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Real-time analysis of cleavage and religation activity of human topoisomerase 1 based on ternary fluorescence resonance energy transfer DNA substrate

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ABSTRACT

Human topoisomerase 1B is a ubiquitous and essential enzyme involved in relaxing the topological state of supercoiled DNA to allow the progression of fundamental DNA metabolism. Its enzymatic catalytic cycle consists of cleavage and religation reaction. A ternary fluorescence resonance energy transfer biosensor based on a suicide DNA substrate conjugated with three fluorophores has been developed to monitor both cleavage and religation Topoisomerase I catalytic function. The presence of fluorophores does not alter the specificity of the enzyme catalysis on the DNA substrate. The enzyme-mediated reaction can be tracked in real-time by simple fluorescence measurement, avoiding the use of risky radioactive substrate labeling and time-consuming denaturing gel electrophoresis. The method is applied to monitor the perturbation brought by single mutation on the cleavage or religation reaction and to screen the effect of the camptothecin anticancer drug monitoring the energy transfer decrease during religation reaction. Pathological mutations usually affect only the cleavage or the religation reaction and the proposed approach represent a fast protocol for assessing chemotherapeutic drug efficacy and analyzing mutant's properties.

1. Introduction

DNA topological problems, appearing during DNA duplication, transcription and chromatin remodeling, can be resolved by DNA topoisomerase [1]. Human topoisomerase 1B (hTop1) relaxes the supercoiled DNA in the absence of energy through direct transesterification reaction [2]. HTop1 binds to supercoiled DNA to form the enzyme-DNA non-covalent complex, through the active site tyrosine residue (Tyr723) [3]. HTop1 resolves DNA torsional stresses by allowing controlled rotation of one cleaved DNA strand through the intact one. The enzymatic catalytic cycle is completed by religation of the cleaved 5′-hydroxyl group to the tyrosine-DNA phosphodiester, resealing the DNA backbone and releasing the enzyme [4]. The reversible DNA cleavage complex is not only a critical step for strand passage during catalytic cycle, but also the unique target of clinical anticancer drugs, which belong to camptothecin (CPT) family [5]. Under normal condition, hTop1 cleavage complex is transient and even not detectable, but it can be specifically and reversibly trapped by CPT and its derivatives, leading to the complex accumulation and cell death [6]. Several mutations in hTop1 have been shown to impact enzymatic function and CPT efficacy *in vitro*, in cultured cells or in patients [7]. A rapid and efficient analysis of hTop1 enzymatic activity and drug sensitivity is required for a clinical treatment and an anti-cancer drug screening.

The conventional biochemical analyses of hTop1 activities are based on the detection at limited and selected time points of radiolabeled oligonucleotide, when the produced DNA fragments are separated by gel electrophoresis to calculate the reaction kinetics [8]. The radioactive risk, the time and labor consumption and the requirement of specialized skill limit the extensive application of this method. Solutions based on unlabeled [9] or fluorescently labeled oligonucleotides

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[10–15] have been proposed for safe and sensitive detection of hTop1 activity. Rolling circle amplification based measurement have the advantage to detect hTop1 activity at very low enzyme concentration, but the method detects the amplified signal after the topoisomerization reaction and doesn't permit to follow the reaction process in real time [10,11]. Quantum dot based nanosensor has been proposed to solve this issue, however, it can only measure the religation process [12]. Fluorophore-quencher pairs based on molecular beacon have been also proposed, but this protocol cannot distinguish between the cleavage and the religation reaction [13–15]. The possibility to selective detects the cleavage and religation reaction is useful since several mutations affect only one of these processes [16]. In clinics this may be even more important in order to asses' chemotherapeutic drugs efficacy.

In this work a ternary fluorescence resonance energy transfer (FRET) biosensor based on a DNA substrate with three fluorophores to analyze both cleavage and religation reactions is proposed. This multiplexed system allows us to track cleavage and religation procedures under non-denaturing condition and to characterize anti-cancer drugs reactivity.

2. Results and discussion

2.1. Design of FRET substrate with three fluorophores for detection of the enzyme activity

A DNA substrate having three fluorophores is designed to directly monitor the hTop1-mediated cleavage and religation reactions using fluorescence (FL) measurement. The substrate is composed by cleavage strand (CL14), complementary strand (CP25) and religation strand (R11) able to complementary interact one with the other (Fig. 1A). The

