Inhibition of HeLa cell DNA topoisomerase I by ATP and phosphate

Robert L.Low* and Joseph A.Holden

Departments of Pathology and Biological Chemistry, Washington University School of Medicine, St. Louis, MO 63110, USA

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ABSTRACT

The relaxation activity of DNA topoisomerase I from HeLa cell nuclei is strongly inhibited by a variety of purine nucleotides in the presence but not absence of 1 mM potassium phosphate. For ATP, 3-4 mM causes nearly complete inhibition. The 2'- and 3'-AMP isomers are active as well in the presence of 1 mM phosphate, but the 5'-AMP isomer and adenosine are inert. At 3 mM ATP, the titration curve for phosphate is sigmoidal with inhibition beginning abruptly at about 0.5 mM. The negatively-supercoiled DNA isolated from an "inhibited" reaction is relaxed as well as the standard DNA template in the absence of ATP and phosphate suggesting that inhibition does not result from an alteration of the template which protects against its relaxation. Relaxation of positively-supercoiled DNA is also inhibited. Catalysis by *E. coli* DNA topoisomerase I and HeLa DNA topoisomerase II is not inhibited at concentrations of ATP and phosphate sufficient to cause 80-90% inhibition of HeLa type I enzyme.

INTRODUCTION

DNA topoisomerases are ubiquitous and likely essential for DNA replication and gene expression (for reviews, see ref. 1-5). Both procaryotes and eucaryotic cells contain two basic types of enzymes, called DNA topoisomerases I and II. Type-1 enzymes change the linking number in steps of one through transient nicking and ligation of one strand of the helix. In distinction, type-2 DNA topoisomerases require ATP and change the linking number in steps of two via transient double-strand cleavage and strand passage. Unlike the prototype type-2 enzyme of *E. coli* (gyrase), the eucaryotic type-2 topoisomerases all fail to introduce negative-supercoils into relaxed DNA in vitro and therefore resemble the type-2 enzyme of phage T4.

Several enzymatic differences have been noted between the procaryotic and eucaryotic type-1 enzymes which suggest a rather fundamental difference in their mechanism of action (6). The eucaryotic enzymes relax both negatively- and positively-supercoiled DNAs to completion (7) while the

bacterial enzymes fail to relax positively-twisted DNAs and only partially relax negatively-supercoiled templates (8). During catalysis, the bacterial enzymes form a covalent intermediate with the 5'-phosphoryl at the site of strand cleavage (9) while the eucaryotic enzymes attach via the 3'-phosphoryl (10,11). The bacterial enzymes show a strong preference for catalysis in regions of single-stranded (SS) DNA. *E. coli* DNA topoisomerase I (protein ω) catenates circles which contain small gaps at least two orders of magnitude better than nicked circles (12) while DNA topoisomerase I of HeLa cells catenates poorly whether or not gapped circles are provided (J.A. Holden and R. Low, manuscript submitted). This difference in template preference may account for the finding that SS DNA is a more potent inhibitor of the bacterial relaxation activity than that of the eucaryotic enzyme (11). Studies of the cleavage of SS DNA using SS and gapped templates also indicate that the procaryotic and eucaryotic enzymes prefer quite different cleavage sites in terms of sequence and potential secondary structure (6,13-17). While the bacterial enzymes cleave at SS regions, the eucaryotic enzymes appear to seek regions of duplex structure (18), and hence during strand passage may be less adept at bridging the enzyme-induced break (5,6) than protein ω (15, 19).

DNA topoisomerase I of HeLa cell nuclei has been previously purified and partially characterized (20,21). Unlike HeLa DNA topoisomerase II, the HeLa type-1 enzyme has not been known to interact with ATP. In this study, we report that HeLa cell DNA topoisomerase I is strongly inhibited by ATP and a variety of other purine nucleotides provided that low levels of inorganic phosphate are present. In contrast, *E. coli* protein ω and HeLa DNA topoisomerase II are immune. This inhibitory effect of ATP is reminiscent of a previous report by Rowe et al (22) that the type 1 DNA topoisomerase of the lower eucaryote *Ustilago maydis* is strongly inhibited by 5mM ATP, ADP, and by nonhydrolyzable analogues of ATP. With the *U. maydis* enzyme, other purines are apparently inactive and although no mention of a requirement for phosphate was described (22), we note that assays used a potassium phosphate buffer.

