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Methyl Mercury Stimulates Chain Elongation by Purified HeLa RNA Polymerase II

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SUMMARY

Methyl mercury (MeHg) inhibited the overall RNA synthetic reaction of HeLa RNA polymerase II. However, when RNA chain initiation was allowed to occur in its absence, MeHg stimulated the rate of the subsequent elongation stage of the reaction. Chain elongation with both double-stranded and single-stranded DNA templates was stimulated. This stimulatory effect was specific for MeHg; both *p*-hydroxymercuribenzoate and $HgCl_2$ inhibited chain elongation (to about the same degree as they inhibited the overall reaction). The stimulatory effect was also specific for the HeLa polymerase; with *Escherichia coli* RNA polymerase, MeHg inhibited elongation (to the same degree as it inhibited the overall reaction).

The most characteristic effect of mercury compounds in biological systems is inhibition (1). Many of the toxic properties of these compounds, at both the organismic and cellular levels, have been ascribed to their ability to inhibit a wide variety of enzymes (1). Cellular macromolecular synthesis is no exception to this generalization. There have been many reports of the inhibition by mercury compounds of DNA, RNA, and protein synthesis in a variety of organisms and cells (2–11), and mercury compounds have been shown to directly inhibit DNA and RNA polymerases (7, 12–14).

Notwithstanding this generalization of mercury compounds as inhibitors, there have been a number of reports of the stimulation of enzymes by mercury compounds (1) (although none to our knowledge of the stimulation of the activity of an RNA polymerase). We previously reported that MeHg specifically stimulates the RNA synthesis in isolated HeLa nuclei that is catalyzed by RNA polymerase II (6). With the purified RNA polymerase II, we found that the overall enzymatic reaction was inhibited by MeHg, but the elongation stage of the reaction was not (6). Although some concentrations of MeHg appeared to stimulate elongation, the effect was small and variable. We have now reexamined the effect of MeHg on the purified enzyme, using a modified assay that is more specific for elongation. In this paper we describe our finding that MeHg does have a significant stimulatory effect on the rate of chain elongation by RNA polymerase II.

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Experimental Procedures

Materials. Unlabeled ribonucleoside triphosphates were purchased from P.L. Biochemical (Milwaukee, WI) and [^{3}H]UTP from NEN (Boston, MA). MeHg and $HgCl_2$ were purchased from Alfa (Danvers, MA), and *p*HMB from Calbiochem (La Jolla, CA).

DNA and enzymes. Calf thymus DNA was purchased from Cooper Biomedical (Malvern, PA). DNA was alkali-denatured by incubation in 0.1 N NaOH for 10 min at room temperature, followed by neutralization in the cold with 1 M Tris·HCl (pH 4.5). *Escherichia coli* RNA polymerase was purchased from NEN. RNA polymerase II was extracted from HeLa cells by the procedure of Hossenlop *et al.* (15) and purified by DEAE-cellulose chromatography. Further purification of the HeLa enzyme by phosphocellulose chromatography was carried out as follows: 3.8 mg of bovine serum albumin were added to a solution containing 1.2 mg of the enzyme, which was then diluted in 50 mM Tris·HCl buffer (pH 7.9) containing 100 μM EDTA, 1 mM dithiothreitol, and 25% (v/v) ethylene glycol, to yield a final concentration of 0.05 M ammonium sulfate and 0.2 mg/ml bovine serum albumin. The enzyme was loaded on a 1-ml phosphocellulose column (Whatman, Clifton, NJ), which had been equilibrated with the same buffer containing 50 mM ammonium sulfate and 0.2 mg/ml bovine serum albumin. The column was washed with 2.5 ml of the same buffer and then successively with 2.5-ml portions of the same buffer containing 0.2, 0.3, and 0.4 M ammonium sulfate. Most of the polymerase activity was eluted in the 0.2 M wash. The fractions containing the activity were pooled and dialyzed against 50 mM Tris·HCl buffer (pH 7.9) containing 0.1 M NaCl and 50% glycerol. The degree of additional purification achieved was difficult to quantitate because the level of protein in the eluate was below the level of detection. However, using the minimum detectable protein as a maximum value for the protein in the eluate, the additional purification achieved can be calculated to be at least 3–5-fold.

