation of supercoiled DNA with the metal complexes leads to the formation of relaxed circular DNA and, eventually, at high [metal complex]/[DNA] ratios, to the formation of linear DNA (FIG. 2). This latter event is strongly concentration dependent, and, therefore it cannot be due to a simultaneous double strand break operated by the complex, but to statistical accumulations of adjacent single strand breaks along the DNA chain, generated by unrelated cleavage events.

The efficiency of the process is strongly dependent on the nature of the metal ion and on the ligand structure. TACI complexes are generally the most effective in cleaving the nucleic acid, followed by TACH and TMCA, irrespective of the nature of the metal ion coordinated to the ligand. Taking as reference the Cu(II) complexes, after 3 hours of incubation in the presence of 60 μM TACI, only cleaved DNA is detected while in the presence of 120 μM

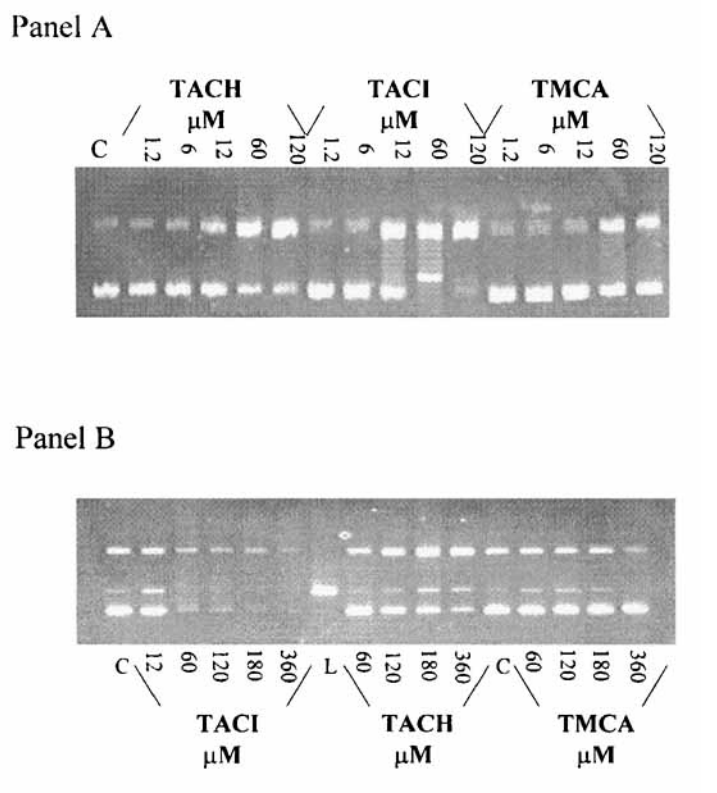


Fig. 2 : Effect of ligand structure and metal ion nature on DNA cleavage

Panel A : pBR 322 cleavage in the presence of increasing amounts of Cu(II) metal complex of test ligands in Hepes 10 mM, pH 8.1, T = 35°C. ($[DNA]_{bp}$ = 12 μM, incubation time 3 h.). Lane C is the control reaction in absence of metal complexes.

Panel B : pBR 322 cleavage in the presence of increasing amounts of Zn(II) metal complex of test ligands in Hepes 20 mM, pH 8.0, T = 35°C. ($[DNA]_{bp}$ = 12 μM, incubation time 24 h.). Lane C: control reaction in absence of metal complexes. Lane L: linear DNA.

TACH and TMCA a sizeable amount of supercoiled DNA is still present. The same trend is also observed within the Zn(II) complexes. Since only a modest difference in hydrolytic efficiency was observed using the model phosphoric ester bond (see TABLE 1), the effects apparent with the nucleic acid are reasonably affected by the affinity of the hydrolytic complex for the nucleic acid. In this connection it is interesting to note that the more prominent DNA cleaving activity is that of the ligand that is likely to bind to the nucleic acid by efficient hydrogen bonding through its three hydroxyl groups.

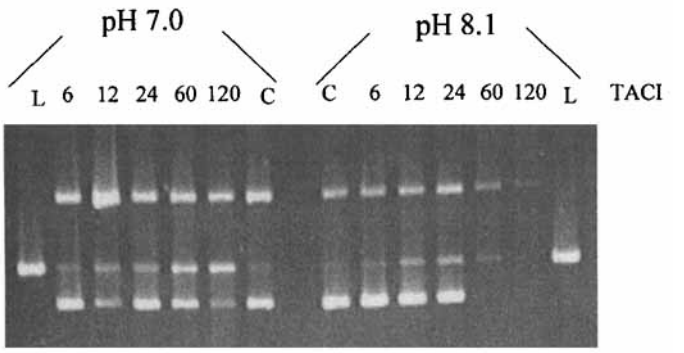


FIG. 3 : Effect of pH on DNA cleavage efficiency promoted by TACI-Zn(II) 1:1.

Panel: pBR 322 cleavage in the presence of increasing amounts of TACI-Zn(II) complex in Hepes 20 mM, T = 35°C, ($[DNA]_{bp}$ = 12 μM, incubation time 24 h.). Lane C: control reaction in absence of metal complexes. Lane L: linear DNA.

Also the nature of the metal ion affects the DNA damage to a remarkable extent, the Cu(II) complexes being more active than the Zn(II) ones. Using 60 μM TACI as ligand, the supercoiled DNA is completely degraded to forms II and III in 3 h with Cu(II) and in 24 h with Zn(II). With the latter metal ion, which lacks of a important red-ox chemistry, a purely hydrolytic mechanism of ester bond cleavage should be operative. This is confirmed by the effect of pH on the reaction rate: upon increasing the pH and, as a consequence, the fraction of metal ion bound hydroxide the amount of DNA cleaved substantially increases (FIG. 3). On the other hand, it is known that the Cu(II) derivatives can damage DNA by an oxidative other than an hydrolytic mechanism[4]. Hence, the increased DNA-cleaving efficiency shown by copper complexes when compared to the Zn(II) counterparts, could be due, at least in part, to the simultaneous occurrence of the two mechanisms. However, different observations point to a prevalence of the hydrolytic over the oxidative pathway. In particular, we observed the same reactivity trend within the two series of metal ion complexes and this, taken together with the fact that the oxidative mechanism has been ruled out for the TACH·Cu(II) complex[7], clearly indicates that the hydrolytic mechanism should be operative also for the TACI and TMCA complexes. Moreover our experiments are performed in the absence of any reductive agent and it has been shown that in the case of a triamine macrocyclic ligand only a small fraction, if any, of the DNA cleavage can be ascribed to oxidation due to adventitious reducing species[12].

In conclusion the test triaminocyclohexane complexes, with Cu(II) and Zn(II) metal ions, promote efficient hydrolysis of model phosphoric esters and of polynucleotides substrates, TACI being the most effective ligand in the case of DNA structures. This fact suggests that, besides optimizing hydrolytic properties of the complex by using appropriate ligands and transition metal ions, the DNA-binding properties of the complex must be taken in due consideration when designing effective DNA-cleaving agents. Besides increasing the overall hydrolysis rate, ligand molecules bearing specific recognition elements for DNA could act selectively at specific spots along the nucleic acid chain, in this closely resembling natural restriction enzymes. Work in this direction would yield extremely powerful tools for chemical manipulation of genetic material.

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