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Synthetic nucleases triggered by mild acidification: a new type of biologically active compounds

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Alkylcobalt chelates possessing tridentate Schiff bases display nuclease activity upon proton-assisted homolytic decomposition, due to the formation of both alkyl free radicals and a cobalt complex catalysing the autooxidation of organic substrates.

Here we report on the nuclease activity of alkylcobalt(III) chelates with tridentate Schiff bases **1**. The main feature of these complexes is their ability to generate alkyl free radicals due to the action of protons [reaction (1)] in the dark, under very mild conditions. Typically, the reaction rate rises with increasing acidity in an 'S'-shaped manner within the pH range 7 to 5 at 0–50 °C.[1–3]

$$\mathrm{RCo^{III}}\ldots \xrightarrow{\mathrm{H}^{+}} \mathrm{R}^{\bullet} + \mathrm{Co^{II}}\ldots \tag{1}$$

We have observed a non-specific, *i.e.* resulting in the statistical distribution of products, cleavage of the 5S RNA *T. Thermophila*[†] upon treatment with complexes **1** in the dark at pH 5.0, *i. e.* upon proton-assisted homolytic decomposition. Table 1 summarizes data obtained with the most thoroughly tested complex **1a**, R = Pr^{i}.[‡] It should be pointed out that no cleavage has occurred at pH 7.5 at which the complex is still stable in solution.

Of course, cleavage of nucleic acids (NA) effected by active free radicals, in particular hydroxyl and alkyl radicals, has been generally known: *e.g.*, refs. 7 and 8. Nevertheless, and

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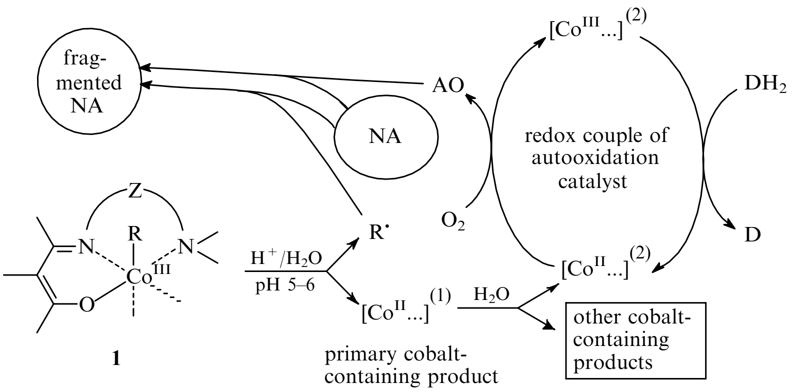
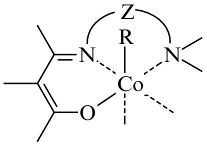
[†] The 5′-^{32}P-labelled preparation was obtained by phosphorylation of the corresponding 122-chain commercial product of Sigma (USA) using T4 polynucleotidekinase and [γ-^{32}P]-ATP.[4]



Scheme 1 Suggested mechanism of nuclease action of alkylcobalt chelates with tridentate Schiff bases.



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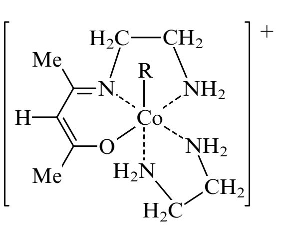
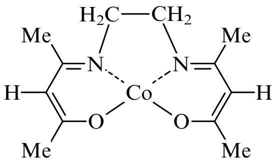


1a ($[RCo(acacen)(en)]^+$)



2 ($[Co(acac_2en)]$)

despite low conversions observed in the case of less complicated anaerobic systems, the reaction found by us merits attention since it is evidently controlled by pH and can be triggered by increasing acidity within the physiological area of conditions. Therefore it may be considered as a model for the selective action of complexes **1** on acidified tissues in living organisms, which is of interest to biology and medicine.[9,10]

The conversion of RNA is not affected by the presence of dioxygen when formation of peroxyalkyl radicals is possible. Thus, while the latter species may in principle cause a radical-chain oxidation of a substrate, such a pathway is not essentially involved in the cleavage of RNA. Introduction of ascorbic acid, which is known to inhibit radical chain oxidation processes,[11] would otherwise have suppressed cleavage of RNA rather than favoured it.