RNA polymerase reactions. The standard reaction mixture contained 50 mM Tris·HCl buffer, pH 7.9, 22 mM $MnCl_2$, 100 mM ammo-

ABBREVIATIONS: MeHg, methyl mercury; *p*HMB, *p*-hydroxymercuribenzoate.

nium sulfate, 0.33 mM each of ATP, GTP, and CTP, 1.4 μM [^{3}H]UTP (final specific activity, 24 Ci/mmol), and 77 μg/ml calf thymus DNA and enzyme. After incubation for 30 min at 37°, the reaction mixture was chilled and 0.2 ml of salmon sperm DNA (0.25 mg/ml) and 0.5 ml of 7% perchloric acid were added. The precipitate was collected on a Whatman GF/C filter and washed with 6% trichloroacetic acid containing 0.1 M sodium pyrophosphate. The filter was dried and counted in a liquid scintillation counter. All results are presented are the means of duplicate determinations: individual determinations varied less than 10% from the mean.

In experiments to measure the rate of the overall polymerase reaction, mercury compounds were added to the reaction before the addition of the enzyme. In experiments to measure the rate of elongation, the enzyme was preincubated for 10 min at 37° with all of the above reaction components (unless otherwise indicated) except for the [^{3}H] UTP. (Unlabeled UTP was present at a concentration of 0.5 μM.) Heparin was then added (final concentration, 100 μg/ml), after which the mercury compound and [^{3}H]UTP were added and the incubation continued for 30 min at 37°. [It should be noted that, in our previous studies on elongation (6), the post-incubation was carried out in the absence of heparin. We subsequently found that with HeLa polymerase II a significant (and variable) amount of initiation continues to occur after preincubation. Because the stimulatory effect of MeHg on elongation tends to be masked by its inhibition of this initiation, in the experiments described here, heparin was present during all elongation reactions.]

Results

MeHg is a potent inhibitor of RNA synthesis by RNA polymerase II of HeLa cells, when it is present before the start of the reaction (Fig. 1, *open circles*). The overall RNA synthesis reaction can be divided into two principal stages, initiation of a new RNA chain, followed by its elongation (16). Because the rate-limiting step of the overall reaction is the initiation of the RNA chain (16), the inhibition of the overall reaction by MeHg is indicative of inhibition of initiation. It is possible to measure the effect of MeHg specifically on the elongation stage of the reaction by allowing initiation to occur in its absence (see Experimental Procedures) and blocking further initiation by the addition of heparin (17). The result of such an experiment (Fig. 1, *closed circles*) demonstrates that, in contrast to the overall reaction, the elongation stage was stimulated by MeHg.

The results of a kinetic experiment (Fig. 2) demonstrate that MeHg increased the initial rate of the elongation reaction.

In the above experiments, because the enzyme was preincubated with the DNA template and the four ribonucleoside triphosphates, the initiation stage of the reaction was completed and the elongation stage had begun before MeHg was added. The initiation stage of the reaction can itself be divided into two substages, binding of the enzyme to the DNA and initiation of the RNA chain (i.e., the synthesis of the first few phosphodiester bonds) (16). In order to determine which of these substages must take place for the subsequent reaction to be stimulated by MeHg, we examined the effect of MeHg on heparin-resistant synthesis after preincubation of the enzyme with the DNA template without the ribonucleoside triphosphates. The result of this experiment (Fig. 3) shows that, after preincubation of the enzyme with only the DNA, the subsequent reaction was not stimulated by MeHg (although it was less sensitive to inhibition). This indicates that MeHg does not stimulate the initiation of the RNA chain, only the subsequent addition of nucleotides to the growing chain.

Elongation requires localized denaturation of the DNA template in advance of the polymerase (18), and MeHg had been shown to induce denaturation of double-stranded DNA (19–23). Thus, it is conceivable that MeHg stimulates elongation not by interacting with the polymerase but rather by inducing localized denaturation of the template DNA. In order to investigate this possibility, we examined the effect of MeHg on chain elongation by the polymerase with denatured DNA as a template. The results (Fig. 4) show that chain elongation with the denatured DNA template was also stimulated by MeHg, arguing against a template denaturation mechanism for the stimulation.

We have also investigated the effect of MeHg on chain elongation by *E. coli* RNA polymerase. With this enzyme, elongation was not stimulated by MeHg and, in fact, was inhibited to the same extent as the overall reaction (Fig. 5).