This inhibitory effect with the HeLa type-1 enzyme was first discovered while assaying low levels of DNA topoisomerase I activity in a phosphate containing buffer used during purification by phosphocellulose chromatography. Here, we summarize our initial studies of these inhibitory effects using the purified enzyme.

MATERIALS AND METHODS

Nucleotides and chemicals

Purified grades of ATP free of vanadate and calcium were purchased from Sigma and P.L. Biochemical Co. ATP- γ -S and AMPPNP were from Boehringer-Mannheim. All other nucleotides were obtained from P.L. Biochemical Co. Adenosine 3', 5'-diphosphate was further purified by filtration through Sephalex G10. Agarose was bought from MCB, ethidium bromide and Hepes were from Sigma, dithiothreitol from Calbiochem. Mono- and dibasic salts of potassium phosphate were purchased from J.T. Baker.

Nucleic Acids and Enzymes

M13 mp9 RF DNA which contains a Hind III-EcoRI 900-bp fragment of human mitochondrial (mt) DNA (23), and ϕ X 174 DNA were prepared and purified by described methods (24).

Positively-supercoiled M13 RF DNA was prepared by incubating relaxed DNA in the presence of stoichiometric levels of gyrase A and B subunits of *E. coli* plus catalytic levels of HeLa DNA topoisomerase I (25). The reaction mixture contained in a total vol. of 400- μ l: 40 mM Hepes (pH 8.0), 10 mM MgCl₂, 100 mM KCl, 2 mM dithiothreitol, 0.5 mM EDTA, 0.25 mg per ml *E. coli* tRNA, 6 μ g of relaxed M13 RF DNA, 20 μ g of gyrase A subunit, 12 μ g of gyrase B subunit, and 20 ng of HeLa DNA-topoisomerase I. After a 15 min incubation at 30°C, the DNA was phenol extracted, ethanol precipitated and resuspended in 20- μ l of 10 mM Hepes (pH 8.0), 0.5 mM EDTA.

E. coli DNA topoisomerase I (protein ω) was purified to near homogeneity from an overproducing strain (26) kindly purified by Professor James Wang (Harvard).

HeLa DNA topoisomerase I was purified according to the method of Liu and Miller (20). Analysis of the final fraction on a silver-stained, SDS-polyacrylamide gel showed a single band of 100,000-D.

Relaxation reactions

The 20- μ l reaction contained 40 mM Hepes (pH 8), 100 mM KCl, 0.25 mg per ml bovine serum albumin, 10 mM MgCl₂, 2 mM dithiothreitol, 0.5 mM EDTA, 300 ng of supercoiled DNA template and 0.5 ng of HeLa DNA topoisomerase I unless otherwise stated. Reactions were run 15 min at 30°C, terminated by the addition of 1% (w/v) SDS, 20 mM EDTA, 0.01% bromphenol blue and run on a 0.8% (w/v) agarose gel usually overnight, at 1.5 V/cm in 40 mM Tris-acetate (pH 8), 2 mM EDTA. Gels were stained with 0.5-1.0 μ g/ml ethidium bromide for 60 min at ambient temperature, destained 20 min in H₂O and photographed using 300 nm illumination and Polaroid type 57 or 55 film.

Enzyme was diluted in 40 mM Hepes (pH 8), 100 mM KCl, 0.20 mg per ml bovine serum albumin, 2 mM dithiothreitol, 0.5 mM EDTA, 40% (v/v) glycerol and 6% (w/v) polyethylene glycol (27) unless otherwise stated.

The abbreviations used are: Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; RF, circular double-stranded replicative form; SDS, sodium dodecyl sulfate; EDTA, [ethylenedinitrilo]tetraacetic acid; SS DNA, single-stranded DNA; AMPPNP, 5'-adenylylimidodiphosphate.

