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Interleukin-2 Induces the Activities of DNA Topoisomerase I and DNA Topoisomerase II in HuT-78 Cells

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Abstract

The induction by interleukin-2 of DNA topoisomerase I and DNA topoisomerase II activities in the human T cell line HuT 78 was investigated. HuT 78 cells were treated with 1,000 units of interleukin-2/ml, and extracts of the HuT 78 nuclei were prepared over a 24 hour period. The extracts were assayed quantitatively for the activities of DNA topoisomerase I and DNA topoisomerase II. Three concomitant, transient increases of three- to eleven-fold in the specific activities of both DNA topoisomerase I and DNA topoisomerase II were observed following treatment with IL-2 at 0.5, four, and 10 hours after treatment with interleukin-2. The specific activities of both enzymes returned to base-line values after each of these transient increases. These results reveal that the activities of DNA topoisomerase I and DNA topoisomerase II are highly regulated in HuT 78 cells upon treatment with IL-2.

Keywords

DNA topoisomerase I; DNA topoisomerase II; interleukin-2

Introduction

The objectives of this study are to characterize the activities of DNA topoisomerase I and DNA topoisomerase II in interleukin-2 activated human leukocytes. The activation of T cells by interleukin-2 (IL-2) has served as a model for cell growth [1]. IL-2 is an extensively characterized cytokine [2] that is required for growth of both normal and neoplastic human T cells [3,4]. IL-2 binds to a high affinity receptor comprised of α , β , and γ subunits [5]. IL-2 treatment of human T cells promotes transcription, DNA replication, and ultimately cell division [6]. The pathways of signal transduction activated by the binding of IL-2 to its receptor have been studied extensively [7]. IL-2 activates protein kinases whose substrates include transcription factors that promote gene expression [8]. It is possible that other mechanisms of signal transduction to the nucleus may be activated by IL-2 such as the activation of enzymes required for DNA metabolism and/or modifications of chromatin [9]. DNA topoisomerases

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are plausible candidates for activation by IL-2 because they appear to be required for both transcription and DNA replication in human cells, and they are activated by phosphorylation [10-13]. In this study the activities of DNA topoisomerase I and DNA topoisomerase II were measured quantitatively in extracts prepared from nuclei of HuT 78 cells at various times after treatment with IL-2.

Materials and methods

Cell culture

HuT 78, a human cutaneous T cell lymphoma cell line [14], was obtained from the American Type Culture Collection. HuT 78 cells were grown in Iscove's Modified Dulbecco's Minimal Medium (IMDM) supplemented with 10-20% fetal bovine serum, 4.0 mM glutamine, and 100 units penicillin/ml, 100 µg streptomycin/ml at 37° C in a humid environment containing 5% CO₂. A total of 2×10^7 cells were treated with IL-2. The cells were centrifuged at 2,000 g and resuspended at a concentration of 10^7 cells/ml in supplemented IMDM and incubated at 37° C. IL-2 (Invitrogen) was added to the suspension to a final concentration of 1,000 international units/ml.

Preparation of nuclear extracts from HuT 78 cells

Extracts of HuT 78 cell nuclei were prepared by a modification of a published method [15]. Aliquots containing 4×10^6 HuT 78 cells were removed at time intervals of 0, 0.5, 1, 2, 4, 6, 8, 10, 12, and 24 hours after treatment with IL-2 and centrifuged at 2,000 g for 5 min. The cell pellets were washed twice with cold phosphate buffered saline (PBS) and washed with buffer A (5.0 mM potassium phosphate, 2.0 mM MgCl₂, 150 mM NaCl, 1.0 mM EGTA, pH 7.0). The cell pellets were resuspended in buffer B [buffer A + 0.3% Triton X-100 and 1.0 mM phenylmethylsulfonyl fluoride (PMSF)] and incubated at 4° C for 30 min. The suspension was centrifuged at 2,000 g for 10 min. at 4° C to pellet the nuclei which were resuspended in three volumes of buffer C (buffer A supplemented with 0.35 M NaCl, 2.0 mM dithiothreitol, 1.0 µM leupeptin, 1.0 µM pepstatin, 1.0 mM PMSF). The suspension was incubated at 4° C for 15 min., and then centrifuged at 12,000 g for 5 min. The supernatant fraction was adjusted to 50% glycerol and stored at -20° C (HuT 78 nuclear extract). The activities of both DNA topoisomerase I and DNA topoisomerase II were stable for at least six months upon storage at -20° C.