The HeLa RNA polymerase II that we used in these experiments is only a partially purified preparation. In order to rule out the possibility that the specificity of the stimulation with this enzyme is due to the presence of a contaminating protein, we subjected the enzyme to an additional purification step (see Experimental Procedures) and examined the effect of MeHg

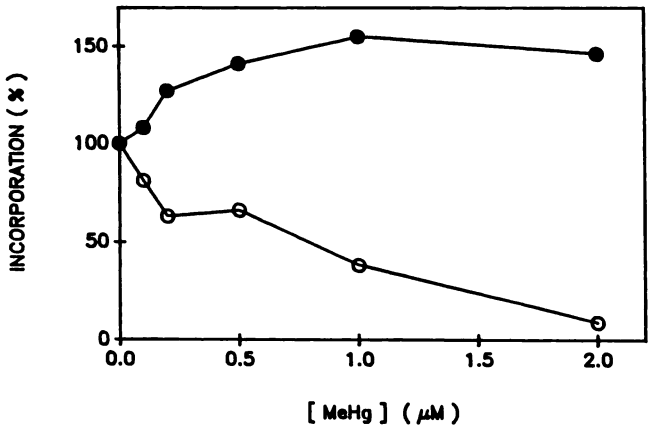

Fig. 1. Effect of MeHg on RNA synthesis by HeLa RNA polymerase II. The rate of the overall reaction (○) or the rate of elongation (●) was measured in the presence of the indicated concentration of MeHg, as described in Experimental Procedures. The results are presented as the percentage of the incorporation in the absence of MeHg (100% = 4700 cpm for the overall reaction, 4800 cpm for the elongation reaction).

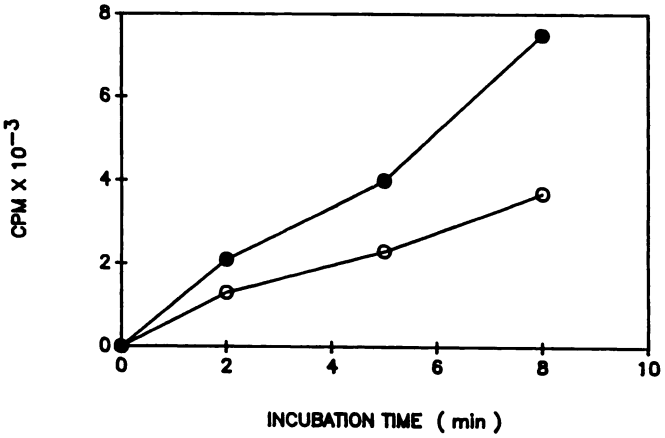

Fig. 2. Effect of MeHg on elongation by HeLa RNA polymerase II. The elongation reaction was carried out either in the absence (○) or presence (●) of 1 μM MeHg as described in Experimental Procedures, except that acid-precipitable radioactivity was determined after incubation at 37° for the indicated times (rather than after 30 min).

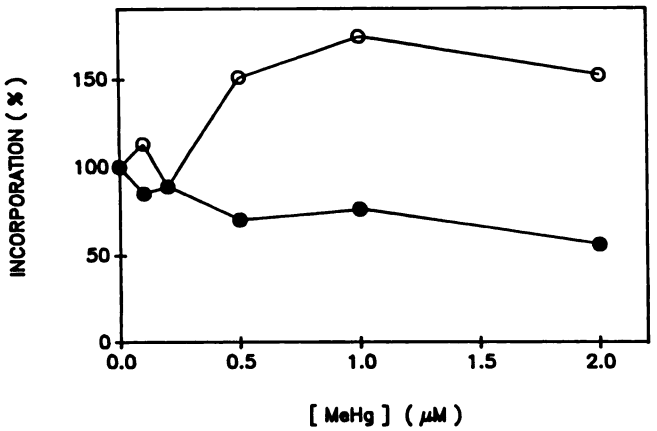

Fig. 3. Effect of MeHg on heparin-resistant RNA synthesis by HeLa RNA polymerase II after preincubation with only some reaction components. The enzyme was preincubated with either complete reaction mixture (as described in Experimental Procedures) (○), or the complete mixture minus the ribonucleoside triphosphates (●). After the preincubation, heparin, the indicated concentration of MeHg, the missing reaction components, and [^{3}H]UTP were added. The incubation was continued for 30 min at 37°, and acid-precipitable radioactivity was measured as described in Experimental Procedures. The results are presented as the percentage of the incorporation in the absence of MeHg (100% = 3700 cpm for complete preincubation, 2300 cpm for preincubation without the triphosphates).

