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Full Length Research Paper

Research on prokaryocyte expression and biological activity of the core region of Bloom's Syndrome protein

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To establish an effective approach for inducing expression of the RecQ core of Bloom's Syndrome protein (BLM$^{642-1290}$) and assaying its biological activity *in vitro*, BLM$^{642-1290}$ recombinant protein was expressed with IPTG at room temperature in *Escherichia coli*, and then the expressed product was assayed using SDS-PAGE and western blotting. After purification via affinity chromatography, DNA binding activity and unwinding activity of the protein were assessed by fluorescence polarization. Furthermore, the ATPase activity of the protein was also assayed using ultraviolet spectrophotometry based on PiColorLock Gold reagent. An effective expression method was established for BLM protein in *E. coli*. The obvious bioactivities of the protein were observed in binding to ssDNA or dsDNA, unwinding the dsDNA in the presence of ATP, as well as catalyzing ATP hydrolysis in the presence of ssDNA *in vitro*. The prokaryocyte expression method of BLM$^{642-1290}$ was established successfully and the protein with biological activity was obtained from recombinant *E.coli*. This would be significant to provide a better understanding on BLM protein and facilitate the elucidation of mechanism of pathopoiesia in Bloom's Syndrome.

Key words: BLM$^{642-1290}$ protein, induced expression, enzymatic activity.

INTRODUCTION

Bloom's Syndrome (BS) is a rare, autosomal recessive genetic disorder (German et al., 1994). BS patients display similar biological properties such as clinical manifestations, pre- and postnatal growth retardation, sun-sensitive facial erythema, subfertility in females and infertility in males, immunodeficiency, and a predisposition to most types of cancers (German, 1993; Chisholm et al., 2001; Hanada, 2007). This disease is caused by mutation of the Bloom's Syndrome gene (BLM) and the gene is located on chromosome 15q26.1 (Mathew et al., 1993). Cells from BS patients show many cytogenetic abnormalities including translocation, chromosome breakage, telomeric association and increased rates of sister-chromatid exchange (SCEs), which can be used as a molecular diagnostic for the disease (Chaganti et al., 1974; Bryant et al., 1979; German et al., 1974; Furuichi, 2001).

The BLM gene encodes a protein called Bloom Syndrome protein (BLM) comprising 1417 amino acid, which is a member of the RecQ family helicase and plays an important role in the maintenance of genomic stability (German, 1993; German et al., 1994; Hickson et al., 2001). The RecQ helicase family is an important member of DNA helicases including the BLM, WRN, RECQ1, RECQ4, RECQ5, *Saccharomyces cerevisiae* Sgs1p, and

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Abbreviations: BS, Bloom's Syndrome; **BLM,** Bloom Syndrome protein; **BLM$^{642-1290}$,** the RecQ core of Bloom's Syndrome protein; **SCEs,** sister-chromatid exchange; **HRDC,** helicase RNase D conserved domain; **IPTG,** isopropyl-1-thio-α-D-galactopyranoside; **RecQ-Ct,** RecQ Conserved -Terminal region; **BSA,** bovine serum albumin; **PMSF,** phenylmethanesulfonyl fluoride; **ssDNA,** single-stranded DNA; **dsDNA,** double-stranded DNA; **ATP,** adenosine triphosphate; **SDS-PAGE,** sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

Schizosaccharomyces pombe Rqh1p proteins. Among which the BLM, WRN, and RECQ4 are defective in Bloom's, Werner's and Rothmund-Thomson Syndromes in humans (Karow et al., 2000; Furuichi, 2001). Mutations in human RecQ genes induced genomic instability and cancer (Novotny et al., 2003; Sharma et al., 2005; Mounira, 2006; Rigu et al., 2008). As most RecQ helicases, BLM helicase shows DNA-dependent ATPase activity and ATP-dependent 3' - 5' DNA helicase activity (Karow et al., 1997; Bachrati and Hickson, 2006), BLM can unwinds a variety of DNA structures including the holliday junction, the triple helix, the D-loop and the highly stable G-quadruplex structure (Lohman and Bjornson, 1996; Karow et al., 1997; Wu et al., 2001; Dutertre et al., 2002). The eukaryotic ssDNA binding protein, replication protein A, stimulates the unwinding activity of BLM helicase, which was necessary for unwinding reinitiation (Brosh et al., 2000; Yodh et al., 2009). BLM helicase can interact with Topo IIIa (topoiso-merase IIIa) and stimulate its helicase activity (Wu and Hickson, 2002). Recent research showed that telomerase-associated protein 1 (TEP1), heat shock protein 90 (HSP90) and topoisomerase Iiα (TOPOIIα) associate with BLM helicase and modulate its helicase activity using telomeric DNA substrates in immortalized cells (Bhattacharyya et al., 2009). Although there have been many intensive studies for BLM protein, the pathologic mechanism of Bloom's Syndrome is still poorly under-stood (Sharma et al., 2005; Rigu et al., 2008). Elucidation of the molecule mechanism of the BLM protein may provide the opportunity to develop effective anti-cancer strategies (Yang et al., 2010).

