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Effect of removal of zinc on alfalfa mosaic virus RNA-dependent RNA polymerase

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The necessity of coat protein for infection of plants by alfalfa mosaic virus (AIMV) and other ilarviruses distinguishes this virus group from other plant virus groups. Recently, the presence of both a zinc-finger type motif and zinc in AIMV coat protein was described [(1989) Virology 168, 48–56]. We studied the effect of a zinc chelator on viral RNA synthesis. Strong inhibition of AIMV RNA-dependent RNA polymerase (RdRp) by ortho-phenanthroline (OP) was observed.

Alfalfa mosaic virus; Zinc; RNA polymerase inhibition

1. INTRODUCTION

Alfalfa mosaic virus (AIMV) requires a few copies of its coat protein bound to the genomic RNAs to initiate infection in host plants. This phenomenon called genome activation distinguishes AIMV and other ilarviruses from all other plant viruses. Coat protein binds preferentially to the homologous 3′-terminal regions of genomic RNAs (for review see [1]. It has been postulated that the coat protein is involved in viral RNA synthesis (for review see [1]). The isolation of an RNA-dependent RNA polymerase (RdRp) which is capable of initiation de novo on added templates [2] provides a tool to elucidate the role of the coat protein in RNA synthesis. The recent report on the presence of both a putative 'zinc-finger' type domain and zinc itself in the coat proteins of the ilarviruses AlMV and tobacco streak virus [3] and the rapidly accumulating data on the importance of 'zinc-finger' domain-containing proteins in eukaryotic transcription regulation [4] prompted us to investigate the effect of removal and addition of zinc on AlMV RdRp activity in vitro.

2. METHODS AND MATERIALS

AlMV RdRp was prepared as described previously [2]. RNA synthesis by this enzyme preparation is fully dependent on added templates, even when micrococcal nuclease treatment was omitted (data not shown).

To 5 μl of the enzyme preparation $ZnCl_2$, ortho-phenanthroline (OP) or combinations of both were added as indicated in the legends of the figures. The volume was adjusted to 50 μl with buffer (final concentrations: 50 mM Tris-HCl, pH 8.2; 10 mM $MgCl_2$; 10 mM dithiothreitol (DTT); 15% (v/v) glycerol). Enzyme preparations were incubated with zinc-ions and chelator for 20 min at 25°C. Subsequently, 50 μl reaction buffer (50 mM Tris-HCl, pH 8.2; 10 mM $MgCl_2$; 10 mM DTT; 2 mM ATP, CTP, GTP; 20 μM UTP; 10 μCi [α-^{32}P]UTP, 400 Ci/mmol; 400 μg/ml template RNAs) was added. Reactions were carried out at 30°C for 30 min.

Reaction products were processed, treated with nuclease S1 and analyzed by gel electrophoresis as described previously [4].

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3. RESULTS AND DISCUSSION

Ortho-phenanthroline is a well-defined chelator of zinc and proved to be effective in systems in which other metal chelators were ineffective [5]. In addition to OP we tested the chelators ethyleneglycolbis(β-amino-ethyl ether)*N,N′*-tetraacetic acid (EGTA) and ethylenediaminetetraacetic acid (EDTA) for their capability to inhibit AlMV RdRp activity. No significant inhibition of AlMV RdRp activity by EGTA and EDTA at a concentration of 5 mM was observed (data not shown). High concentrations (20 mM) of EDTA did inhibit RdRp activity probably by removal of Mg^{2+} ions essential for polymerase activity. OP has a strong inhibitory effect on AlMV RdRp activity (Fig. 1, lane 2; Fig. 2). OP at a concentration of 2 mM inhibits AlMV RdRp activation for more than 50% while complete inhibition of RdRp activity is observed at concentrations 6 mM and higher (Fig. 2). The inhibition is not observed if zinc ions are added simultaneously with the chelator (Fig. 1, lane 3) or just prior to incubation with AlMV RNAs and nucleosidetriphosphates (Fig. 1, lane 5). This indicates that the inhibition is caused by the removal of zinc from the RdRp preparation and is not due to impurities in the OP preparation. AlMV RdRp is slightly stimulated by the addition of zinc ions (Fig. 1, lane 5). Addition of divalent metal ions: Ca^{2+}, Co^{2+}, Cu^{2+}, Mg^{2+} and Mn^{2+} did not restore the activity of