RESULTS

HeLa DNA topoisomerase I is inhibited by ATP in the presence of inorganic phosphate

The relaxation activity of a highly purified fraction of DNA topoisomerase I from HeLa cell nuclei is markedly inhibited by ATP when low levels of inorganic phosphate are present. In assays containing 300 ng of supercoiled template, about 80-90% inhibition of relaxation is seen at 2 mM ATP and 1 mM potassium phosphate (Fig. 1A). Little significant inhibition occurs at levels of phosphate and ATP lower than 0.4 mM and 0.5 mM respectively (lanes 3-5 and 11-13), or with either component alone: 2 mM ATP itself appears inert (lane 2) as well as 1 mM phosphate (lane 10). Using 600 ng of supercoiled template, the titrations for phosphate and ATP inhibition were similarly carried out and for each point, the extent of inhibition was quantitated by scanning the photograph negatives. As shown in Fig. 1B, using 3 mM ATP, the phosphate titration is sigmoidal and inhibition occurs abruptly around 0.5 mM. Keeping phosphate at 1 mM, 50% inhibition occurs at 0.75 mM ATP and nearly complete inhibition at 3 mM (Fig. 1C). Sodium pyrophosphate in the range 0.5-2.0 mM does not substitute for inorganic phosphate and above 5 mM causes marked inhibition (>80%) in the absence of ATP (not shown).

Kinetic experiments have shown that the rate of relaxation of the negatively-supercoiled DNA template appears unaffected by either 2 mM ATP or 1 mM potassium phosphate alone but that in the presence of both components, inhibition is sustained even when incubation times are extended to at least 90 min at 30°C (not shown). These inhibitory effects are seen with different commercial preparations of ATP, including a highly purified lot free of vanadate and calcium. Varying the KCl concentration from 12 to 100 mM in the reaction mixture or increasing the MgCl₂ to 15 or 20 mM fails to prevent inhibition. When relaxed templates plus the HeLa topoisomerase I are incubated with 2 mM ATP and 1 mM phosphate, no change in the pattern of

Figure 1: Inhibition of HeLa DNA topoisomerase I requires ATP and phosphate. Relaxation reactions contained 0.5 ng of purified DNA topoisomerase I and were carried out and processed as described in "Materials and Methods". A, reactions each have 300 ng of supercoiled DNA and in lanes 2-8 contained 2 mM ATP and 0.0 to 1.8 mM P_i as indicated. Reactions for lanes 10-16 contained 1 mM P_i and 0.0 to 4.0 mM ATP as indicated; minus enzyme controls are denoted C as shown in lanes 1 and 9. B, reactions contained 600 ng of DNA, 3 mM ATP and 0.0, 0.13, 0.25, 0.5 or 1.0 mM P_i. Photograph negatives (Polaroid type 55 film) were scanned using a microdensitometer (Helena Laboratories). The peak tracing corresponding to the band of supercoiled DNA was cut out and weighed. For each lane, the percent inhibition was calculated based on the amount of DNA still supercoiled relative to a minus enzyme control. C, reactions contained 600 ng of DNA, 1.0 mM P_i and 0.5, 1.0, 2.0 or 4.0 mM ATP. Percent inhibition at each level of ATP was calculated from tracing data as described above.

Figure 2: Excess purified or crude topoisomerase I activity can overcome the inhibition by 2 mM ATP and 1 mM phosphate. Relaxation reactions contained 300 ng of DNA and were carried out and processed as described in "Materials and Methods". A, titration of purified topoisomerase I in the absence (lanes 2-8) or presence (lanes 10-16) of 1 mM P_i and 3 mM ATP. B, titration of topoisomerase I activity in a crude nuclear fraction in the absence (lanes 2-5) or presence (lane 6-9) of 1 mM P_i and 3 mM ATP.

relaxed topoisomers is seen indicating that ATP and phosphate do not elicit a gyrase activity (not shown).