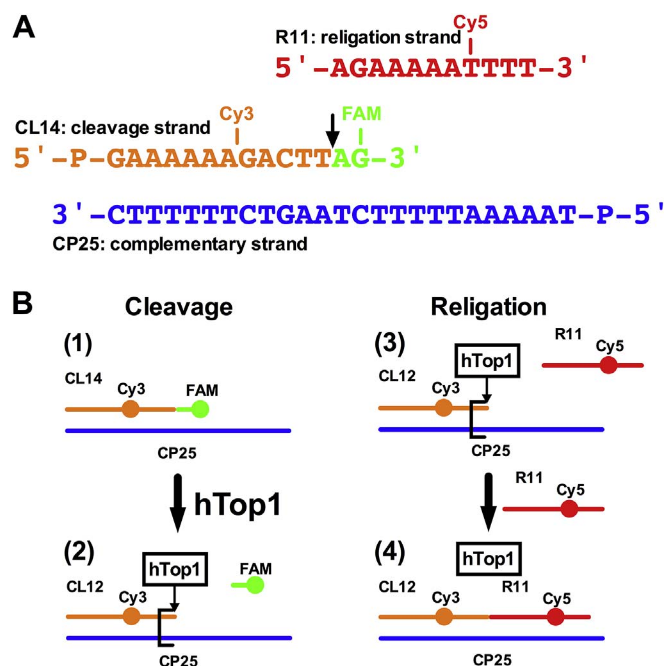


Fig. 1. DNA FRET biosensor with three fluorophores. (A) Design of the fluorescent DNA strands. A cleavage strand (CL14, orange and green) phosphorylated at 5′-end is synthesized with an hTop1 preferred cleavage site, indicated by an arrow, flanked by a FRET pair, FAM (green) and Cy3 (orange). The complementary strand (CP25, blue) is phosphorylated at 5′-end. The religation strand (R11, red) is conjugated with Cy5 (red) fluorophore. (B) Schematic representation of the FRET based hTop1 activity analysis. (1) CL14/CP25 substrate labeled with FAM/Cy3 FRET pairs; (2) The cleavage reaction resulting in the formation of covalent DNA-enzyme complex and dissociation of AG-3′ dinucleotide fragment (green); (3) Addition of complementary R11 oligonucleotide labeled with Cy5 (red); (4) Religation reaction bringing Cy3 and Cy5 FRET pairs at a close distance. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

CL14 and CP25 oligonucleotides are conjugated with fluorophores as indicated in Fig. 1A and are phosphorylated at their 5′-end to avoid nonspecific religation events [17]. The CL14 strand is annealed to CP25 to obtain CL14/CP25 suicide substrate (SS), containing a hTop1 preferred cleavage site indicated by an arrow (Fig. 1A). The CL14 strand is labeled with FAM and Cy3 dyes (Fig. 1B, panel 1), to permit an efficient FRET in the intact SS that is reduced upon occurrence of hTop1 cleavage reaction (Fig. 1B, panel 2). FAM and Cy3 are separated by 7 nucleotides, for an estimated distance of about 24 Å that permits an efficient FRET since the distance for 50% FRET efficacy is 44 Å [18]. HTop1 cuts the SS at the site indicated by the arrow, releasing the AG-3′ dinucleotide fragment that is too short to be religated and the enzyme remains covalently bound to 12-oligonucleotide 3′-end [19].

After the cleavage reaction, excess of a complementary R11 oligonucleotide having a Cy5 fluorophore labeled almost in the middle of the oligonucleotide is added to trigger hTop1 mediated religation reaction (Fig. 1B, panel 3). A new FRET system is obtained after religation, consisting of Cy3 on cleaved substrate and Cy5 on R11 strand that are at close a distance (Fig. 1B, panel 4). Cy3 and Cy5, on the religated DNA substrate, are separated by 13 nucleotides with an estimated distance of about 45 Å that permits an efficient FRET since the distance for 50% FRET efficacy is about 60 Å [20]. As result, both the cleavage and religation reaction can be followed by a FRET experiment.

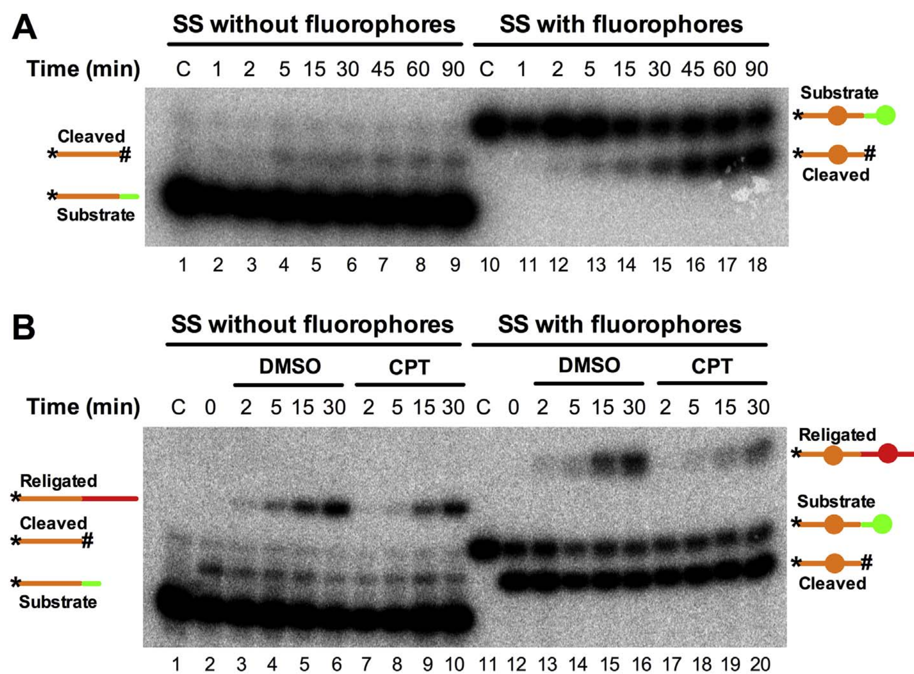
2.2. Comparison of the cleavage and religation reaction with an unlabeled or fluorescently labeled DNA substrate