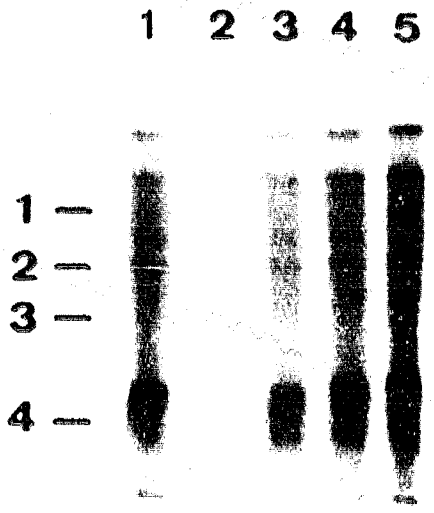

Fig. 1. Autoradiogram of double-stranded products synthesized in reaction mixtures containing: untreated AlMV RdRp (lane 1); AlMV RdRp preincubated with 10 mM OP (lane 2); AlMV RdRp preincubated with 10 mM OP and 10 mM $ZnCl_2$ added simultaneously prior to RNA synthesis (lane 3); AlMV RdRp preincubated with 10 mM OP, 10 mM $ZnCl_2$ was added just prior to RNA synthesis (lane 4); AlMV RdRp preincubated with 10 mM $ZnCl_2$ (lane 5). Positions of AlMV virion RNAs are indicated.

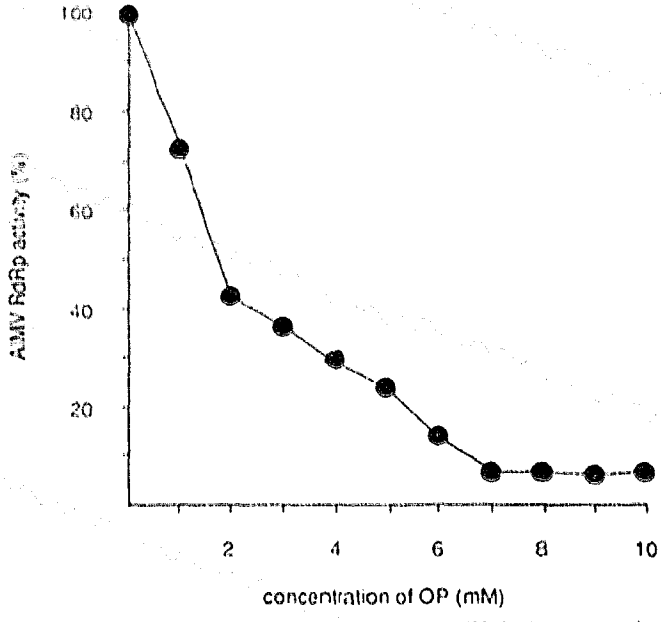

Fig. 2. Inhibition of AlMV RdRp by OP. AlMV RdRp was preincubated with OP at concentrations indicated in the graph. Incorporation of [^{32}P]UMP in double-stranded products synthesized by OP-treated AlMV RdRp was determined. Incorporation of [^{32}P]UMP by non-treated AlMV (11 547 cpm) was set to 100% activity.

OP-treated AlMV RdRp or stimulated AlMV RdRp activity (data not shown). Since DTT is a weak chelator of zinc the experiments were repeated using β-mercaptoethanol as a reducing agent. However, in none of the experiments a difference was observed using this agent instead of DTT (data not shown).

From the data presented in this paper it is clear that a zinc-dependent factor is involved in AlMV RNA synthesis. Since no double-stranded reaction products of any size were detected after removal of zinc from AlMV RdRp either an early event in viral RNA synthesis e.g. initiation is inhibited or the structural integrity is reversibly disrupted by zinc removal. It is tempting to conclude that removal of zinc from the AlMV coat protein is the cause of the inhibition of RdRp activity. However, it is equally well possible that removal of zinc from another protein in the crude enzyme preparation is responsible for the inhibition of RdRp activity. Further purification and characterization of the AlMV RdRp should provide more information on the nature of the zinc-dependent factor present in the AlMV RdRp complex.

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