Inhibition is reversed by increasing enzyme levels. In reactions which contain 300 ng of DNA (0.13 pmol of circles), about 0.25 ng to 0.5 ng of DNA topoisomerase I (2.5 to 5 fmol of enzyme) fully relaxes the template in 15 min at 30°C (Fig. 2A). In the presence of 2 mM ATP and 1 mM phosphate, significant inhibition is observed (lanes 12 and 13). By increasing the level ten-fold, complete relaxation of the template can be achieved (lanes 15 and 16). The inhibitory effect of ATP and phosphate is not a peculiarity of the enzyme in its purified state. As shown in Fig. 2B, limiting levels of topoisomerase I activity identified in a crude nuclear fraction are also significantly and similarly inhibited by 2 mM ATP and 1 mM potassium phosphate. As noted above for the purified enzyme, higher enzyme levels

Figure. 3: Inhibition by ATP and phosphate shows no template specificity, occurs at several DNA levels and affects relaxation of positively-supercoiled DNA. Relaxation reactions were otherwise carried out and processed as described in "Materials and Methods". A, reactions contained either 300 ng of a chimeric mp9 RF DNA (lanes 1-3) or ϕ X174 RF DNA (lane 4-6). Addition of 1 mM P_i and 3 mM ATP are as indicated for lanes 3 and 6. Minus enzyme controls (lanes 1 and 4) are denoted C. B, reactions contained 0.5 ng of enzyme and 75 to 900 ng of DNA as indicated and were carried out in the absence (lanes 1-5) or presence (lanes 6-10) of 1 mM P_i and 2 mM ATP. Minus enzyme control, lane 11. C, reactions contained 300 ng of positively-supercoiled DNA and 1 or 2 ng of topoisomerase I in the absence (lanes 2 and 3) or presence (lanes 4 and 5) of 1 mM P_i and 3 mM ATP.

completely relax the template. These higher levels of the crude nuclear fraction likely contribute little DNA topoisomerase II activity. Catenation and relaxation studies indicate that in this nuclear fraction, there is about ten times more DNA topoisomerase I than II and that for the relaxation activity, the turnover number for the type I enzyme is at least ten-fold greater than that for the type II enzyme (J.A. Holden and R. Low, unpublished results).

Inhibition of relaxation activity is seen with different negatively-supercoiled DNAs and affects relaxation of positively-supercoiled templates as well.

Inhibition shows no obvious need for a specific template. In the

presence of 3 mM ATP and 1 mM phosphate it occurs equally well (>80% inhibition) with a chimeric (negatively-supercoiled) M13 RF DNA which contains a cloned, 0.9 Kb insert of human mitochondrial DNA (Fig. 3A, lanes 1-3) and with ϕ X174 RF DNA (Fig. 3A, lanes 4-6). A DNA titration with the former template indicates that significant inhibition is observed over a wide range of levels from 75 ng to 900 ng, amounts of template that are typically used to assay relaxation activity (Fig. 3B). Inhibition requires the presence of DNA. Preincubating topoisomerase I in diluent containing 3 mM ATP and 1 mM potassium phosphate (-DNA) prior to assay in relaxation reactions lacking either phosphate or ATP is without effect. No inhibition is seen (not shown).

Relaxation of a positively supercoiled DNA is similarly inhibited (Fig. 3C). About 2 ng of topoisomerase I will largely relax 300 ng of a positively-supercoiled template in 30 min at 30°C (Fig. 3C, lane 3). Inclusion of 2 mM ATP plus 1 mM phosphate significantly blocks the relaxation (Fig. 3C, lane 5).

Negatively-supercoiled DNA isolated from an inhibited reaction appears unaltered.