The RecQ core of BLM is consisted of the DEAH, RecQ Conserved-Terminal (RecQ-Ct) region, helicase and RNAseD C-terminal (HRDC) domains (Janscak et al., 2003). DEAH helicase domain is consist of seven amino acid sequence motifs that are responsible for coupling of ATP hydrolysis to DNA binding, translocation and unwinding (Dillingham et al., 2001). The HRDC domain is distal to the C-terminus and it is important to modulate the helicase function via auxiliary contacts to DNA (Liu et al., 1999). The RecQ-Ct domain is unique to the RecQ family of helicases and located just after the conserved seven signature motifs. To date, the investigation in structure and function of BLM in BS patients is insufficient, which leads to the mechanism of pathopoiesia in Bloom's Syndrome remains elusive. A full description of the detailed mechanism of pathopoiesia in the disease requires a deeper understanding about the BLM protein. The biological study of the helicase will provide valuable information towards elucidating the mechanism of pathopoiesia. To this end, the purification of BLM protein and unraveling of its biological activity were the foundation of further study on the mechanism of pathopoiesia. At present, the expression methodology of the BLM protein includes prokaryocyte expression and eukaryotic expression and the former is normally in *E. coli* cells (Janscak et al., 1996), while the latter is in yeast cells (Hickson et al., 2001). In order to uncover the character of the BLM protein, the protein with the biological activity was purified using nickel chelate affinity chromatography and size exclusion chromatography *in vitro* (Guo et al., 2005). Several modern methods have been developed to measure the biological activity of the protein. The electrophoretic mobility shift assay, smFRET (single-molecule fluorescence resonance energy trans-fer), fluorescence polarization assay and radiometric assay were used to analyze DNA-binding activity and helicase activity of the protein (Xu et al., 2003; Yodh et al., 2009; Dou and Xi, 2010; Yang et al., 2010). The analytical methods of ATPase activity include measuring the radioactive γ^{-32} Pi liberated during hydrolysis ATP and the release of inorganic phosphate by a colorimetric assay (Janscak et al., 2003; Guo et al., 2007). In the current study, we have established the effective method that expressed $BLM^{642-1290}$ recombinant protein in *E.coli* BL21 and obtained higher purity of recombinant protein. Then, DNA-binding activity, DNA unwinding activity and ATPase activity of the protein had also been assayed, employing different methods by which higher biological activity of protein might be obtained. The results would be significant to provide a better understanding on BLM protein and facilitate the elucidation of mechanism of pathopoiesia in Bloom's Syndrome.

MATERALS AND METHODS

Chemicals

All chemicals used in this study were analytical grade. Bovine serum albumin (BSA), isopropyl-1-thio-α-D-galactopyranoside (IPTG), Triton X-100, imidazole, Phenylmethanesulfonyl fluoride (PMSF) and Tween 20 were obtained from Solarbio (China). Ampicillin and chloramphenicol were provided by Sigma (USA). Tryptone and Yeast extract were provided by Oxoid (England). Supports of chromatography used for $BLM^{642-1290}$ recombinant protein purification: Sephadex G-200 and fast flow affinity chromatography column were purchased from GE Healthcare (USA).

Construction of pET15b-$BLM^{642–1290}$ expression vector

The recombinant plasmid pET15b-$BLM^{642–1290}$ was donated by Professor XuGuang Xi in Curie institute of the 11th university in Paris, France. A plasmid for expression of core region of Bloom's Syndrome protein was generated by inserting the core region of *BLM* gene between the NdeI and XhoI sites of pET15b expression plasmid (Novagen, Germany). The expression product of the core region of *BLM* gene was consisted of the DEAH, RecQ-Ct and HRDC domains (amino acid residues 642-1290). In this study, BLM was fused in frame with an N-terminal peptide containing six tandem histidine residues. The expression of his-tagged fusion was driven by a T7 RNA polymerase promoter. The recombinant plasmid was transformed into *E. coli* B21 in accordance with the previous report (Guo et al., 2005).

DNA substrates preparation

The PAGE-purified oligonucleotides (Table 1) were purchased from Shanghai biological engineering technology service Co., LTD

Table 1. Oligonucleotide sequences used in this study.

Substrate	Length/mer	Structure and sequence
A1	63	5'AATCCGTCGAGCAGagttagggttagggttagggttagttttttttttttttttttttttttt3'
A2	14	3'FAM-TTAGGCAGCTCGTC5'
B1	45	5'AATCCGTCGAGCAGAGTTAGGttaggttaggttagttttttttt3'
B2	21	3'FAM-TTAGGCAGCTCGTCTCAATCC5'
C1	56	5'AATCCGTCGAGCAGAGTTAGGGTTAGGGTTAGGGTTagttttttttttttttttt 3'
C2	36	3'FAM-TTAGGCAGCTCGTCTCAATCCCAATCCCAATCCCAA5'
D1	20	5' TGACCATCagttttttttt 3'

Fa represents the fluoresce in chemical group.