To investigate whether the presence of fluorophores alters the cleavage and religation reaction, the hTop1 activity has been followed using a substrate labeled at 5′-end of CL14 strand with and without fluorophores conjugation, as illustrated in Fig. 2. An excess of hTop1 enzyme is incubated with the two SS substrates to obtain cleaved DNA fragments that are resolved on a denaturing polyacrylamide gel, as shown in Fig. 2A. The CL14 strand conjugated with two fluorophores runs slower than unlabeled CL14 (Fig. 2A compare lane 1 to lane 10) due to the larger molecular weight of the initial substrate. The enzyme is able to cut the SS for both the unlabeled and fluorescently labeled substrates, releasing a dinucleotide and remaining trapped over it. In the case of the oligonucleotide labeled with fluorophores, the FAM dye is released with the dinucleotide so that the cleaved labeled oligonucleotide reaches a molecular weight comparable to that of the unlabeled oligonucleotide (Fig. 2A). In both cases, the cleaved products increase as a function of time indicating that the enzyme is able to cleave the substrate either in absence or in presence of fluorophores. Quantification of the cleavage percentage shows that the presence of the fluorophores slightly slows down the kinetics of the cleavage reaction without changing the qualitative trend (Fig. 2C).

After formation of the cleavage complex, the R11 oligonucleotide is added to start the religation reaction in the presence of dimethyl sulfoxide (DMSO) or 60 μM CPT. The religated strand appears in the upper part of the gel and again the bands run differently due to the different molecular weight of the labeled and unlabeled oligonucleotides (Fig. 2B). Also in this case the presence of the fluorophores slightly decreases the religation rate without changing the qualitative trend (Fig. 2D). In both cases, addition of the anticancer drug CPT slows down the religation rate (Fig. 2B compare the lanes 7–10 to lanes 3–6, and the lanes 17–20 to lanes 13–16), the rate being slower for oligonucleotide labeled with fluorophores (Fig. 2B and D).

2.3. FRET analysis of hTop1 cleavage reaction using a fluorescent substrate

The fluorescence spectrum of the two fluorophores individually inserted in the DNA substrates has been initially measured to evaluate their emission spectra. The emission spectrum of FAM (Fig. 3A, green full line) and the excitation spectrum of Cy3 (Fig. 3A, orange dashed line) extensively overlap at around 520 nm, indicating that they may



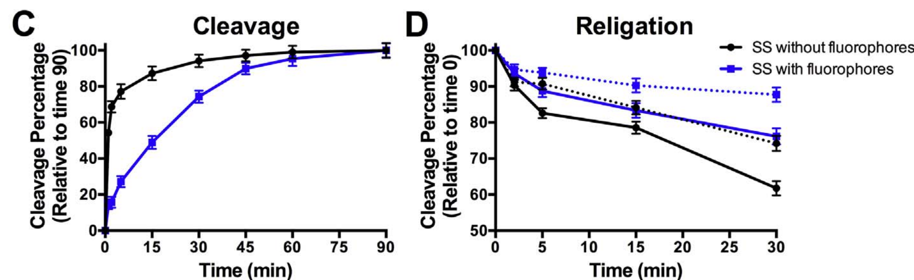


Fig. 2. Analysis of cleavage and religation kinetics using unlabeled (left) or fluorescently labeled (right) SS radiolabeled (*) at 5′-end of CL14 strand. (A) Cleavage kinetics. Time course of cleavage reaction of hTop1 carries out with unlabeled (lanes 2–9) or fluorescently labeled substrate (lanes 11–18). (B) Religation kinetics. Time course of religation reaction upon addition of R11 complementary substrate to hTop1 covalent complex in the absence (lanes 3–6 and lanes 13–16) or presence of 60 μM CPT (lanes 7–10 and lanes 17–20). Time 0 represents the covalent complex before addition of R11 strand (lanes 2 and 12). "C" indicates the substrate without the protein (lanes 1 and 10 in A, lanes 1 and 11 in B). "Cleaved" identifies the strand cleaved by enzymes at the preferred cleavage site. "Religated" indicates the resealed DNA substrate. (C) and (D) Percentage of cleaved fragment, normalized to time 90 or time 0 for cleavage and religation reaction respectively, plotted against time for fluorescent (blue line) or not fluorescent substrate (black line) in the absence (full line) and presence of CPT (dashed line). $n = 3$. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

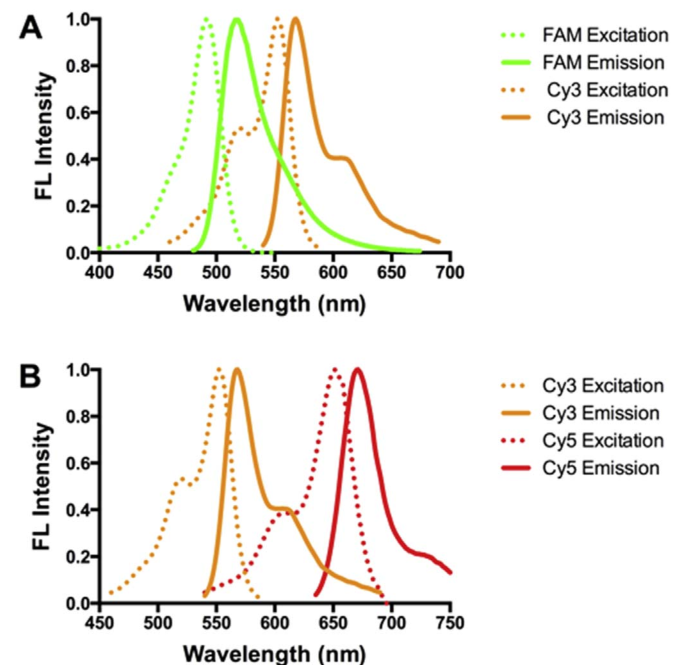


Fig. 3. Fluorescence spectrum analysis of three fluorophores. (A) FL excitation and emission spectra of FAM (green), Cy3 (orange) and Cy5 (red) individually labeled DNA substrate. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

