RNA POLYMERASE, MANGANESE AND RNA METABOLISM OF ZINC SUFFICIENT AND DEFICIENT E. GRACILIS

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Summary

The RNA from zinc sufficient (+Zn) and deficient (-Zn) E. gracilis have been isolated and the three major RNA classes separated by affinity chromatography on oligo-(dT) and DBAE celluloses. The total RNA content and the ribosomal and transfer RNA fractions are the same in (+Zn) and (-Zn) cells. In (-Zn) cells, the messenger RNA fraction doubles and its base composition is altered, resulting in a two-fold increase in the G+C/A+U ratio. We have examined the role of Mn in determining these changes in RNA, since the intracellular content of this metal increases in (-Zn) cells. Increasing the Mn (II) content from 2 to 10 mM in assays with RNA polymerase from (-Zn) cells increases the incorporation of GMP relative to UMP from 1.0 to 3.5. Further, the ratios of CMP/GMP incorporation are 1.0, 2.2 and 1.2 in assays with 2, 5, 10 mM Mn (II), respectively. Thus, Mn (II) concentration can significantly alter function of RNA polymerase from (-Zn) E. gracilis cells.

Introduction

Zinc deficiency markedly alters the metabolism of DNA, RNA, protein, Mn (II) and other metals in E. gracilis (1-4). Zinc deficient (-Zn)[†] E. gracilis cells contain twice as much DNA but the same amount of RNA as zinc sufficient (+Zn) cells. However, we now find that the mRNA fraction of (-Zn) cells is increased and its base composition is altered significantly.

The two RNA polymerases found in (+Zn) cells (5,6) are replaced by an unusual RNA polymerase which contains 2 gram atoms of Zn/700,000 grams of protein in (-Zn) cells. Its purification and detailed characteristics will be reported (2). Using this enzyme as a function of the concentration of Mn (II) in a cell free system, we have obtained RNA products whose base composition is

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[†]Zinc deficient and sufficient cells were grown in media containing 10^{-7} and 10^{-5} M Zn (II) and will hereafter be referred to as (-Zn) and (+Zn), respectivel;

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also changed dramatically, emulating those which are the consequence of zinc deficiency in E. gracilis.

Materials and Methods

Euglena gracilis, strain Z, was grown as described previously (3,4). All cultures were harvested in early stationary phase.

The total RNA from both (+Zn) and (-Zn) E. gracilis was isolated by a standard phenol-ethanol extraction (7). Chromatography of the extract on oligo-(dT)-cellulose (Collaborative Research, Waltham, MA) removes mRNA (8) and further, chromatography on dihyroxyboryl amino ethyl (DBAE) cellulose (Collaborative Research, Waltham, MA) removes tRNA (9), leaving ribosomal RNA.

Each RNA fraction was lyophilyzed and hydrolyzed using 0.1 ml 72% $HClO_4$ per 5 mg RNA and a 3 μl sample of the hydrolysate was subjected to high pressure liquid chromatography (Micromiretics Model 7115-24, Micromiretics, Norcross, GA) on a double column of Partisil 10 SCX (Whatman, Inc., Clifton, NJ). The bases were eluted using a gradient varying from 0.05 M to 0.08 M $NH_4H_2PO_4$, pH 3.35. Elution was monitored with a Hewlett Packard 7130A recorder (Hewlett Packard, Palo Alto, CA), and peak areas were integrated with a Hewlett Packard 3350 Lab Administration Data System. Standard mixtures of uracil, guanine, cytosine and adenine were used at concentrations ranging from 0.06 to 1 μg. For each base the peak areas were directly proportional to the amounts of base applied.

The RNA polymerase from (-Zn) cells was isolated by methods used previously to purify the RNA polymerases I and II from (+Zn) cells (5,6). Assay solutions contained 10 μg of this purified RNA polymerase; 10 μmol Tris HCl, pH 8.0; 0.1 μmol dithiothreitol; and 20 μg heat denatured calf thymus DNA in a total volume of 0.1 ml (5). The concentrations of Mn (II) in the assay mixture varied from 2 to 10 mM. The effect of different concentrations of Mn (II) on the incorporation of ^{3}H-UMP, ^{3}H-GMP or ^{3}H-CMP was examined by assaying with 5 μCi of ^{3}H-UTP (25 Ci per mM) plus 5 x 10^{-3} μmol each of GTP, ATP, and CTP or with 5 μCi of ^{3}H-GTP (20 Ci per mM) plus 5 x 10^{-3} μmol each of UTP, ATP, and CTP, or with ^{3}H-CTP (21 Ci per mM) plus 5 x 10^{-3} μmol each of GTP, ATP, and UTP.

Results and Discussion

The total amount of RNA in (+Zn) and (-Zn) E. gracilis cells is virtually the same. However, (-Zn) cells contain slightly less rRNA, the same amount of tRNA, and twice as much mRNA as (+Zn) cells (Table I).