(China). Briefly, two kinds of single-stranded DNA (ssDNA) were equally mixed and were denatured in buffer (20 mM Tris-HCl, pH 7.2, and 100 mM NaCl) by heating in water bath for 5 min at 85°C. Then the denatured DNA was slowly annealed at room temperature, the annealed products were used as fluorescence-taged DNA substrates to assay the biological activity of $BLM^{642-1290}$ recombinant protein according to Xu's report (Xu et al., 2003).

Induced expression of $BLM^{642-1290}$ in *Escherichia coli* BL21

The 50 µg/ml ampicillin and 30 µg/ml chloramphenicol were added to the Luria–Bertani (LB) liquid medium (pH 7.0) containing 10 g/L Tryptone, 5 g/L Yeast extract, and 10 g/L NaCl. 1 mL *E. coli* BL21 bearing pET15b-$BLM^{642-1290}$ plasmid was added to 1 L LB liquid medium. The mixed solution was cultured at 37°C and 200 rpm until the A_{600} reached 0.5 ~ 0.6. The cultured bacterium fluid was incubated with shaking at 17°C for 20 h by the addition of IPTG to 0.45 mM, protein production was induced by IPTG. The bacterias were harvested by centrifugation at 4,000 rpm for 15 min, and then stored at -80°C.

Separation and purification of $BLM^{642-1290}$ recombinant protein

The bacterias were thawed and resuspended in buffer A containing 20 mM Tris-HCl (pH 7.9), 500 mM NaCl, 10% (v/v) glycerol, 0.1% (v/v) Triton X-100, 5 mM imidazole, and 1 mM PMSF. The suspended liquid was cracked by ultrasonic breakage (Sonics, USA) to reduce viscosity. The insoluble part of the lysate was removed by centrifugation at 13,000 rpm for 40 min. The supernatant was applied to a 24 ml fast flow affinity chromatography column of Chelating Ni^{2+} Sepharose pre-equilibrated with buffer A. Then the column was washed with the following buffer: 10 column volume (CV) of buffer A, 10CV of buffer B containing 20 mM Tris-HCl (pH 7.9), 500 mM NaCl, 10% (v/v) glycerol, 0.1% (v/v) Triton X-100, 60 mM imidazole, and 1 mM PMSF. Finally, the target protein retained on the column was eluted by 10 CV of buffer C containing 20 mM Tris-HCl (pH 7.9), 500 mM NaCl, 10% (v/v) glycerol and 500 mM imidazole. The whole process was monitored by the FPLC system AKTA purifier100 (GE Healthcare, USA). High concentration fractions were loaded onto FPLC size exclusion chromatography Superdex 200. The column was eluted by buffer D containing 20 mM Tris-HCl (pH 7.9), 150 mM NaCl and 10% (v/v) glycerol. The fractions with target protein were pooled, and the mixed liquor was concentrated by one amicon membrane to desirable concentration. Then, the target protein was stored at -80ºC. The final protein concentration was determined by Bradford dye method (Bradford, 1976) with BSA as the standard. Purity of the protein was determined by 10% (v/v) SDS-PAGE.

Western blotting assays

A total of 20 ml of per fraction liquid was analyzed by 10% (v/v) SDS-PAGE for western blotting analysis in every lane. Firstly, the gels were electroblotted onto NC membrane (Solarbio, China) which was blocked in phosphate-buffered saline containing 0.05% (v/v) Tween 20 and 5% (w/w) skim milk. Secondly, the membrane was incubated with 6 × his-tag rat monoclonal antibodies (dilution 1:1,000) for 120 min (Beyotime, China), then, washed in phosphate buffer containing 0.1% (v/v) Tween 20. Thirdly, the membrane was incubated in blocking buffer containing anti-mouse immunoglobulin peroxidase (dilution 1:1,000) for 120 min (GE Healthcare, USA). Finally, the membrane was washed again by phosphate buffer (0.1% v/v) Tween 20) and detected for the target protein by ECL chemiluminescence kit (GE Healthcare, USA).

DNA-binding activity assays

The DNA-binding activity was analyzed using a Beacon fluorescence polarization spectrophotometer (PanVera, USA) as the previous report (Xu et al., 2003). The 100 nM $BLM^{642-1290}$ recombinant protein was added to 150 µl binding reaction buffer containing 2 nM of different lengths of fluorescein-labeled double-stranded DNA (dsDNA) and ssDNA. Each sample was incubated for 5 min at 25°C before being measured by fluorescence polarization. The change of anisotropy was recorded every 8 s until it was stabilized. The dissociation constants (apparent K_d values) of the protein for DNA-binding were calculated by equations (1) and (2) according to Dou's report (Dou et al., 2004),

$$\alpha D_T = NP_T \frac{\alpha}{1-\alpha} + K_d \quad (1)$$

where, D_T and P_T are the total molar concentrations of DNA and protein used, respectively.

$$\alpha = \frac{A_{\max} - A}{A_{\max} - A_{\min}} \quad (2)$$

A is the fluorescence anisotropy at a given concentration of protein, A_{max} is the anisotropy at saturation, and A_{min} is the initial anisotropy.