serve as a donor/acceptor pairs for a FRET system (Fig. 3A). Indeed the DNA substrate labeled with both FAM and Cy3 fluorophores, as described in Fig. 1A, gives rise to an efficient FRET effect (Fig. 4A, 0 min). Upon addition of hTop1, the DNA substrate is cleaved with the consequent release of FAM, resulting in an increase in the intensity at 518 nm and a decrease at 568 nm due to the reduction of the FRET event (Fig. 4A 15–90 min). This experiment indicate that the reaction can then be followed plotting as a function of time the decrease of the intensity at 568 nm or the intensity increase at 518 nm (Fig. 4A).

2.4. FRET analysis of hTop1 religation process using a fluorescent substrate

The emission and excitation spectra of Cy3 (Fig. 3B, orange full line) and Cy5 (Fig. 3B, red dashed line) extensively overlap at around 610 nm, indicating they can be used as a donor/acceptor pairs in a FRET system (Fig. 3B). Addition of a labeled Cy5-R11 strand to enzyme-DNA complex labeled with Cy3 results in a significant increase of the fluorescence intensity at 670 nm and a decrease at 570 nm as a function of time (Fig. 4B). The efficient FRET process is due to the religation of the R11 strand that brings the two fluorophores to a close distance (Fig. 4B, 0–30 min). The process can be followed plotting as a function of time the intensity increase at 670 nm or the intensity decrease at 570 nm (Fig. 4B).

Both the cleavage and religation experiments have been done at an ionic strength of 150 mM KCl and at a SS concentration of 16 pmol, that were found to be the best condition to have an optimal signal as shown in the experiments at different ionic strengths (Fig. 5A) and substrate concentrations (Fig. 5B).

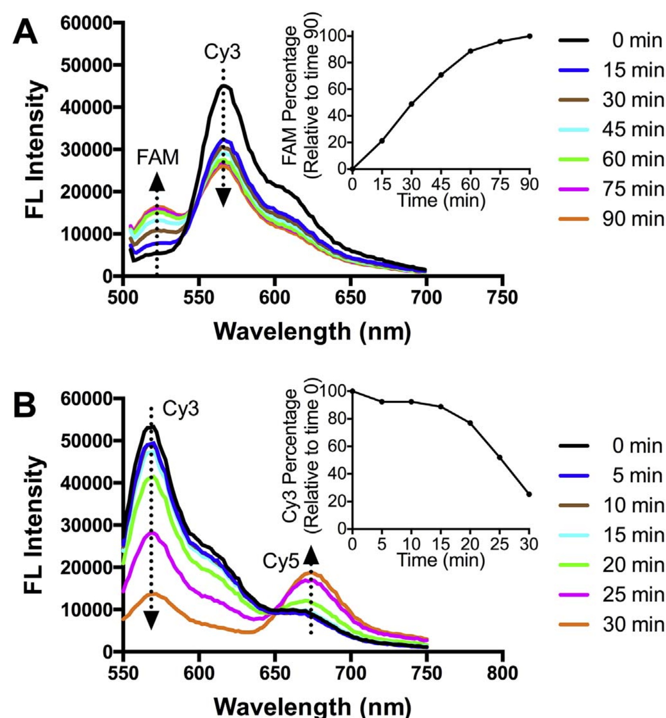


Fig. 4. FRET efficiency analysis of cleavage and religation reactions. (A) Fluorescent spectra (500–700 nm) monitoring cleavage reaction as function of time of a fluorescently labeled SS in presence of hTop1 upon excitation at 488 nm. Insert: time course of FL emission (FAM) at 518 nm. (B) Fluorescent spectra (550–750 nm) monitoring the religation reaction due to the addition of R11 upon excitation at 518 nm. Insert: time course of FL emission (Cy3) at 568 nm.

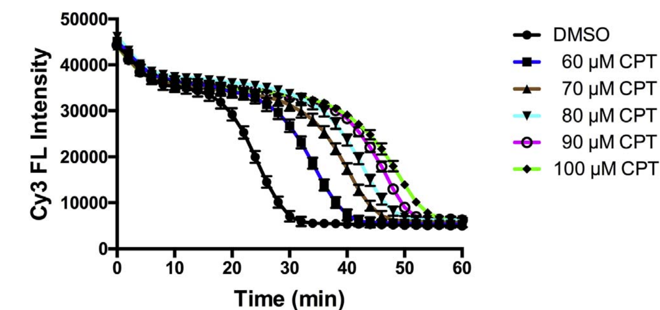


Fig. 6. CPT reversibly slows down the religation reaction. Time course of FL emission of Cy3 donor after adding of R11 strand, in the absence and presence of 60–100 μM of CPT, to the cleaved complex, under an excitation of 518 nm. $n = 3$.

