ETHAMBUTOL-MEDIATED ALTERATIONS OF THE ESR OF Cu(II)–POLYNUCLEOTIDES COMPLEXES

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1. Introduction

As part of its considerable antituberculous activity [1], ethambutol has been shown to cause significant modifications in the nucleic acid metabolism of mycobacterial cells [2–4]. Spectroscopic studies [5] have also revealed that ethambutol can form chelates with metal ions. We have now further examined the nature of the interaction of the drug with Cu(II) ions by the electron spin resonance technique, and have found that the drug–Cu(II) complex becomes significantly modified by polynucleotides.

Previous studies in our laboratory have dealt with complex formation between Cu(II) and polynucleotides [6] and have also shown the existence of an interaction between ethambutol and nucleic acids [3, 4]. In order to define the nature of the interaction that takes place when Cu(II) is exposed to the presence of both ethambutol and polynucleotides, we have undertaken the ESR studies to be described.

2. Materials and methods

Calf thymus DNA was obtained from Worthington Biochemical Corp. and used without further purification. Poly d(A–T) and dG:dC were purchased from Miles Laboratories, Inc. Ethambutol was obtained from Lederle Cyanamid International Corporation. Electron spin resonance (ESR) was measured on a Varian V-4500 spectrometer operating at around 9200 MHz. All measurements were performed at liquid nitrogen temperatures [6].

Samples were prepared in aqueous solution at 2 mM concentrations of ethambutol and of $CuCl_2$ and at 4 mM concentrations of the polynucleotide phosphates.

3. Results and discussion

The first series of experiments involved the measurements of the g-values of Cu(II) in complexes with DNA, with ethambutol and with DNA plus ethambutol as a function of pH (fig. 1). It is apparent from the figure that at pH 7 the value of g_m is practically identical in the Cu–ethambutol and Cu–ethambutol–DNA complex. g_m stands for the value of the spectroscopic splitting factor at maximum absorption, very close to $g_\perp$ [7]. At lower pH's, the values of g_m for these three complexes differ considerably, indicating that a triple complex has formed in which Cu(II) has magnetic characteristics which differ from those in the double complexes with either ethambutol or DNA. This agrees with spectroscopic data [3] showing also a more pronounced interaction at pH 5 than at pH 7 between ethambutol and DNA at a low ratio of the drug to the polynucleotide phosphates. Hence, we chose to perform the following experiments also at pH 5.

Since the nuclear spin of Cu(II) is $\frac{3}{2}$ the complete ESR Cu(II) spectrum in frozen solution consists of four hyperfine lines characterized by $g_\parallel$ as well as the $g_\perp$ line. These four lines appear only with the magnetic field directed parallel to the symmetry axis ($\parallel$) because the hyperfine splitting constant, $A_\parallel$ is about an order of magnitude larger than $A_\perp$; in consequence the splitting of the line with the magnetic field in the direction perpendicular to symmetry axis ($\perp$) is not

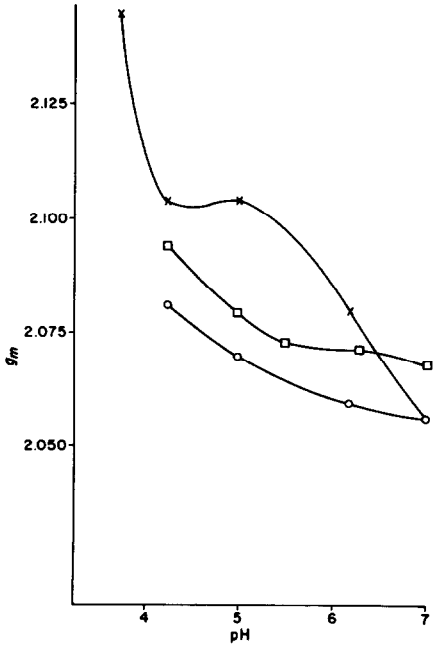

Fig. 1. The values of g_m of Cu(II) as a function of pH at T = 77°K. (x—x—x): Cu(II)–Ethambutol (1:1); (□—□—□): Cu(II)–DNA (1:2); (○—○—○): Cu(II)–Ethambutol–DNA (1:1:2).

resolved. The magnitudes of $g_{\|}$ and $A_{\|}$ provide a more sensitive indication of changes in Cu(II) binding that g_m hence we have plotted in fig. 2, $g_{\|}-2.000$ versus $A_{\|}$ and interpret the variation in these values according to Kivelson [8]. This interpretation assigns a higher degree of covalency to the $g_{\|}$ values to the left of the diagram, while higher values of $g_{\|}$ correspond to increased ionicity. What is however more important to us than the test of the validity of this interpretation, is the experimentally observed variation among the complexes under study.

The important fact is that at pH 5, Cu(II) in DNA–Cu(II)–ethambutol and in dG:dC–ethambutol shows almost identical characteristics, which differ considerably from those of Cu(II) in its complexes with d(A–T)–ethambutol, whereas the d(A–T)–drug–Cu(II) and d(A–T)–Cu(II) were rather comparable. The triple complexes of Cu(II)–drug to either dG:dC or to DNA were however clearly obvious at pH 5 (fig. 2), suggesting that such triple complexes involve preferentially G–C pairs.

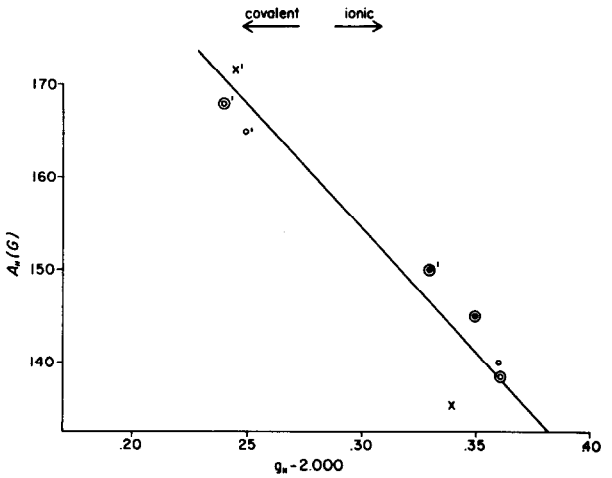

Fig. 2. Hyperfine splitting constant, $A_{\|}$ versus $g_{\|}-2.000$ of Cu(II) at pH 5.0 and T = 77°K in: ⊛, Cu(II)–DNA (native); X, Cu(II)–DNA (denatured); ◦, Cu(II)–dG:dC; and ⊛, Cu(II)–d(A–T). The primes indicate the above complexes with addition of ethambutol. Molarities as in fig. 1.

We have furthermore examined Cu(II) spectra in alkali denatured DNA both with and without ethambutol. The $g_{\|}$ and $A_{\|}$ in these series were practically identical (fig. 2) to the values obtained in native DNA. The similarity in the behavior of native and denatured DNA described above is consistent with the hypochromicity previously observed [4], according to which the ethambutol–copper chelate interacts preferentially with the bases, promoting the opening of the helix in native DNA.

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