Helicase activity assays

The helicase activity was measured as Xu's report (2003). An appropriate quantity (2 nM) of different lengths of fluorescein-labeled

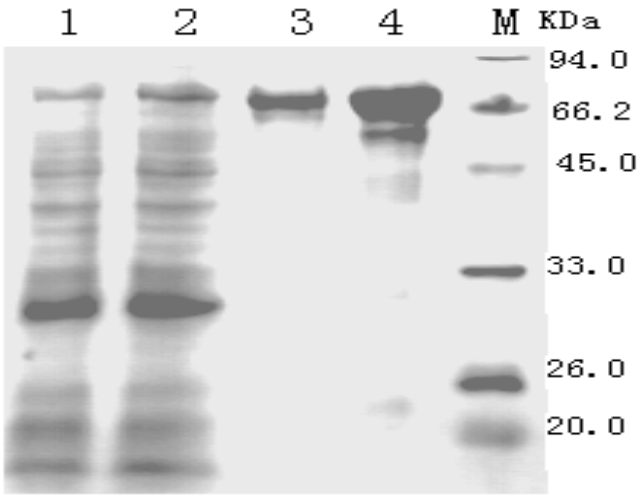

Figure 1. Purification of BLM$^{642-1290}$ recombinant protein was resolved in 10% (w/w) SDS-PAGE and stained with coomassie blue. lane1, bacteria fluid; lane2, supernatant fluid; lane3, pure enzyme solution; lane4, pooled fractions from chitin column; M, marker proteins (in kDa)

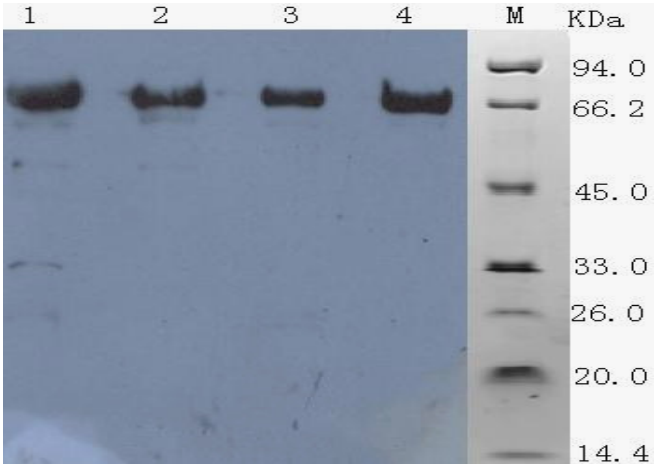

Figure 2. Western blotting assay of BLM$^{642-1290}$ recombinant protein. Lane 1, the bacterias liquid; lane 2, the supernatant pooled; lane 3, pooled fractions from chitin column; lane 4, the pure enzyme solution; M, marker proteins (in kDa).

dsDNA was added to the unwinding buffer (150 ml total) containing 25 mM Tris-HCl (pH 7.4), 25 mM NaCl, 3 mM $MgCl_2$, and 0.1 mM DTT (dithiothreitol) in a temperature-controlled cuvette at 25°C. The anisotropy was continuously measured until it was stabilized. After that, the BLM$^{642-1290}$ recombinant protein was added to the cuvette. Then, the change of anisotropy was assayed immediately. When the anisotropy became stable again, 1 mM adenosine triphosphate (ATP) was added quickly to the reaction buffer. The change of anisotropy was recorded every 8 s until stabilized.

ATPase activity assays

The DNA-dependent ATPase activity was measured using ultraviolet spectrophotometry based on PiColorLock Gold reagent (Innova Biosciences, England). The theory is based on a colorimetric estimation of inorganic phosphate (Pi) produced by ATP hydrolysis (Chan et al., 1986). The reactions were carried out at 25°C in buffer containing 0.5 M Tris-HCl (pH 7.4), 0.1 M $MgCl_2$, 2 nM ssDNA (20, 45, and 63 mer) cofactors and initiated by adding 10 mM ATP. 200 µl reactions served as the samples were removed from the total reactions at 2.5, 5, 7.5, 10, 15, 20, 25, and 30 min. After that, 50 µl Gold mix was added in order to terminate ATP hydrolysis. Two minutes later, every sample was got steady by adding 20 µl stabiliser, then every sample was read at wavelength 650 nm in ultraviolet spectrophotometer after 30 min. The $A_{activity}$ (units/ml) was defined as one unit which represented the amount of enzyme that catalysed the hydrolysis of 1 µmol substrate per minute. The activity of undiluted enzyme sample was given by the following equation (3) that comes from the instruction of ATPase assay kit,

$$A_{activity} = \frac{A \times C}{500\,B} \quad (3)$$

where, A is concentration of Pi (µmol) determined from the standard curve; B is assay time in minutes; C is reciprocal of the enzyme dilution factor.