2.5. Screening of anti-cancer drug by FRET analysis

HTop1 is the unique molecular target of a class of anticancer compounds belonging to the CPT family [21,22]. CPT binds to hTop1-DNA covalent complex to generate CPT-hTop1-DNA ternary complex, slowing down the religation and leading to hTop1 cleavage complex accumulation. The efficacy of the proposed FRET system to detect CPT inhibition has been investigated during religation process. HTop1 is initially incubated with SS substrate at 37 °C for 90 min to obtain the enzyme-DNA cleavage complex. The Cy3 and Cy5 FRET system has been followed upon addition of labeled R11 complementary strand in the absence and presence of increasing CPT concentration from 60 to 100 μM. In the absence of CPT, the religation reaction is completed within 30 min (Fig. 6, black line). The increase of CPT concentration gradually delays the religation process and, in the presence of 100 μM CPT, it is delayed up to about 1 h (Fig. 6, green line). These results indicate that the proposed FRET system represents an easy and efficient way to monitor the drug response and can be proposed for a high throughput screening of new cancer drug targeting hTop1.

2.6. Characterization of hTop1 mutants with the proposed FRET method

As a control the proposed FRET method has been tested on the inactive Tyr723Phe mutant that can non-covalently bind to supercoiled DNA with an affinity identical to the wild type enzyme, but it is not able to carry out the cleavage process [23]. In line a negligible FL intensity changes is observed for both cleavage (Fig. 7A) and religation (Fig. 7B)

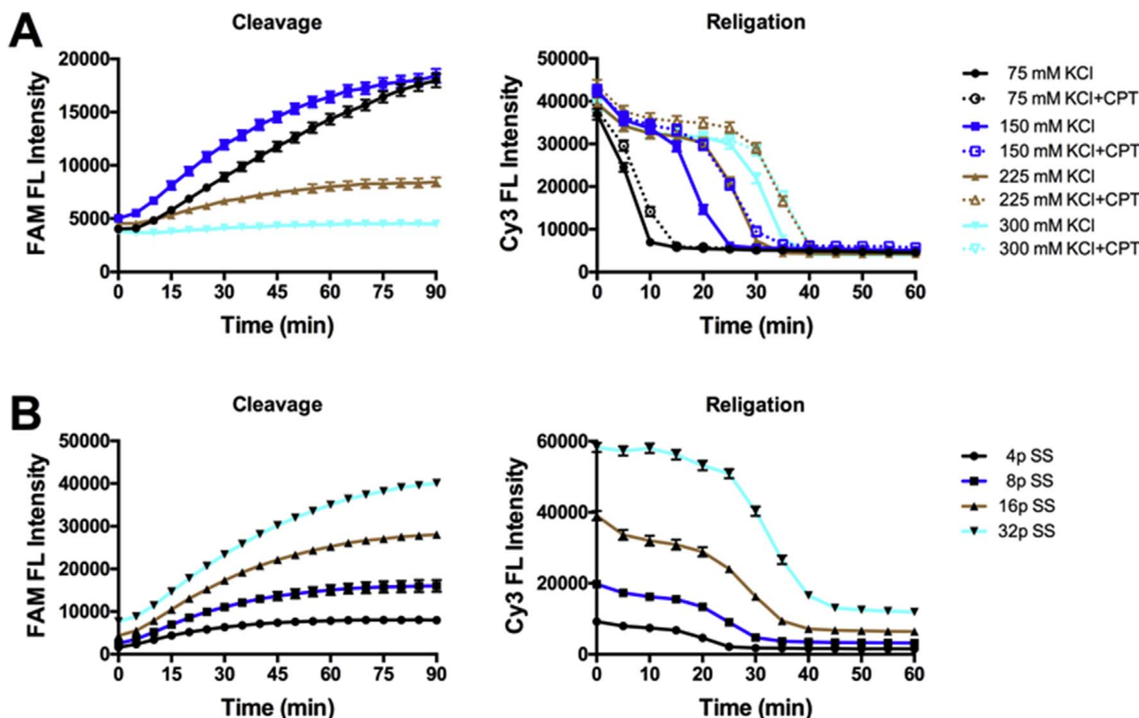


Fig. 5. Effect of ionic strength (A) and SS concentration (B) on fluorescence emission.