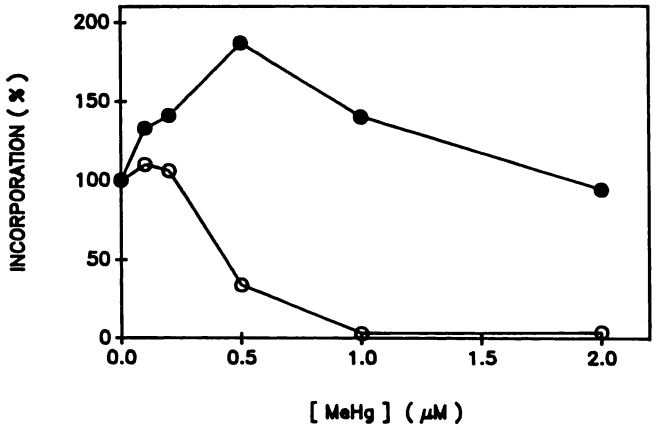

Fig. 4. Effect of MeHg on RNA synthesis by HeLa RNA polymerase II with single-stranded DNA as template. The experiment was as described in Fig. 1 except that the reaction contained 23 μg of alkali-denatured calf thymus DNA, instead of the native DNA that is in the standard reaction mixture. The *symbols* are the same as in Fig. 1 (100% = 17,500 cpm for the overall reaction, 13,100 cpm for the elongation reaction).

on elongation by the more purified enzyme. The results (Fig. 6) show that the additional purification did not affect the degree of stimulation of elongation by MeHg.

We also examined the effect of other mercury compounds on the overall RNA polymerase reaction and on elongation. The results (Fig. 7) show that neither an inorganic mercury compound ($HgCl_2$) nor an aromatic organic mercury compound (*p*HMB) had the same stimulatory effect as MeHg on elongation. In fact, both compounds inhibited chain elongation by the polymerase to approximately the same degree as they inhibited the overall polymerase reaction.

Discussion

Our results have shown that there are several elements of specificity in the stimulatory effect of MeHg on elongation by

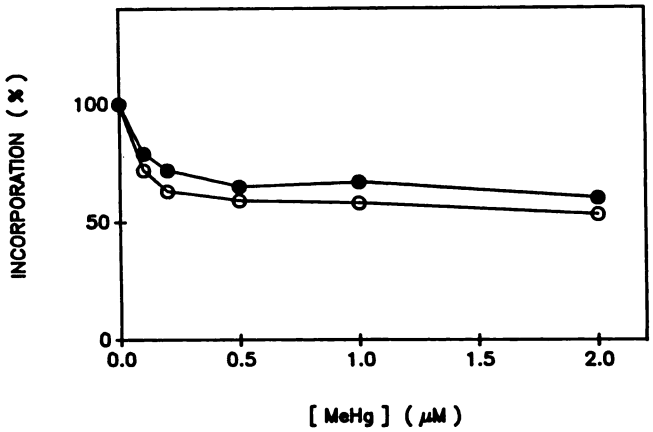

Fig. 5. Effect of MeHg on RNA synthesis by *E. coli* RNA polymerase. The experiment was carried out as in Fig. 1 (100% = 24,000 cpm for the overall reaction, 10,600 cpm for the elongation reaction).

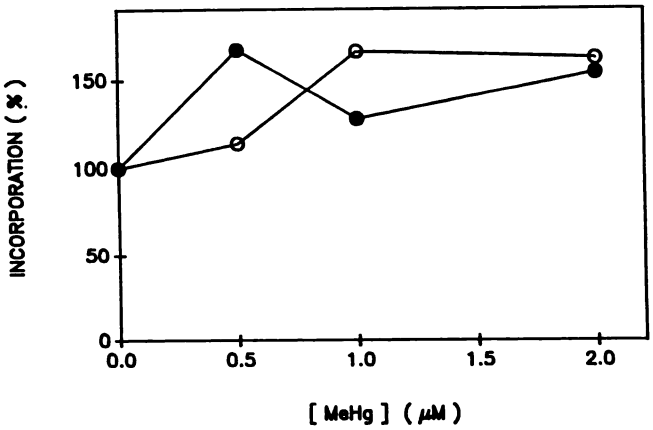

Fig. 6. Effect of MeHg on the rate of elongation by more highly purified HeLa RNA polymerase II. The rate of elongation by the standard enzyme (○) or by enzyme that had been further purified by phosphocellulose chromatography (●) (see Experimental Procedures) was measured in the presence of the indicated concentration of MeHg. The results are presented as the percentage of the incorporation in the absence of MeHg (100% = 4000 for the standard enzyme, 2200 for the phosphocellulose-purified enzyme).

RNA polymerase. First, the HeLa polymerase exhibits the stimulation but the *E. coli* enzyme does not. Second, MeHg stimulates elongation by the HeLa enzyme but $HgCl_2$ and *p*HMB do not. These results suggest that the stimulation requires structural specificity in both the enzyme and the mercury compound; this supports the idea that the stimulatory effect results from a specific interaction between MeHg and the enzyme.

