REVERSE TRANSCRIPTASE FROM AVIAN MYELOBLASTOSIS VIRUS: A ZINC METALLOENZYME

D. S. Auld,* H. Kawaguchi,* D. M. Livingston† and B. L. Vallee*††

*The Biophysics Research Laboratory, Department of Biological Chemistry, Harvard Medical School, Division of Medical Biology, Peter Bent Brigham Hospital, Boston, Massachusetts and the Viral Lymphoma and Leukemia Branch, National Cancer Institute, National Institute of Health, Bethesda, Maryland, 20014

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Previous postulates of a relationship between a zinc enzyme and the leukemic process (1,2) have led to the identification of the RNA dependent DNA polymerase -- reverse transcriptase -- of avian myeloblastosis virus as a zinc metalloenzyme. Microwave induced emission spectrometry provides a microanalytical system capable of measuring precisely 10^{-11} to 10^{-14} g atoms of metal in microgram amounts of enzyme, orders of magnitude more sensitive than other, conventional methods. The chromatographic fraction with highest enzymatic activity contains 1.5×10^{-11} g atoms of zinc per 1.4 µg of protein, corresponding to 1.7 to 1.9 g atoms of zinc per mole of enzyme for a molecular weight previously determined either as 1.6 or 1.8×10^{5}. The Zn/activity ratio is constant in the active fractions. Copper, iron and manganese are absent, i.e., at or below their limits of detection, 10^{-13} to 10^{-14} g atoms. Agents known to chelate zinc inhibit the enzyme while their nonchelating isomers do not. The data underline the participation of zinc in nucleic acid metabolism and bear importantly upon the lesions which accompany leukemia and zinc deficiency.

Introduction

Differences in zinc metabolism of normal and leukemic leukocytes first led to the postulate that disturbance of a zinc dependent enzyme is critical to the pathophysiology of myelogenous and lymphatic leukemia (1,2). Many enzymes (3) including the DNA and RNA polymerases of E. coli (4,5) are now known to contain zinc essential for their catalytic activities. The existence of an RNA dependent DNA polymerase -- reverse transcriptase -- in avian myeloblastosis virus (AMV) and other RNA tumor viruses (6,7) has given new perspectives regarding a biochemical basis of leukemic and other malignant transformations. The earlier indications of a role for zinc in the metabolism of normal and leukemic leukocytes (1,2) led to

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†Present address: Children's Cancer Research Foundation, Boston, Massachusetts.

††To whom correspondence should be addressed.

the present kinetic and spectrographic studies of RNA dependent DNA polymerase of AMV. The data demonstrate that it is a zinc metalloenzyme.

Materials and Methods

AMV polymerase was prepared from the purified virus by a modification of the procedure of Ross et al (8). EDTA was not used in any of the purification steps. Concentrations of agents in the standard 100 µl assay are: $MnCl_2$, 0.2 mM; poly rA, 0.1 µM; oligo dT_{12-18}, 1 µM; ^{3}HTTP, 2.4 µM; DTT, 2 mM; KCl, 0.10 M; Tris-Cl, pH 7.8, 0.11 M. Approximately 0.2 µg of protein were added to initiate the reaction which was terminated at 60 min with 10% trichloroacetic acid.

Poly rA and oligo dT_{12-18} were obtained from Collaborative Research, Waltham, Mass., and ^{3}HTTP, 70 C per mole, from New England Nuclear Corporation. All chemicals were reagent grade. Protein was measured on 15 µl aliquots using the Lowry method (9). Microgram quantities of enzyme, obtained by gel exclusion chromatography, were analyzed for zinc, copper, iron and manganese on 5 µl aliquots according to principles discussed previously (10), but using instrumental facilities designed by Dr. George Wooten, Monsanto Research Corporation, Dayton Laboratories, Dayton, Ohio. The validity and accuracy of the method was established by determination of zinc in a series of zinc metalloenzymes of known metal content and stoichiometry.

Results and Discussion

With poly rA as the template and oligo dT_{12-18} as the initiator, the rate of the AMV polymerase catalyzed incorporation of TMP at 25° pH 7.8 is linear over a 20-fold range of enzyme concentration. Concentrations of DTT, KCl and Mn^{2+} were chosen to result in optimal conditions for the assay (see methods). The K_m for TTP is 1×10^{-5} M and the V_{max} is 12 pmoles TTP incorporated per minute per µg of protein at 25°.