To determine whether the inhibition of relaxation activity resulted from an alteration of the negatively-supercoiled template, the DNA from an "inhibited" reaction was purified by phenol extraction and gel filtration, and assayed. As shown in Fig. 4, this purified DNA is now relaxed by topoisomerase I with the same efficiency (lanes 2-5) as the parent, negatively-supercoiled template (lanes 7-10). With both DNAs, 0.5 ng of topoisomerase I relaxes 300 ng of template in 15 min at 30°C. Three additional tests confirm that the supercoiled template in the inhibited reaction remain negatively twisted (not shown). First, the purified, inhibited DNA bands with negatively-supercoiled DNA in a CsCl-ethidium bromide equilibrium density gradient. Second, this DNA is efficiently relaxed by *E. coli* protein ω , a DNA topoisomerase that relaxes negatively- but not positively-supercoiled DNAs. Third, when the purified "inhibited" DNA is partially relaxed and run on a two-dimensional agarose gel system, (one dimension run with the intercalating agent chloroquine) (28), a ladder of negatively-supercoiled topoisomers is visualized.

DNA topoisomerase I of *E. coli* (protein ω) and HeLa DNA topoisomerase II are not inhibited by ATP plus phosphate.

As shown in Fig. 5A, a limiting amount of purified protein ω (lane 3) is not significantly inhibited by either 2 or 4 mM ATP alone (lanes 11 and 12)

Figure 4: DNA isolated from an "inhibited" reaction is relaxed as efficiently as the standard negatively-supercoiled template. Reactions contained 300 ng of either standard supercoiled DNA (lanes 1-5) or supercoiled DNA isolated and purified from an inhibited reaction (lanes 6-10). See "Materials and Methods" for preparation of the "inhibited" template. Each reaction contained 0.15 to 10 ng of purified topoisomerase I as indicated and was carried out and processed as detailed in "Materials and Methods".

or if supplemented with 1 or 2 mM potassium phosphate (lanes 7-10). Results with HeLa DNA topoisomerase II are similar (Fig 5B). With catalytic levels of enzyme, no inhibition of relaxation is seen with 1 or 3 mM ATP in the presence of either 1 or 2 mM potassium phosphate (lanes 7-10). As expected for this topoisomerase activity, no relaxation occurs in the absence of ATP (Fig 5B, lane 6).

A variety of purine nucleotides and their derivatives can substitute for ATP.

In the presence but not in the absence of 1 mM potassium phosphate, a wide variety of purine nucleotides and their derivatives inhibit the relaxation activity of HeLa DNA topoisomerase I as well as ATP does (Table

Figure 5: ATP and phosphate do not inhibit *E. coli* protein ω or HeLa DNA topoisomerase II. A, relaxation reactions contained 300 ng of DNA and were carried out 15 min at 30°C. Lane 1-4: 0, 0.5, 1 or 2 U of purified protein ω . Lanes 5-12, each reaction contained 1 U of protein ω with additions of either P_i and ATP, or both as indicated. B, relaxation assays were as above except additions of enzyme, P_i and ATP as indicated. Lane 1: no enzyme control; lanes 2 and 3, 2.5 ng at HeLa topoisomerase I in the absence or presence of 1mM P_i and 1mM ATP, respectively; lane 4-10, 2u of purified HeLa topoisomerase II and with additions of P_i and ATP as indicated.

I). Titration studies indicate that several of these cause about 80% inhibition of relaxation at concentrations of 1-2 mM of nucleotide. This group includes in addition to ATP: ADP, dATP, dGTP and NAD. The titration data for dATP and dGTP in the presence of 1 mM phosphate is shown in Fig. 6A; maximal inhibition occurs at about 1 mM and 0.5 mM respectively. Adenosine and 5'-AMP appear inert whether or not 1 mM phosphate is present. Less than 5% inhibition is seen at concentrations up to 2 mM (not shown). For the 2'- and 3' isomers of AMP and for 3', 5'-cAMP however a phosphate-dependent inhibitory effect is obtained and substantial inhibition is seen by 0.25 mM. As shown in Fig. 6B, these low levels of 5'-AMP (67 to 250 μ M, lanes 2-7) are without effect whether or not phosphate is included. For the 3'-isomer, inhibition begins with as little as 67 μ M provided that 1 mM phosphate is added (lanes 11-13). A similar effect for the 2'-isomer is