Concentration of protein

The concentration of protein in the HuT 78 nuclear extracts was determined using the Bio-Rad reagent by a modification of a published method [16].

Assay of DNA topoisomerase I

Aliquots of 1-4 µl of HuT 78 nuclear extracts diluted 1:100 with buffer C were assayed for the catalytic activity of DNA topoisomerase I in a total volume of 25 µl containing 10 mM potassium phosphate, 200 mM NaCl, 10 mM EDTA, 0.1 mg/ml BSA, pH 7.4 and 0.8 µg of pBR322 or pHC624 supercoiled DNA. The reactions were incubated at 37° C for 30 min. Reactions were terminated by the addition of 5 µl of stop/loading solution (1 mg/ml proteinase K, 1% SDS, 125 mM EDTA, 0.1% bromophenol blue, 10% glycerol). The samples were incubated at 37° C for 15 min., and then electrophoresed at 33 volts for 4 hrs. on a 1% agarose gel in 2× Tris-acetate EDTA (TAE) buffer (80 mM Tris, 40 mM sodium acetate, 2.0 mM EDTA, pH 8.3) plus 0.5 µg/ml ethidium bromide as described [17]. Supercoiled and relaxed DNAs migrate as discrete bands under these conditions rather than as Gaussian distributions of topoisomers, and the bands for supercoiled and relaxed DNA are well separated from nicked DNA.

Assay of DNA topoisomerase II

DNA topoisomerase II was assayed quantitatively by the formation of cleavable complexes as described [18]. Assays were performed in a reaction volume of 20 μ l containing 10 mM Tris-HCl, pH 7.9, 50 mM NaCl, 50 mM KCl, 0.1 mM EDTA, 5.0 mM MgCl₂, 2.5% glycerol, 2.0 mM ATP, and 0.8 μ g of pBR322 supercoiled DNA. The reaction was initiated with the addition of 1.0–4.0 μ l HuT 78 nuclear extract, and samples were incubated at 30° C for six min. The reactions were terminated by the addition of 2.0 μ l of 4.0 mg/ml proteinase K and incubation at 45° C for 30 min. An aliquot of 2.0 μ l of loading buffer (10 mM Tris-HCl, pH 7.9, 60% sucrose, 0.5% bromophenol blue, 0.5% xylene cyanol) was added to each assay followed by incubation at 70° C for two min. The samples were electrophoresed on 1% agarose gels in TAE buffer (40 mM Tris-HCl, pH 8.3, 20 mM sodium acetate, 1.0 mM EDTA) + 0.2 μ g/ml ethidium bromide at 50 V for 3.5 hrs.

Photography and scanning microdensitometry

Agarose gels were photographed on a Photodyne UV transilluminator using a Canon Power Shot A590 digital camera. The images were imported into a computer, and the bands were quantified by scanning densitometry with the use of the Un-Scan-It software (Silk Scientific, Inc.). The conversion of supercoiled DNA to relaxed DNA was quantified for assays of the catalytic activity of DNA topoisomerase I. The conversion of supercoiled DNA to full-length linear DNA and smaller linear DNA products was quantified for DNA topoisomerase II in assays of the formation of cleavable complexes.