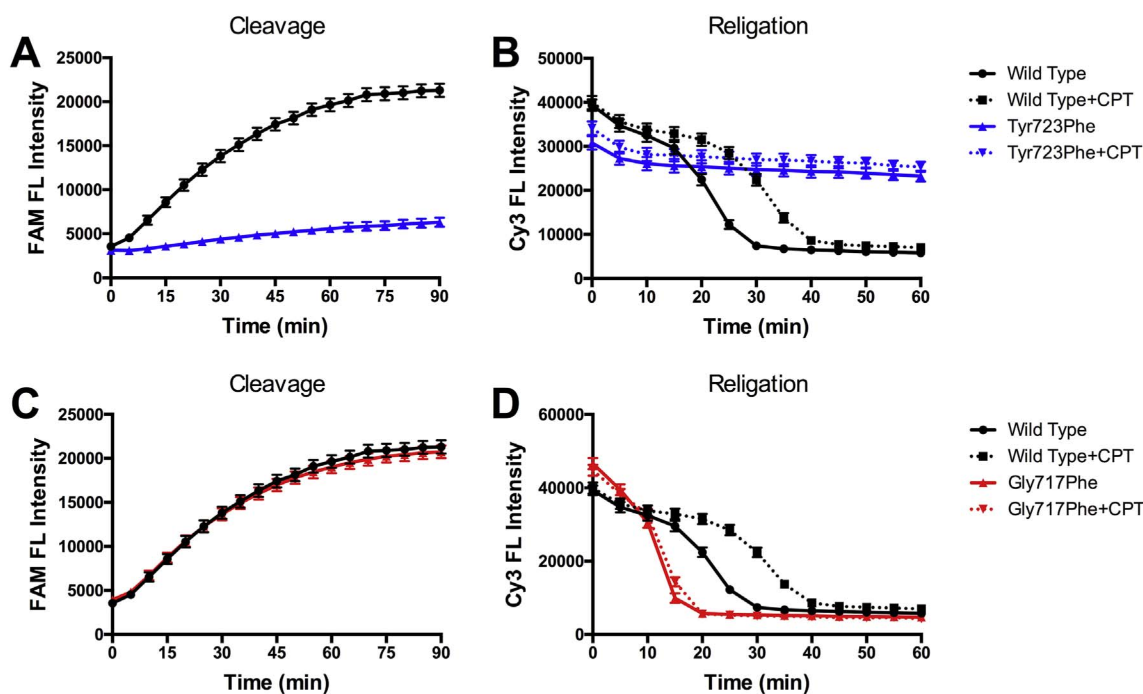


Fig. 7. Analysis's of the cleavage and religation reactions of hTop1 mutants. Time course of the cleavage (Panel A and C) and religation (Panel B and D) reactions to monitor the wild type (black), inactive mutant Tyr723Phe (blue) and CPT resistant mutant Gly717Phe (red). Full or dashed line indicate the reaction in the absence or presence of 60 μM CPT. $n = 3$. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

reactions and in this latter case the addition of CPT does not induce any significant fluorescent difference. The method has been also tested on the Gly717Phe mutant that has been previously shown to have a fast religation rate that is only partially reduced in the presence of CPT [24]. In line the FRET experiments indicate that the mutant shows a cleavage kinetics comparable to the wild type (Fig. 7C) and a very fast religation rate that is unaffected by CPT (Fig. 7D). These results show the potentials of the designed FRET system for a fast analysis of the mutant activity.

3. Conclusions

These results indicate that the proposed fluorescence approach can be used to test the hTop1 cleavage and religation activity. The DNA substrate labeled with three fluorophores permits to analyze the enzyme cleavage and religation kinetics following the FL spectrum. The method can be applied, *in vitro*, to monitor the function of hTop1 mutants with clinical significance following the reactivity toward the CPT anticancer drug and it is suitable for an efficient high throughput screening of drugs targeting hTop1.

4. Methods

4.1. Reagents and apparatus

CPT (Sigma-Aldrich) was dissolved in 99.9% dimethyl sulfoxide (DMSO, Sigma-Aldrich) to a final concentration of 4 mg/ml (11.5 mM) and stored at −20 °C. T4 polynucleotide kinase was provided from New England BioLabs. [γ-32P] ATP (3000 Ci/mmol) was supplied by PerkinElmer. All oligonucleotides were produced by Sangon biotech (Shanghai) Co., Ltd. All other reagents were analytical grade and used as received. The FL emission was detected using a Tecan Infinite M200 Pro. microplate reader.

4.2. HTop1 enzyme purification

HTop1 enzyme with an N-terminal FLAG tag was expressed by single copy plasmid YCpGAL1-FLAG-hTop1 under galactose inducible promoter transformed in *Saccharomyces cerevisiae* Top1 null strain EKY3 (ura3-52, his3Δ200, leu2Δ1, trp1Δ63, top1::TRP1, MATα) and purified by anti-FLAG M2 monoclonal affinity gel (Sigma-Aldrich) with FLAG peptide (Sigma-Aldrich) competitive elution as we previously described [25]. Mutants of hTop1 Tyr723Phe and hTop1 Gly717Phe were generated by oligonucleotide-directed mutagenesis of YCpGAL1-FLAG-hTop1 by QuikChange site-directed mutagenesis kit (Agilent technologies). The levels and integrity of both wild type and mutant enzymes were assessed by immunoblot with monoclonal anti-FLAG M2 antibody (Sigma-Aldrich).

4.3. Synthetic DNA substrates

Fluorescently labeled cleavage strand (CL14, 5′-P-GAAAAAA/Cy3/GACTTAG-FAM-3′), complementary strand (CP25, 5′-P-TAAAAATTTT TCTAAGTCTTTT-TTC-3′) and religation strand (R11, 5′-AGAAAAA/Cy5/TTTT-3′) were produced by Sangon biotech (Shanghai) Co., Ltd and verified by mass spectrum. The 5′-end of CL14 and CP25 were phosphorylated to avoid nonspecific religation.

To prepare the radiolabeled substrate, T4 polynucleotide kinase was used to transfer the radio phosphoryl donor of [γ-32P] ATP to the 5′-end of CL14 strand. CL14 with a unique 5′ radiolabel was purified by gel filtration using an Illustra G-25 column (GE Healthcare). To assemble the suicide substrate by hybridization, equal amount of CL14 and CP25 strands were heated to 95 °C for 5 min, cooled slowly to room temperature and kept at 4 °C until use in TE buffer containing 10 mM Tris-HCl pH 7.5 and 1.0 mM EDTA.

