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A biochemical analysis of topoisomerase II α and β kinase activity found in HIV-1 infected cells and virus ☆

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Abstract

Human topoisomerase II plays a crucial role in DNA replication and repair. It exists in two isoforms: topoisomerase II alpha (α) and topoisomerase II beta (β). The α isoform is localized predominantly in the nucleus, while the β isoform exhibits a reticular pattern of distribution both in the cytosol and in the nucleus. We show that both isoforms of topoisomerase II are phosphorylated in HIV infected cells and also by purified viral lysate. An analysis of the phosphorylation of topoisomerase II isoforms showed that extracts of HIV infected cells at 8 and 32 h. post-infection (p.i.) contain maximal phosphorylated topoisomerase II α, whereas infected cell extracts at 4 and 64 h p.i. contain maximum levels of phosphorylated topoisomerase II β. In concurrent to phosphorylated topoisomerase II isoforms, we have also observed increased topoisomerase II α kinase activity after 8 h p.i and topoisomerase β kinase activity at 4 and 64 h p.i. These findings suggest that both topoisomerase II α and β kinase activities play an important role in early as well as late stages of HIV-1 replication. Further analysis of purified virus showed that HIV-1 virion contained topoisomerase II isoform-specific kinase activities, which were partially isolated. One of the kinase activities of higher hydrophobicity can phosphorylate both topoisomerase II α and β, while lower hydrophobic kinase could predominantly phosphorylate topoisomerase II α. The phosphorylation status was correlated with catalytic activity of the enzyme. Western blot analysis using phosphoamino-specific antibodies shows that both the kinase activities catalyze the phosphorylation at serine residues of topoisomerase II α and β. The catalytic inhibitions by serine kinase inhibitors further suggest that the α and β kinase activities associated with virus are distinctly different.

Keywords: Topoisomerase II α; Topoisomerase II β; Topoisomerase II phosphorylation; HIV-1 infection; HIV-1-associated kinase activity; Topoisomerase II kinase activity

Human topoisomerase II[1] is present in two isoforms, alpha (α) (170 kDa) and beta (β) (180 kDa) [1]. The α isoform is expressed in all proliferating cells and tissues, while the β isoform is constitutively expressed in all cell types. The α isoform is predominantly localized in the nucleus, while the β isoform shows a reticular pattern of distribution [2]. Both isoforms in vitro exhibit catenation and decatenation, knotting and unknotting, and

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[1] *Abbreviation used:* topoisomerase II, topoisomerase II; PKC, protein kinase C; CK-II, casein kinase II; dNTP, deoxynucleotide triphosphate; ddNTP, dideoxynucleotide triphosphate; p.i, HIV-1 post-infection period; PBS, phosphate-buffered saline; TBS, Tris buffer saline, SDS, sodium dodecyl sulphate; NBT, nitro blue tetrazolium; BCIP, 5-bromo, 4-chloro 3-indolyl phosphate; UCS, uninfected cell supernatant.

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relaxation of supercoiled DNA. Various reports implicate the activities of these isoforms in DNA replication, repair, and recombination [3].

Topoisomerase II binding and cleavage sites are present in the HIV-1 LTR [4,5]. Additional topoisomerase II binding and cleavage sites have been identified at 850 bp upstream from an HIV-1 integration site in human DNA [4]. Inhibition of topoisomerase II expression in HIV-1 infected cells can decrease HIV-1 replication by fivefold, suggesting a role for topoisomerase II in HIV-1 replication [6]. The activity of topoisomerase II during cell cycle is tightly regulated through phosphorylation and the phosphorylated form being an active form of enzyme. It is unknown whether the activity of topoisomerase II during viral infection is also regulated through phosphorylation. Since topoisomerase II has been reported to be present in phosphorylated form in HIV-1 infected cells [7], the rationale of present investigation is to find the levels of topoisomerase II α and β phosphorylation immediately after virus infection and to identify the factors that promote the phosphorylation of topoisomerase II isoforms during the course of HIV-1 infection, such an information would help in identifying the pathways that regulate activation of topoisomerase II isoforms through phosphorylation and HIV-1 replication. Our results show that topoisomerase II isoforms undergoes phosphorylation in cell extracts infected by HIV-1. Topoisomerase II β kinase activity is stimulated in the early stages of infection, whereas topoisomerase II α kinase activity is stimulated at later stages. Further, we show that both isoforms of topoisomerase II are phosphorylated by serine kinase present in purified HIV virion, which can be partially isolated using an ion exchange chromatography.