Although there have been reports of enzyme stimulation by mercury compounds, the effect of MeHg on the HeLa RNA polymerase appears to be unique in that there is inhibition of one stage of the reaction and stimulation of another. There is evidence that there is a change in the conformation of the polymerase upon transition from the initiation to the elongation mode (24). One model to explain the different effects of MeHg on initiation and elongation by the HeLa enzyme is that this transition results in a different sulfhydryl group becoming accessible to MeHg. Interaction of MeHg with the SH group that is accessible in the enzyme before initiation results in inhibition, whereas interaction of MeHg with the SH group that is accessible during elongation results in stimulation. The

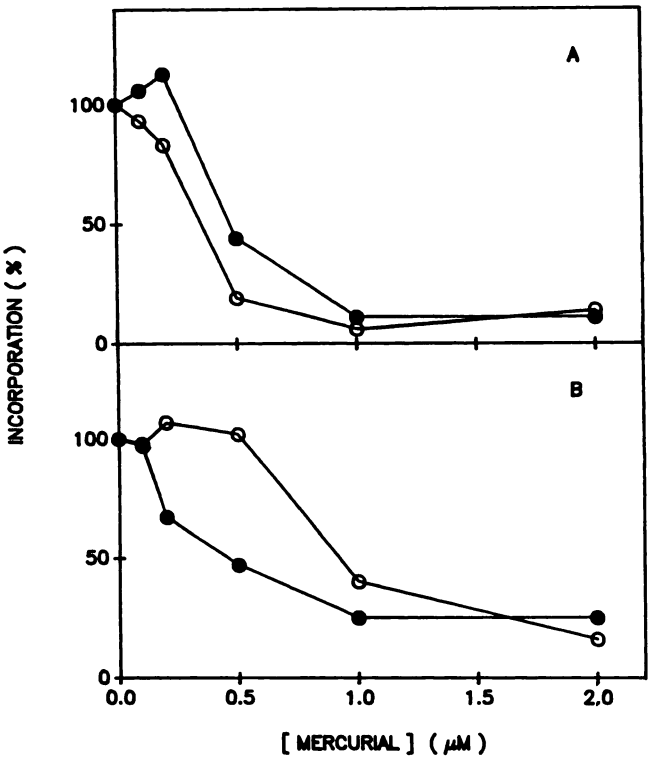

Fig. 7. Effects of other mercury compounds on RNA synthesis by HeLa RNA polymerase II. The *symbols* are the same as in Fig. 1. The results are presented as in Fig. 1. A, $HgCl_2$ (100% = 3500 cpm for the overall reaction, 9100 cpm for the elongation reaction); B, *p*HMB (100% = 8300 cpm for the overall reaction, 4500 cpm for the elongation reaction).

model further suggests that with the *E. coli* enzyme, in contrast, there is no change in SH group accessibility during the transition from initiation to elongation. Thus, with this enzyme MeHg interacts with the same SH group in both cases and inhibits both initiation and elongation. An alternative model is that, even in the case of the HeLa polymerase, MeHg interacts with the same SH group during both initiation and elongation. However, because the enzyme conformation is different during the two stages of the reaction, this interaction results in inhibition of the former and stimulation of the latter. We are currently investigating these alternative models.

The molecular mechanism by which MeHg stimulates elongation is not yet clear. It could be the result of an actual increase in the rate of addition of nucleotides to the growing RNA chain. However, there have been reports that have indicated that the measured rate of elongation is actually a composite of two rates, the "normal" rapid rate of nucleotide addition and the very slow rate during periods of "pausing" by the polymerase (16). Thus, a decrease in these pause periods would be expected to result in an increase in the measured rate of elongation. In fact, evidence has been obtained that the HeLa transcription factor TFII-S stimulates the rate of elongation by such a mechanism (25, 26). Conversely, the drug 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole has been shown to inhibit elongation by increasing pausing (27). The possible effect of MeHg on pausing is under investigation.

Webb (1) has suggested that the stimulation of enzymes by mercury compounds could be an important mechanism of their effects on cellular metabolism. This is a reasonable possibility because, in principle, an increase in the activity of an enzyme that is part of a metabolic pathway can have as much of a disruptive effect on metabolism as a decrease in that activity. This concept is particularly attractive in the case of RNA polymerase, because stimulation of transcription could have an effect on the level of many cellular enzymes. Thus, further investigation of the stimulatory effect of MeHg on RNA polymerase will be important for achieving a better understanding of its toxicity *in vivo*.

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