4.4. Monitor hTop1 activities based on suicide substrate with three fluorophores

The excitation and emission spectra of DNA substrates individually labeled with FAM, Cy3 or Cy5 were measured to verify the spectral overlap (Fig. 3A and B). The FRET pairs for cleavage were FAM as donor and Cy3 as acceptor, which were conjugated to CL14 and further hybridized with CP25 to build suicide substrate (SS). Equal and excess

amount of hTop1 wild type or mutant enzyme was incubated with 16 pmol SS in standard hTop1 reaction buffer containing 20 mM Tris–HCl pH 7.5, 0.1 mM Na_2EDTA, 10 mM $MgCl_2$, 5 μg/ml acetylated bovine serum albumin and 150 mM KCl. The reaction was performed at 37 °C and followed by FL measurement of FAM and Cy3 under the excitation at 475 nm for 90 min. The enzyme cuts SS releasing the dinucleotide labeled with FAM and remains covalently conjugated to SS to form the enzyme-DNA cleavage complex. The FRET dyes for religation were composed by a Cy3 fluorophores present on the cleavage complex as donor and Cy5 labeled on the religation strand R11 as acceptor. The religation reaction was performed adding 40 pmol of R11 strand to the cleavage complex in the presence of DMSO or 60 μM CPT. The reaction was continually monitored at 37 °C and following the FL intensity of Cy3 and Cy5 under the excitation wavelength of 520 nm. Key factors influencing the reaction efficiency such as ionic strength and SS concentrations had been optimized to have a strong signal (Fig. 5).

4.5. Detect hTop1 activity using radiolabeled SS

The cleavage strand CL14 was radiolabelled with [γ-32P] ATP at its 5′-end [24]. The complementary strand CP25 was phosphorylated at its 5′-end with unlabeled ATP. Equal amount of CL14 and CP25 strands were annealed to obtain the CL14/CP25 SS, containing hTop1 preferred cleavage site. Identical amounts of wild type or mutant hTop1 enzyme was incubated with 16 pmol SS in standard hTop1 reaction buffer at 37 °C. A 5 μl aliquots were removed at indicated time points and the reaction was stopped with 0.5% (w/v) SDS. For the religation reaction, equal amount of hTop1 wild type or mutant enzyme was incubated with 16 pmol SS for 90 min at 37 °C. After the formation of the cleaved complex, a 5 μl aliquot was removed and used as point at time 0. Subsequently, 40 pmol of R11 oligonucleotide was mixed with the cleavage complex to trigger the religation reaction in presence of DMSO or 60 μM CPT. A 5 μl aliquots were removed at the indicated time points and the reaction was stopped with 0.5% (w/v) SDS. Both the cleaved and religated products were precipitated by ethanol and resuspended in 5 μl of 1 mg/ml trypsin, followed by incubation at 37 °C for 60 min. The samples were analyzed by denaturing 7 M urea/20% polyacrylamide gel electrophoresis in running buffer containing 48 mM Tris, 45.5 mM boric acid and 1 mM EDTA.