Calculation of specific activities

One unit of activity for DNA topoisomerase I is defined as the amount of enzymatic activity that results in 50% conversion of the supercoiled DNA to relaxed DNA under the standard assay conditions described above. The volume of extract corresponding to 1.0 unit of activity was determined by assays of varying amounts of nuclear extracts. The results were graphed as the percentage of conversion of supercoiled to relaxed DNA versus amount of extract added to the assay. The specific activity of DNA topoisomerase I was calculated as units of activity/mg protein. One unit of activity for DNA topoisomerase II is defined as the amount of strand breakage activity that results in 50% conversion of the supercoiled to linear DNA under standard conditions for the assay as described above. The volume of extract corresponding to 1.0 unit of activity was determined by assays of varying amounts of nuclear extracts, and it was determined from direct extrapolation of the assay yielding the greatest percentage of conversion of supercoiled to linear DNA products. The specific activity of DNA topoisomerase II was calculated as units of strand breakage activity/mg protein.

Results

The activity of DNA topoisomerase I was measured in nuclear extracts prepared from HuT 78 cells at various times after treatment with 1,000 units of IL-2/ml as described above. The activity of DNA topoisomerase I was quantified by titrations of supercoiled pBR322 DNA with varying amounts of extracts in order to determine precisely the volume of nuclear extract that yields 50% conversion of supercoiled to relaxed DNA (1.0 unit of activity) as described [17]. The concentration of protein in the extracts was determined by the Bio-Rad method. The specific activities of DNA topoisomerase I in the HuT 78 nuclear extracts were calculated in units/mg and plotted versus the time after IL-2 treatment at which the cells were harvested (Fig. 1). In addition, the activity of DNA topoisomerase II in the same extracts was measured by the formation of cleavable complexes [19]. The assays for DNA topoisomerase II were performed as titrations of nuclear extracts, and the volume of extract corresponding to 50% conversion of supercoiled to linear DNA (1.0 unit of activity) was determined. The specific

activities of DNA topoisomerase II in the HuT 78 nuclear extracts were calculated in units/mg protein and plotted as a function of time after IL-2 treatment (Fig. 1).

There were three concomitant, transient increases in the specific activities of DNA topoisomerase I and DNA topoisomerase II in the HuT 78 nuclear extracts following treatment of the cells with IL-2 as shown in Fig. 1. There was a seven- to eleven-fold increase in the specific activities of both enzymes 30 min. after treatment by IL-2. Subsequently, three- to nine-fold increases in the activities of both DNA topoisomerase I and DNA topoisomerase II were observed at four hours after treatment with IL-2. Furthermore, two peaks of activity for DNA topoisomerase II at 0.5 and 4 hours following treatment with IL-2 were observed in catalytic assays of DNA topoisomerase II employing the unknotting of bacteriophage P4 DNA, an assay that is highly specific for DNA topoisomerase II [20] (Santangini and Foglesong, unpublished data). Six-fold increases in the specific activities of DNA topoisomerase I and DNA topoisomerase II were observed at 10 hours after treatment of the HuT 78 cells with IL-2 (Fig. 1). In addition, the activity of DNA topoisomerase II in HuT 78 nuclear extracts was assayed using the decatenation of kinetoplast DNA and observed to be optimal at 10-12 hours after treatment of the cells with IL-2 (Jaiswal, Singh, and Foglesong, unpublished data).

The results reported here reveal by quantitative assays that the activities of DNA topoisomerase I and DNA topoisomerase II are highly regulated in IL-2-activated HuT 78 cells with three prominent, concomitant peaks observed for both enzymes within 12 hours of treatment of HuT 78 cells with IL-2. After each peak, the activities of both DNA topoisomerase I and DNA topoisomerase II return to baseline values.