The mRNA base composition and the G+C/A+U ratios of (+Zn) and (-Zn) cells, (Table II), determined by high pressure liquid chromatography, (Figure 1), are highly reproducible, varying by about 1-2% in multiple experiments. The results obtained for (+Zn) cells are in agreement with those reported by others for E. gracilis mRNA isolated under identical conditions (10). The data for mRNA from (-Zn) cells differ strikingly. Cytosine increases from 26 to 49%

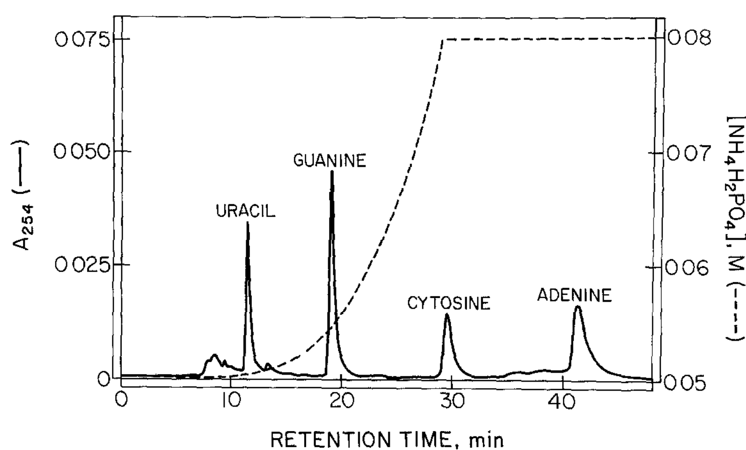

Figure 1 The purine and pyrimidine content of *E. gracilis* mRNA was analyzed by high pressure liquid chromatography. A three μl sample of an acid hydrolysate was used. The retention time for each base differs allowing for their separation. The peak areas for each base are proportional to the amount of base applied. The fraction of the total for each base is shown in Table II.

Table I: RNA Content of Zinc Sufficient (+Zn) and Deficient (-Zn) *E. gracilis*.

	Total RNA μg x 10^6/cell	Ribosomal %	Transfer %	Messenger %
(+Zn)	20 ± 5	79 ± 5	15 ± 3	6 ± 2
(-Zn)	19 ± 5	74 ± 4	15 ± 3	11 ± 3

Total RNA of a known number of *E. gracilis* cells was isolated from a cellular homogenate in 0.1 M Tris HCl, pH 7.6. The various RNA classes were separated as described in the text. Total RNA content was measured by an orcinol method. Each value is the mean ±1 s.d. (n=5).

of the total base content, while guanine and adenine decrease from 35 and 21% to 25 and 10%, respectively; uracil is essentially constant.

We have previously discussed various mechanisms through which zinc deficiency could affect RNA and DNA metabolism (5,11). In particular, we have emphasized the essentiality of zinc for the function of both DNA and

Table II: Base Composition of E. gracilis Messenger RNA

Base	(+Zn)	(-Zn)
Cytosine	26	49
Guanine	35	25
Adenine	21	10
Uracil	18	16
G+C/A+U	1.6	2.9

The data for each base are expressed as a percent of total. The values are the mean of three separate analyses of messenger RNAs. The base composition and the G+C/A+U ratio of (+Zn) cells are in agreement with those reported earlier from E. gracilis mRNA isolated at pH 7.6. (10)

RNA polymerases and have also noted the importance of Mn (II) (or Mg (II)) for the activity of these enzymes. However, the possible synergism and/or antagonism of these and other metals in nucleic acid polymerase action has not been examined critically. One consequence of zinc deficiency in E. gracilis (3, 4) is the 35-fold increase in intracellular Mn and the 5-fold increase in Mg content. This, together with earlier experiments utilizing E. coli RNA polymerase (12) prompted us to examine the role of these metals in determining the composition of the products (Table II). Thus, we have determined the effects of varying Mn (II) concentrations on the incorporation of bases into RNA produced by the single, unusual RNA polymerase from (-Zn) E. gracilis. These experiments were carried out in cell-free systems, and the incorporation of GMP, UMP or CMP served as the criterion of the base composition of the resultant RNA. Increasing the concentration of Mn (II) in the mixture used for assaying the single RNA polymerase from (-Zn) cells from 2 to 10 mM, progressively increases the GMP/UMP ratio from 1.0 to 3.5.

Table III: RNA Polymerase from (-Zn) _E. gracilis_: Effect of [Mn] on GMP/UMP and CMP/GMP Incorporation

[Mn] mM	GMP/UMP	CMP/GMP
2	1.0	1.0
5	1.1	2.2
10	3.5	1.2

Mn (II) was the only activating metal in the assay mixtures.

The CMP/GMP ratio is 1.0 in assays with 2 mM Mn (II). It increases to 2.2 and decreases to 1.2 in assays with 5 and 10 mM Mn (II), respectively (Table III). These data demonstrate that the base composition of the product is markedly dependent on the Mn (II) concentration. Such data, together with those in Table II, suggest that the changes in mRNA composition observed for (-Zn) cells may, in fact, be a consequence of their increased Mn--or other metal--content.

It is apparent that a metal, such as Mn, can have a pronounced effect on the transcription of endogeneous DNA template by RNA polymerase, a fact which may prove of considerable importance in understanding the role of metals in RNA (and DNA) polymerase action to which Mn (or Mg) have long been known to be essential. The indispensibility of zinc was recognized much later, and in fact, subsequent to the finding that its deficiency causes the accumulation not only of Mn but also of Mg, Fe, Cr, Ni, Ca, (3,4) and Cu (13). However, the mutual interaction and metabolic interdependence of such metals as well as their possible effects on translation and transcription have not been studied. Metal-metal interrelationships have been recognized in numerous organisms and tissues and described as "imbalance", "antagonism", "synergism" and "conditioned deficiencies" (14-16), much as the biochemical basis of all of these phenomena has remained largely unexplained.

The present system and the capability to isolate and characterize DNA, RNA, and the corresponding polymerases from eukaryotic cells clearly provide an exceptional opportunity to explore the biochemistry of these relationships in a specific case which is of major importance. Thus, the present studies direct attention to novel modes of approach to study the effect of metal metabolism on nucleic acid and protein synthesis with profound implications for their mechanisms.

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