Table I
Inhibition of DNA topoisomerase I by various purine
nucleotides and their derivatives

Nucleotide	Concentration (mM) to cause about 80% inhibition a)	Dependence on 1 mM phosphate
ATP	2 mM	yes
ADP	1-2 mM	yes
3'-AMP	.25 mM	yes
2'-AMP	.25 mM	yes
dATP	1 mM	yes
dGTP	.5 mM	yes
3',5' cAMP	.25 mM	yes
2',3'-cAMP	.5 mM	yes
NAD	1-2 mM	yes
GTP	1-2 mM	yes
Adenosine 3',5'-diphosphate	1 mM	yes
AMPPNP	1 mM	no
ATP- γ -S	.75 mM	no
5'-AMP	----b)	--
Adenosine	----b)	--

a) Relaxation assays were carried out as described in "Materials and Methods". Titrations of each nucleotide were performed both in the presence and absence of 1 mM potassium phosphate. Inhibition values were estimated from visual inspection of agarose gels.

b) Less than 5% inhibition at 2 mM.

seen at 250 μ M (lane 19). Each of the pyrimidine nucleotides CTP, UTP, dCTP and dTTP appear inactive whether or not 1mM phosphate is present. At 1mM nucleotide, less than 10% inhibition was observed (not shown).

In addition, this survey has identified two compounds the nonhydrolyzable analogues AMPPNP and ATP- γ -S which inhibit topoisomerase I activity and in the absence of phosphate (Table I). For each, 80% inhibition requires a concentration of about 1 mM. Addition of 1 mM phosphate does not increase the inhibitory effect of these two nucleotides.

Figure 6: dATP, dGTP, 2'-AMP and 3'-AMP each inhibit HeLa topoisomerase I as well as ATP. Reactions contained 300 ng of DNA and 0.5 ng of purified topoisomerase I and were carried out and processed as described in "Materials and Methods". A, reactions contained 1 mM P_i and 0.07 to 1.0 mM of either dATP or dGTP as indicated. B, lanes 2-4: 67, 125, 250 μ M 5'-AMP respectively; lanes 5-7, same as in lanes 2-4 respectively but plus 1 mM P_i ; lanes 8-10, 67, 125, 250 μ M 3'-AMP respectively; lanes 11-13, as in lanes 8-10 but plus 1 mM P_i ; lanes 14-16, 67, 125, 250 μ M 2'-AMP respectively; lanes 17-19, same as lanes 14-16 but plus 1 mM P_i .

DISCUSSION

DNA topoisomerase I of HeLa cell nuclei is strongly inhibited *in vitro* by a variety of purine nucleotides provided that low levels of inorganic phosphate and DNA template are present. For ATP, a modest level of three to four millimolar causes nearly complete inhibition when 1 mM phosphate is included. The rate of enzyme inhibition must be rapid since in the uninhibited relaxation reaction, relaxed topoisomers begin to accumulate within about one minute at 30°C. The identification of a rather broad spectrum of active purine effectors and the common requirement for phosphate provide only a few clues to the mechanism of inhibition. One model is that the purine effector bound to a specific site on the enzyme greatly increases the enzyme's affinity for phosphate which blocks productive encounters of the enzyme with the DNA template. Whether the purine nucleotide and phosphate groups occupy allosteric sites or a portion of the template binding site is still not clear. The titration curve for phosphate appears quite sigmoidal suggesting that binding of one phosphate group is essential to enhance the binding of a second. Phosphate may not simply competitively inhibit binding to the DNA template since titration studies with phosphate

at two different levels of DNA, 125 ng and 600 ng, indicate the same level of phosphate (0.5 mM) is required for inhibition (unpublished data). Because ADP and 2'- and 3'-AMP are each as potent as ATP, the inhibitory effect of ATP probably does not accompany hydrolysis of a high energy phosphate bond and it seems unlikely that inhibition results from phosphate-dependent autophosphorylation of the enzyme.