Steady-state kinetic parameters K_m and k_{cat} for ATP were measured at a concentration of 400 nM protein. The concentrations of ATP varied from 0.1 mM to 1 mM in these measurements. The K_m and k_{cat} values were derived by fitting data by the Michaelis-Menten equation.

RESULTS

Expression and purification of the BLM$^{642-1290}$ protein

Bacterias-induced expression, supernatant fluid, pooled fractions from chitin column, and pure enzyme solution were analyzed with 10% SDS-PAGE (Figure 1), respectively. The results showed that the molecular weight (MW) of the BLM$^{642-1290}$ recombinant protein was predicted to be 72 KDa in accordance with theoretical value and its purity exceeded 95%. The concentration of BLM$^{642-1290}$ recombinant protein obtained was 3.48 g/L, and around 2.0 - 2.5 mg of pure protein might be obtained from each gram of cell paste.

Identification of the BLM$^{642-1290}$ recombinant protein

Bacterias fluid induced by IPTG, supernatant fluid, pooled fractions from chitin column, and pure enzyme solution were analyzed by Western blotting (Figure 2). The results displayed that four samples had a single band and Mr was consonant with each other, which proved that the purified protein was the target protein and immune responses occurred with the 6 × his-tag rat monoclonal antibodies.

DNA-binding activity assays

The DNA-binding activity of BLM$^{642-1290}$ recombinant

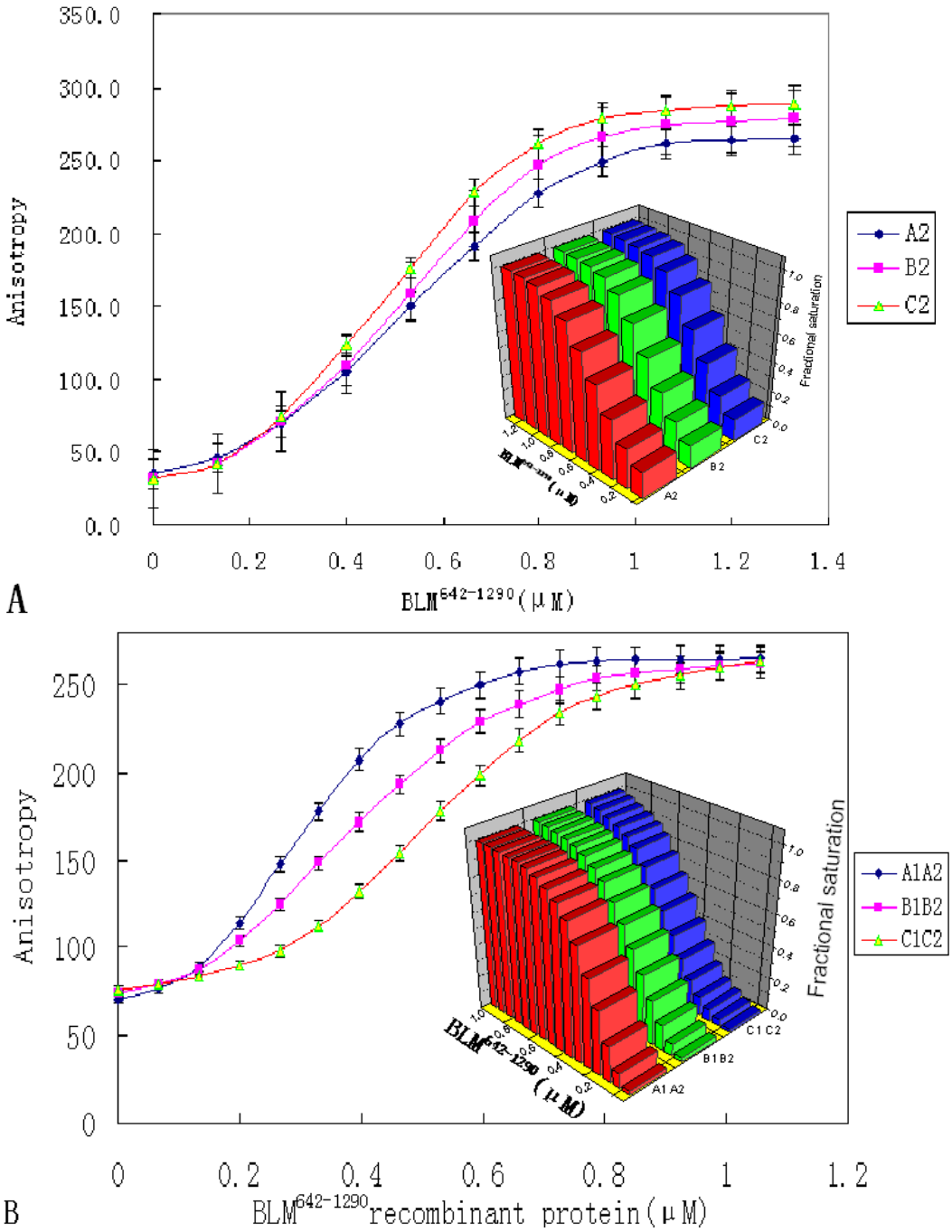

Figure 3. The binding curve of the BLM$^{642-1290}$ recombinant protein with different lengths DNA. Fluorescence anisotropy values were determined as a function of BLM concentration for different lengths of ssDNA (A) and dsDNA (B) substrates. Experiments were performed in DNA-binding activity assay buffer (20 mM Tris-HCl, pH 7.5) at 25℃. A total of 2 nM fluorescein-labelled DNA was titrated with increasing amounts of BLM$^{642-1290}$ recombinant protein. Steady-state fluorescence anisotropy versus the fractional saturation of BLM$^{642-1290}$ recombinant protein (see the staircase chart). The degree of saturation was estimated directly from the plateau region of the titration curve. At saturation, the moles of BLM$^{642-1290}$ recombinant protein binding to DNA are equal to the 2nM different lengths fluorescein-labeled DNA, assuming that no protein-DNA complexes precipitations were formed.