Discussion

These peaks of the specific activities of DNA topoisomerase I and DNA topoisomerase II coincide with increases in phosphorylation of proteins induced by interleukin-2 and transcription of the gene encoding *fos* [7]. *Fos* and *jun* form AP-1[21], a transcription factor that promotes increased expression of responsive genes including the gene encoding IL-2 [22]. The second peak of the specific activities of DNA topoisomerase I and DNA topoisomerase II occurs at 4 hours after IL-2 treatment (Fig. 1) at the same time that increased transcription of *myc* and other genes has been reported [23]. The fact that both DNA topoisomerase I and DNA topoisomerase II are transiently activated at 0.5-4 hrs. after IL-2 treatment suggests that both enzymes function in transcription. The third peak of the specific activities of DNA topoisomerase I and DNA topoisomerase II at 10-12 hours after IL-2 treatment coincides with the onset of DNA replication [6]. The fact that both enzymes are activated at 10-12 hours after treatment with IL-2 provides additional evidence for the involvement of DNA topoisomerase I and DNA topoisomerase II in DNA replication. DNA topoisomerase I and DNA topoisomerase II α are both believed to act as swivelases that allow for efficient movement of the DNA replication fork [24,25,26]. In addition, DNA topoisomerase II α is required for decatenation and may function in the condensation and decondensation of chromatin that accompanies mitosis [27].

The molecular mechanisms by which DNA topoisomerase I and DNA topoisomerase II become activated in IL-2-activated T cells are not yet known. Both enzymes are regulated by phosphorylation and dephosphorylation. Dephosphorylation inhibits the activity of DNA topoisomerase I, and phosphorylation of both enzymes by casein kinase II or protein kinase C α stimulates their activities [28]. Increased phosphorylation of DNA topoisomerase I was observed at four serines residues (10, 21, 112, and 394) in mitotic K562 cells [29]. Phosphorylation of the serine residues in DNA topoisomerase I was demonstrated by casein kinase II (Ser¹⁰), protein kinase C α (Ser²¹), and Cdk1 (Ser¹¹² and Ser³⁹⁴). Phosphorylation of serine²¹ by protein kinase C α , a protein kinase activated by IL-2, resulted in a two-fold increase

in the activity of the enzyme and a stabilization of camptothecin-induced cleavable complexes [29] supporting the hypothesis that phosphorylation can activate DNA topoisomerase I at least modestly. Since protein kinase C α , casein kinase II, and cyclin-dependent kinase are all activated by IL-2 [8], the increased activity of DNA topoisomerase I observed in HuT 78 cells activated by IL-2 may be due at least in part to phosphorylation of the enzyme by protein kinases that are activated by IL-2. Also, the observation that a protein the size of DNA topoisomerase I (92 kDa) is phosphorylated in human T cells within 10 minutes of treatment with IL-2 [6] is consistent with this hypothesis. Therefore, the observed increases in activities of DNA topoisomerase I and DNA topoisomerase II reported here may be due to phosphorylation of the enzymes on several sites by multiple protein kinases. The decreases in the activities of DNA topoisomerase I and DNA topoisomerase II reported here may be due, at least in part, to the action of phosphatase(s) which have yet to be identified.

The magnitude of the enhancement of the activities of DNA topoisomerase I and DNA topoisomerase II observed in this study (3- to 11-fold) is striking and reproducible. Such large modulations of the activities of these enzymes reveal that human cells can rapidly activate and deactivate both DNA topoisomerase I and DNA topoisomerase II in response to a signal molecule, and they suggest that both play important roles in the programs of increased transcription and subsequent DNA replication that are induced by IL-2 in human T cells. The results presented here show that HuT 78 cells treated with IL-2 represent a model system in which regulation of the activities of DNA topoisomerase I and DNA topoisomerase II by interleukin-2 and the roles of DNA topoisomerases in macromolecular biosynthesis can be investigated. Further research is needed to characterize the mechanism of this activation including IL-2-induced covalent modifications of DNA topoisomerase I and DNA topoisomerase II such as phosphorylation and dephosphorylation as well as the isolation and characterization of any ancillary factor(s) required for their optimal activities.

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Figure 1.

The specific activities of DNA topoisomerase I (closed circles) and DNA topoisomerase II (open circles) in units/mg protein are plotted as a function of time after treatment with IL-2 for nuclear extracts prepared 0-24 hrs after treatment of HuT 78 cells with IL-2.