A sharp rise in efficiency of the process when it is carried out under air in the presence of ascorbic acid is particularly noteworthy. It is known that certain redox active transition metal complexes capable of the formation of adducts with dioxygen and catalysing autooxidation of organic substrates (DH_2) such as ascorbic acid, display nuclease effect under similar conditions.[6,12] These reactions involve active oxygen species (AO) including hydroxyl radicals as both intermediates and by-products. Such a mechanism can be also assumed to operate in our case, since under the slightly acidic conditions the starting organocobalt complex **1a** should be the precursor of an autooxidation catalyst. Namely, proton-assisted hydrolytic disproportionation of the primary product of homolysis, *viz.* transient $[Co^{II}(acacen)(H_2O)_2]^+$ complex, must result in formation, among other products, of a cobalt(II) chelate with the related tetradentate Schiff base, $[Co(acac_2en)]^+$ **2**.[3] The nuclease activity of cobalt chelates with tetradentate Schiff bases was established earlier.[13]

Table 1 Efficiency of the cleavage of 5S RNA of *Th. Thermophila* bacteria under the action of the $[Pr^iCo(acacen)(en)]Br$ complex at pH 5.0.[a]

Conditions	RNA conversion, %	
	In the absence of AA	In the presence of AA[b]
Anaerobic	11–15	12–17
Aerobic	12–14	85–88

[a] Reaction solutions which were 10^{-5}, 10^{-2} and 5×10^{-2} mol dm^{-3} in RNA (OE_{260} 0.01), the complex and a potassium phosphate buffer, respectively, were kept at 37 °C in the dark for 1 h. Remaining RNA and products of its cleavage were separated by electrophoresis in polyacrylacetamide gel. Conversion of RNA was calculated as the ratio of the radioactive count of the cleavage products to that of the remaining RNA. [b] 5×10^{-2} mol dm^{-3}.

Hence, it becomes evident that the nucleic acid cleavage in question is ultimately caused by two kinds of active species differing in both nature and origin (Scheme 1). They are (1) an alkyl free radical generated in the homolysis step and (2) AO formed in the course of the autooxidation process catalysed by a cobalt complex resulting from transformations of the other product of homolysis. Thus, organocobalt complexes with tridentate Schiff bases can be regarded as biologically active pH-dependent agents operating in two steps.

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References

1 I. Ya. Levitin, A. L. Sigan, R. M. Bodnar, R. G. Gasanov and M. E. Vol'pin, *Inorg. Chim. Acta*, 1983, **76**, L169.

2 A. D. Ryabov, I. Ya. Levitin, A. T. Nikitaev, A. N. Kitaigorodskii, V. I. Bakhmutov, I. Yu. Gromov, A. K. Yatsimirsky and M. E. Vol'pin, *J. Organometal. Chem.*, 1985, **292**, C4.

3 I. Ya. Levitin, A. K. Yatsimirsky and M. E. Vol'pin, *Metalloorg. Khim.*, 1990, **3**, 865 (*Organomet. Chem. USSR*, 1990, **3**, 442) and references therein.

[‡] This complex was prepared generally by analogy with **1a**, R = Et,[5] and characterized by satisfactory elemental analyses, as well as adequate ^1H NMR and IR spectra, products of acid-induced decomposition and ion-exchange TLC behaviour on SiO_2 (*cf.* refs. 5, 6): Silufol plates (Lachema, Czech Republic), 0.1 M solution of NaOAc in $MeOH–H_2O–Et_3N$ 400 : 100 : 1 (v/v/v) mixture as eluent, −10 °C, in the dark; the only spot of a chromatogram, with $R_f = 0.36$; having been originally orange, it turned grey when exposed to daylight.