protein was analyzed by fluorescence polarization technology (Figure 3). It showed that the protein could bind with different lengths of ssDNA (Figure 3A) and dsDNA (Figure 3B), however, the amounts of the protein that

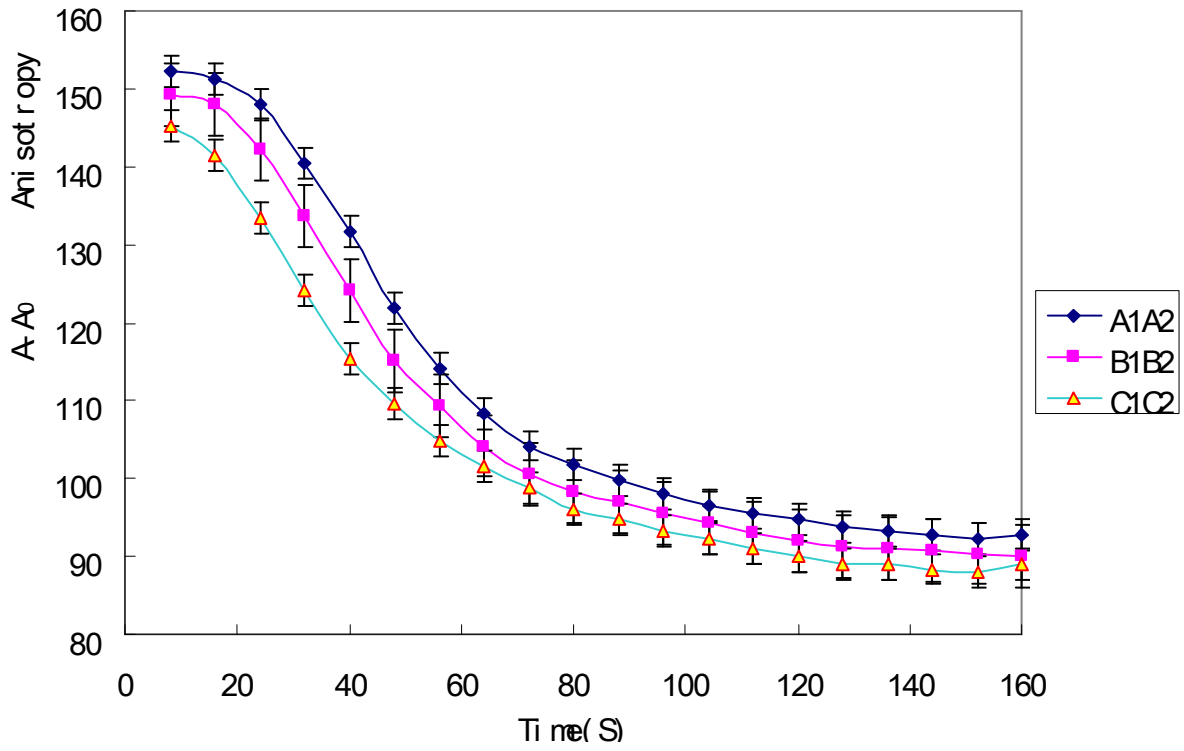

Figure 4. The detection of the DNA unwinding activity of the BLM$^{642-1290}$ recombinant protein. DNA unwinding activity by fluorescence polarization assay. Note: A was the fluorescence anisotropy at time t and A_0 was the fluorescence anisotropy in the presence of the DNA. As described in the 'MATERALS and METHODS' section, 2 nM different lengths dsDNA substrate was pre-incubated with 50 nM BLM$^{642-1290}$ recombinant protein for 5 min at 25°C. The unwinding reaction was added 1 mM ATP.

saturate 2 nM DNA were very different. The fractional saturation of the protein was derived from the anisotropy determined at each concentration during titration (staircase chart), indicating that the fractional saturation was markedly different between the two kinds of DNA substrates. No significant difference in the fractional saturation of the protein bounded with three lengths of ssDNA was investigated, conversely an obvious difference existed when the substrates were dsDNA. The differences were assayed via analysis dissociation constants (apparent K_d values) for two kinds of DNA substrates under equilibrium conditions. The dissociation constants (K_d) of either ssDNA or dsDNA as the substrates were 1.2 ± 0.2 or 25.7 ± 2.4 μM, respectively, suggesting that the BLM$^{642-1290}$ recombinant protein could bind stably with ssDNA or dsDNA. The affinity between the protein and ssDNA was 20 times higher than that with the dsDNA, which indicated that the ability of the protein-binded ssDNA molecules was stronger than dsDNA molecules.