Inhibition does seem to require the presence of the negatively-supercoiled DNA template. If the enzyme is diluted in diluent including 2 mM ATP, 1 mM phosphate and 10 mM $MgCl_2$ but lacking DNA, no detectable inactivation of the enzyme occurs even if the enzyme in this diluent is preincubated for 5 min at 30°C before assaying it for relaxation activity (unpublished data). Inhibition does not appear to result from a physical change in the supercoiled template which protects it against relaxation. The supercoiled DNA that is isolated from an inhibited reaction can be relaxed by either HeLa topoisomerase I (Fig. 4) or protein ω (unpublished data) with the same efficiency as the standard supercoiled DNA template.

So far, the inhibitory effects of ATP and phosphate appear irreversible. When partially inhibited reactions are incubated for extended periods, no further relaxation of the remaining supercoiled template occurs. Also, attempts to relieve inhibition by dilution of the ATP and phosphate effectors have not been successful (unpublished data). This apparent inactivation of the enzyme may be an in vitro artifact. If ATP and phosphate act to block binding of the enzyme to the DNA, the loss of stabilization provided by template binding could be crucial to the survival of dilute, catalytic concentrations of enzyme. Alternatively, ATP and phosphate may instead directly inactivate the enzyme only while its bound to the supercoiled DNA template. Whether such an inactivation process would require a cycle of template relaxation remains uncertain.

In this study, the main focus has been on the inhibitory effect of ATP. Since the intracellular concentrations of ATP and phosphate are estimated to be 4 mM and 3-4 mM, respectively (29,30), a large proportion of the intracellular topoisomerase I would be expected to be inhibited in vivo. The ability to extract and purify substantial amounts of active enzyme suggest that in vivo either inhibition is freely reversible, overcome by excess enzyme, or a factor(s) protects against inactivation or readily restores the activity of inactivated enzyme. Alternatively, chromosomal DNA of HeLa cells may not induce sufficient negative superhelicity to initiate

rounds of relaxation essential for widespread topoisomerase I inactivation.

Current interest in the regulation of eucaryotic topoisomerase I activity has so far focussed on three areas of post-translational modification: proteolysis, poly (ADP)-ribosylation and protein phosphorylation. Proteolysis is currently regarded an artifact of isolation and as already shown, can be largely avoided if measures are taken to suppress or inhibit proteases (1). Remarkably, the major proteolyzed forms of the HeLa enzyme appear to be fully active and no difference in enzymatic properties from those of the intact (100-KD) enzyme have yet been found (20). In this present study, the intact enzyme has been used. Poly (ADP)-ribosylation can alter and thereby inhibit HeLa DNA topoisomerase I in vitro although only a small proportion of the enzyme seems to be so affected in vivo (31,32). Protein phosphorylation (33) may modulate topoisomerase activities in vivo as well. Purified DNA topoisomerase I of Novikoff Hepatoma has been found to be a phosphoprotein and in that study (34), phosphorylation of a serine residue has been correlated with a modest enhancement of relaxation activity. In contrast, viral and cell-encoded tyrosine protein kinases both have been shown to phosphorylate and thereby inactivate calf thymus topoisomerase I in vitro (35). Phosphorylation may affect type 2 enzymes as well. Purified *Drosophila* DNA topoisomerase II has been recently shown to have an intrinsic, protein kinase activity with a variety of protein substrates including itself (36). Whether our preparations of HeLa DNA topoisomerase I contain phosphorylated enzyme will require further examination as well as the possibility that phosphorylation or other kinds of modifications modulate the sensitivity to ATP and phosphate inhibition.

The identification of a marked sensitivity of the HeLa cell enzyme to physiologic levels of ATP and phosphate in vitro suggest that allosteric regulation of this enzyme may be significant in vivo, especially if fluctuations of ATP, phosphate, and other effectors marshall major alterations in gene expression. In contrast, the immunity of protein ω of *E. coli* to ATP and phosphate is rather striking and encourages continued study of these and other type 1 topoisomerases.

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*To whom correspondence should be addressed

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