Metal complexing agents inhibit the AMV polymerase catalyzed reaction both reversibly and also in a time dependent, irreversible manner.

Table I

Effect of Metal Binding Agents on the (AMV) RNA Dependent DNA Polymerase Activity*

Metal Binding Agent	Concentration x 10^4, M	v/v_c** %
1,10-phenanthroline	1	40
	2	4
1,7-phenanthroline	2	101
	8	95
4,7-phenanthroline	3	98
8 hydroxyquinoline-5-sulfonate	1	33
	5	15
EDTA	3	30
	5	2

*All rates measured at 25°. See methods for other conditions.

**Enzyme activities in the absence, v_c, and in the presence of inhibitor, v.

In the absence of such agents the enzyme is completely stable for 60 minutes either at 25° or 37°. At 25°, in the presence of 1,10-phenanthroline, 1 mM, the enzyme is inhibited instantaneously and reversibly with a K_I of 7×10^{-5} M. At 37°, preincubation of enzyme with 1,10-phenanthroline at time intervals up to 60 minutes causes both instantaneous and irreversible decreases in activity.

The metal complexing properties of 1,10-phenanthroline account for the inhibition: its isomers 1,7 and 4,7-phenanthroline neither bind metals

nor do they inhibit the nucleotide polymerization reaction under conditions where 1,10-phenanthroline inhibits it completely (Table I). Moreover, a number of other, structurally different metal binding agents, e.g., EDTA and 8-hydroxyquinoline-5-sulfonate also markedly inhibit the polymerase activity instantaneously (Table I). Such data support the interpretation that these agents bind to a hitherto unidentified functional and/or structurally indispensible transition or group IIB metal. The demonstration of the presence of stoichiometric quantities of such a metal is, of course, essential to the verification of this hypothesis.

Conventional procedures have not been sufficiently sensitive to allow quantitative metal determinations on very small amounts of enzyme. Microwave induced emission spectrometry, however, can extend the usual detection limits by 6 orders of magnitude to 10^{-14} g atoms and, thus, precise quantitative metal analyses on the microgram amounts of enzyme available to us for this purpose seemed feasible by this means. The presence of either zinc, copper or iron could account for the observed inhibition of the AMV polymerase, and these elements and manganese were determined after removal of metal quenching agents and low molecular weight protein contaminants by gel exclusion chromatography. The 45 µl fractions were analyzed for metals, activity and protein. Enzyme activity emerged in a sharp, narrow band with maximal activity, v, of 2.1 pmoles/min/µl enzyme. The elements were measured quantitatively with high precision when absolute amounts vary from 10^{-11} to 10^{-14} g atoms. Zinc is present in stoichiometric quantities in the purified enzyme (Table II), but the transition metals, Cu, Fe and Mn are virtually absent, i.e., the amounts detected, 10^{-13} to 10^{-14} g atoms, correspond to less than 0.06 g atoms per mole of enzyme (Table II). The specific activity, 7.5 pmole/min/µg protein of the most active fraction, No. 19, is three times higher than that of the enzyme placed on the column. This activity is closely similar to that of highly purified preparations of the enzyme employed for molecular

Table II

Analysis of Gel Exclusion Chromatography Fractions (45 µl) Exhibiting Maximal Activity*

Fraction No.	Zn g at x 10^{11}	Protein µg	Zn/Protein g at/mole	Cu	Fe	Mn
				g at/mole		
18	0.7	0.7	1.8	<.001	<.01	.06
19	1.5	1.4	1.9	<.001	<.01	.05
20	1.2	1.1	1.9	<.001	<.01	.05

*Calculations are based on a molecular weight of this polymerase of 1.8 x 10^5 (13). Zinc and protein content are expressed as g atoms or µg per 5 µl aliquot, respectively. Analyses were performed in triplicate.

weight determinations (11-13). The detection of 1.5 x 10^{-11} g atoms of zinc in 1.4 µg of protein (Table II) corresponds to from 1.7 to 1.9 g atoms of zinc per mole of enzyme for molecular weights found to be either 1.6 or 1.8 x 10^5 (11-13). Zinc may either participate directly in catalysis or control structure and affect function indirectly or both (3). For the nucleotidyl polymerases, these alternatives remain to be determined.

The present studies on the reverse transcriptase from AMV show that zinc is essential in the formation of DNA from RNA templates and implicates zinc in a leukemic process, confirming a hypothesis of long standing (1,2). The roles of zinc in nucleic acid metabolism and its bearing on the induction of leukemia are under study.

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