Helicase activity assays

The helicase activity of BLM$^{642-1290}$ recombinant protein was tested using fluorescence polarization technology (Figure 4). The substrates were 2 nM of dsDNA with three different lengths of 3′overhang including 63:14-mer (A_1A_2), 45:21-mer (B_1B_2) and 56:36-mer (C_1C_2). It demonstrated that the fluorescence anisotropy of protein-dsDNA complexes was declined with time in the presence of ATP, which indicated that BLM$^{642-1290}$ recombinant protein could unwind three different lengths of 3′ overhanged dsDNA in the presence of ATP. The helicase activity was no great difference when three lengths of dsDNA as the substrates. Moreover, the anis-otropies just declined to stable values because the protein could bind with the ssDNA.

ATPase activity assays

The ATPase activity of BLM$^{642-1290}$ recombinant protein was examined by ultraviolet spectrophotometry based on PiColorLock Gold reagent (Figure 5). BLM is a DNA stimulated ATPase and an ATP-dependent helicase. The amounts of Pi were generally increased with the time (Figure 5A), which indicated that the ability of the protein catalyzed ATP hydrolysis was time-dependent. Besides, the ATPase activity of the protein was closely related to

the length of ssDNA, indicating that the longer the length of ssDNA cofactors were, the higher the ATPase activity was. Furthermore, the $A_{activity}$ of the $BLM^{642-1290}$ recombinant protein was also generally declined with time via analysis of $A_{activity}$ (Figure 5B). The K_m and k_{cat} for ATPase were measured at a protein concentration of 400 nM and