Materials and methods

Materials

HIV-1 isolates: HIV-$1_{93IN101}$ (Sub type C/NSI&R5) was an Indian isolate obtained from the NIH-AIDS Research and Reference Reagent program, USA; HIV-1_{CRI50}, an Indian subtype C isolate was isolated, propagated, and maintained at the Cancer Research Institute (unpublished information).

Mouse anti-human topoisomerase II α (Clone: 31) and topoisomerase II β (Clone: 40) monoclonal antibodies were from BD Biosciences, USA, these antibodies showed specific reactivity to α and β isoforms, we have not detected any cross reactivity of these antibodies to antigens as per the Western blot analysis. Monoclonal anti-protein kinase C (clone MC5) and anti-casein kinase 2 α, human (clone 1AD9), ATP, PMSF, BSA, and Protein A–agarose, Triton X-100, Ficoll, DTT, were from Sigma. PVDF membrane was obtained from PALL Life Sciences, USA, PEG 8000 from Gibco, Butanol and di-isopropyl ether from s.d.fine-chem limited. Mono S, Mono Q, and phenyl-Sepharose are from Amersham. All other reagents and chemicals are of standard grade.

Buffers

Buffers used: 10× kinase buffer (0.5 M Tris, pH 7.5, 50% glycerol, 0.1 M $MgCl_2$, and 50 mM DTT); RIPA buffer (50 mM Tris–Cl, pH 7.4, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS, 1 mM EDTA, pH 7.0, 150 mM NaCl, 1 mM PMSF, 1% aprotonin 1%, pepstatin 10 μg/ml, 1 mM Na_3VO_4, and 50 mM NaF); 10× CIP buffer (10 mM $ZnCl_2$, 10 mM $MgCl_2$, 100 mM, Tris–HCl, pH 8.3); extraction buffer (20 mM Tris–HCl, pH 7.5, 0.1 mM β-mercaptoethanol, 1 mM $MgCl_2$, 0.1 mM EDTA, 5% glycerol, 0.1% Triton X-100, 0.5 mM KCl, 0.5 mM PMSF, and 1μg/μl pepstatin and leupeptin); and lysis buffer (10 mM Tris–HCl, pH 7.4, 1% Triton X-100); and relaxation buffer (50 mM Tris–HCl, pH 8.0, 120 mM KCl, 0.5 mM EDTA, 0.5 mM DTT, 10 mM $MgCl_2$, 30 μg/ml BSA, and 1 mM ATP).

In situ phosphorylation assay

5×10^7 $CD4^+$ T-cells (SupT1) were incubated with [^{32}P]phosphoric acid for 2 h in RPMI-1640 complete medium at 37 °C in a CO_2 incubator (Forma) with 5% (±0.1) CO_2. The cells were incubated with and without HIV-$1_{93IN101}$ (10 ng of p24 equivalent). Infection was stopped at indicated time points after infection and the cells (5×10^6) were centrifuged at 400*g*. The cell pellet was washed twice with the cold PBS. The washed cells were lysed with PBS containing 1% Triton X-100 followed by sonication at 3 × 10 s pulses. Topoisomerase II α or topoisomerase II β were immunoprecipitated in separate aliquots by addition of monoclonal antibodies and the immunoprecipitates was collected by addition of 6% protein A–agarose. The protein A beads were incubated at 4 °C for 15 min and the supernatants was removed. The beads were washed twice with PBS containing 0.5% Triton X-100. Bound topoisomerase II was eluted by 20 μl of 5% TCA. The eluate was spotted on Whatman No.1 filter paper discs and dried. ^{32}P was measured in scintillation fluid (0.5 g POPOP, 5 g PPO in 1 L of toluene) using a Wallac 1400 DSA scintillation counter. Each experiment was performed in triplicate and all data points represent an average of results from the triplicate experiments.

Western blot analysis of topoisomerase II in cell lysates

Time course experiments were conducted as explained above for infection assays. Protein was solubilized in RIPA buffer. Lysates containing 75 μg of total protein were