Acknowledgements

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References

[1] J.C. Wang, Cellular roles of DNA topoisomerases: a molecular perspective, Nat. Rev. Mol. Cell Biol. 3 (2002) 430–440, http://dx.doi.org/10.1038/nrm831.
[2] K.D. Corbett, J.M. Berger, Structure, molecular mechanisms, and evolutionary relationships in DNA topoisomerases, Annu. Rev. Biophys. Biomol. Struct. 33 (2004) 95–118, http://dx.doi.org/10.1146/annurev.biophys.33.110502.140357.
[3] J.J. Champoux, DNA topoisomerases: structure, function, and mechanism, Annu. Rev. Biochem. 70 (2001) 369–413, http://dx.doi.org/10.1146/annurev.biochem.70.1.369.
[4] L. Stewart, A model for the mechanism of human topoisomerase I, Science (80-.) 279 (1998) 1534–1541, http://dx.doi.org/10.1126/science.279.5356.1534.
[5] B.L. Staker, M.D. Feese, M. Cushman, Y. Pommier, D. Zembower, L. Stewart, A.B. Burgin, Structures of three classes of anticancer agents bound to the human topoisomerase I-DNA covalent complex, J. Med. Chem. 48 (2005) 2336–2345, http://dx.doi.org/10.1021/jm049146p.
[6] Y. Pommier, DNA topoisomerase I inhibitors: chemistry, biology, and interfacial inhibition, Chem. Rev. 109 (2009) 2894–2902, http://dx.doi.org/10.1021/cr900097c.
[7] I. D'Annessa, C. Tesauro, Z. Wang, B. Arnò, L. Zuccaro, P. Fiorani, A. Desideri, The human topoisomerase 1B Arg634Ala mutation results in camptothecin resistance and loss of inter-domain motion correlation, Biochim. Biophys. Acta 1834 (2013) 2712–2721, http://dx.doi.org/10.1016/j.bbapap.2013.09.017.
[8] P. Fiorani, C. Tesauro, G. Mancini, G. Chillemi, I. D'Annessa, G. Graziani, L. Tentori, A. Muzi, A. Desideri, Evidence of the crucial role of the linker domain on the catalytic activity of human topoisomerase I by experimental and simulative characterization of the Lys681Ala mutant, Nucleic Acids Res. 37 (2009) 6849–6858, http://dx.doi.org/10.1093/nar/gkp669.
[9] L. Zuccaro, C. Tesauro, T. Kurkina, P. Fiorani, H.K. Yu, B.R. Knudsen, K. Kern, A. Desideri, K. Balasubramanian, Real-time label-free direct electronic monitoring of topoisomerase enzyme binding kinetics on graphene, ACS Nano 9 (2015) 11166–11176, http://dx.doi.org/10.1021/acsnano.5b05709.
[10] L. Zuccaro, C. Tesauro, B. Cerroni, A. Ottaviani, B.R. Knudsen, K. Balasubramanian, A. Desideri, Rolling circle amplification-based detection of human topoisomerase I activity on magnetic beads, Anal. Biochem. 451 (2014) 42–44, http://dx.doi.org/10.1016/j.ab.2014.02.003.
[11] M. Stougaard, J.S. Lohmann, A. Mancino, S. Celik, F.F. Andersen, J. Koch, B.R. Knudsen, Single-molecule detection of human topoisomerase I cleavage-ligation activity, ACS Nano 3 (2009) 223–233, http://dx.doi.org/10.1021/nn800509b.
[12] M.L. Jepsen, A. Ottaviani, B.R. Knudsen, Y.-P. Ho, Quantum dot based DNA nanosensors for amplification-free detection of human topoisomerase I, RSC Adv. 4 (2014) 2491, http://dx.doi.org/10.1039/c3ra45557b.
[13] H. Jun, J.T. Stivers, Diverse energetic effects of charge reversal mutations of poxvirus topoisomerase IB, Biochemistry 51 (2012) 2940–2949, http://dx.doi.org/10.1021/bi3001903.
[14] E.L. Kristoffersen, L.A. Jørgensen, O. Franch, M. Etzerodt, R. Frøhlich, L. Bjergbæk, M. Stougaard, Y.-P. Ho, B.R. Knudsen, Real-time investigation of human topoisomerase I reaction kinetics using an optical sensor: a fast method for drug screening and determination of active enzyme concentrations, Nanoscale 7 (2015) 9825–9834, http://dx.doi.org/10.1039/C5NR01474C.
[15] L.B. Marcussen, M.L. Jepsen, E.L. Kristoffersen, O. Franch, J. Proszek, Y.-P. Ho, M. Stougaard, B.R. Knudsen, DNA-based sensor for real-time measurement of the enzymatic activity of human topoisomerase I, Sensors (Basel) 13 (2013) 4017–4028, http://dx.doi.org/10.3390/s130404017.
[16] C. Tesauro, B. Morozzo della Rocca, A. Ottaviani, A. Coletta, L. Zuccaro, B. Arnò, I. D'Annessa, P. Fiorani, A. Desideri, Molecular mechanism of the camptothecin resistance of Glu710Gly topoisomerase IB mutant analyzed in vitro and in silico, Mol. Canc. 12 (2013) 100, http://dx.doi.org/10.1186/1476-4598-12-100.
[17] P. Fiorani, G. Chillemi, C. Losasso, S. Castelli, A. Desideri, The different cleavage DNA sequence specificity explains the camptothecin resistance of the human topoisomerase I Glu418Lys mutant, Nucleic Acids Res. 34 (2006) 5093–5100, http://dx.doi.org/10.1093/nar/gkl670.
[18] T. Scientific, The Molecular Probes Handbook, (2010) https://www.thermofisher.com/it/en/home/references/molecular-probes-the-handbook.html , Accessed date: 27 December 2017.
[19] P. Fiorani, A. Bruselles, M. Falconi, G. Chillemi, A. Desideri, P. Benedetti, Single mutation in the linker domain confers protein flexibility and camptothecin resistance to human topoisomerase I, J. Biol. Chem. 278 (2003) 43268–43275, http://dx.doi.org/10.1074/jbc.M303899200.
[20] M.C. Murphy, I. Rasnik, W. Cheng, T.M. Lohman, T. Ha, Probing single-stranded DNA conformational flexibility using fluorescence spectroscopy, Biophys. J. 86 (2004) 2530–2537, http://dx.doi.org/10.1016/S0006-3495(04)74308-8.
[21] A. Coletta, A. Desideri, Role of the protein in the DNA sequence specificity of the cleavage site stabilized by the camptothecin topoisomerase IB inhibitor: a metadynamics study, Nucleic Acids Res. 41 (2013) 9977–9986, http://dx.doi.org/10.1093/nar/gkt790.
[22] B.L. Staker, K. Hjerrild, M.D. Feese, C. a Behnke, A.B. Burgin, L. Stewart, The mechanism of topoisomerase I poisoning by a camptothecin analog, Proc. Natl. Acad. Sci. U. S. A 99 (2002) 15387–15392, http://dx.doi.org/10.1073/pnas.242259599.
[23] Y. Pommier, P. Pourquier, Y. Fan, D. Strumberg, Mechanism of action of eukaryotic DNA topoisomerase I and drugs targeted to the enzyme, Biochim. Biophys. Acta 1400 (1998) 83–105 http://www.ncbi.nlm.nih.gov/pubmed/9748515 , Accessed date: 27 December 2017.
[24] Z. Wang, I. D'Annessa, C. Tesauro, S. Croce, A. Ottaviani, P. Fiorani, A. Desideri, Mutation of Gly717Phe in human topoisomerase 1B has an effect on enzymatic function, reactivity to the camptothecin anticancer drug and on the linker domain orientation, Biochim. Biophys. Acta 1854 (2015) 860–868, http://dx.doi.org/10.1016/j.bbapap.2015.04.017.
[25] B. Arnò, I. D'Annessa, C. Tesauro, L. Zuccaro, A. Ottaviani, B. Knudsen, P. Fiorani, A. Desideri, Replacement of the human topoisomerase linker domain with the plasmodial counterpart renders the enzyme camptothecin resistant, PLoS One 8 (2013) e68404, , http://dx.doi.org/10.1371/journal.pone.0068404.