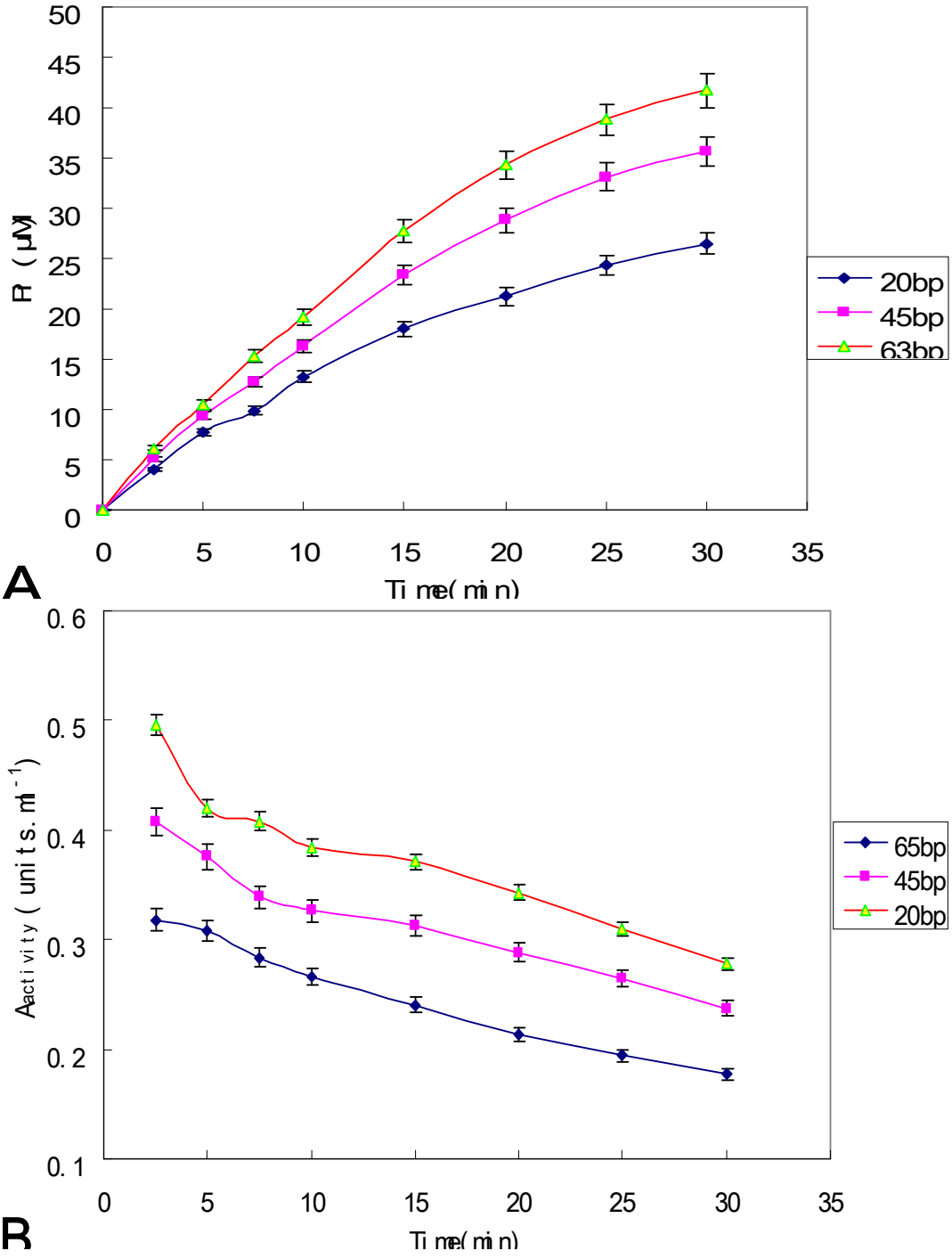

Figure 5. The detection of the ATPase activity of the $BLM^{642-1290}$ recombinant protein. A: Time course of ATP hydrolysis by 400 nM $BLM^{642–1290}$ recombinant protein in the presence of 63-mer, 45-mer, and 20-mer ssDNA. B: Curves of $A_{activity}$ that based on the equation (3) of $BLM^{642–1290}$ recombinant protein in the same conditions. In each reaction, DNA was present at a concentration of 2 nM and ATP was 500 μM. Experiments were performed at 25°C in ATPase activity assay buffer containing 50 mM Tris-HCl (pH 7.5), 2.5 mM $MgCl_2$.

were summarized in Table 2. No remarkable change in K_m of the protein was gained when three lengths ofssDNA were used as the cofactors. However, the V_{max} and K_{cat} were closely related to the length of ssDNA, increased with the length of ssDNA cofactors, which proved the BLM protein was a DNA-dependent helicase.

Table 2. Properties of ATPase activity of $BLM^{642-1290}$ recombinant protein.

$BLM^{642-1290}$ (mer)	V_{max}μM/min	K_mμM	K_{cat} s^{-1}	K_{cat} / $K_m$$s^{-1}$. mM^{-1}
63	146.2 ± 25.6	187.6 ± 2.4	6.08 ± 1.7	32.4 ± 2.0
45	124.3 ± 20.5	185.4 ± 3.8	5.17 ± 1.5	27.9 ± 1.6
20	95.4 ± 15.3	186.9 ± 2.1	3.98 ± 1.2	21.3 ± 1.3

DISSUSSION

In this study, we have characterized recombinant core region (amino acid residues 642-1290) of BLM helicase including the DEAH, RecQ-Ct and HRDC domains. Firstly, the $BLM^{642-1290}$ recombinant protein was expressed in *E. coli* induced by 0.45 mM IPTG, purified by the ion-change chromatography and FPLC size exclusion chromatography, consequently the target protein with the purity of more than 95% was obtained. Target protein of 72 KDa with higher purity was consistent with previous report by Janscak et al. (2003). Rather, biological activity of recombinant protein including DNA-binding activity and helicase activity were confirmed by fluorescence polarization assay. Additionally, ATPase activity was further confirmed using the ultraviolet spectrophotometry *in vitro*. Core region of the BLM helicase is an important part of the RecQ helicase family members (Bachrati and Hickson, 2008). Undoubtedly, biological activity of this core region was very important for the whole BLM helicase. Results verified the region had biological activity for DNA-binding, DNA unwinding and ATPase activity, which suggested that amino acid residues 642 - 1290 still retained the enzymatic activities as the full-size BLM in this study.

In order to obtain enough BLM protein and understand its biological activity *in vitro*, we have established an effective prokaryocyte expression method based on Guo's description (Guo et al., 2007). Our methods yielded typically 2 - 2.5 mg of pure protein per 1 g of cell paste, which was higher than previous study (Janscak et al., 2003). This method shortened the purification period and reduced the damage of protein in contrast with Guo's methods, which was contributed to three gradients elution of imidazole including 5, 60 and 500 mM in ion-change chromatography. The whole process just took five hours, contrarily, the Guo's methods employing five linear gradients of imidazole and several pH gradients take at least seven hours (Guo et al., 2007; Bugreev et al., 2007; Ren et al., 2007). Besides, the whole process was monitored by the FPLC which was completely self-driven instead of the man-made operation, resulting in the reduced the external destruction to the protein.

The property of molecules fluid and reaction property of basic groups of ssDNA were diversified depending on dsDNA. In comparison with dsDNA, the ssDNA has more hydrogen ions (H^+) or hydroxyl ions (OH^-) exposed to the reaction buffer than the dsDNA, which might increase the affinity between the protein and ssDNA. In addition, mutagenesis and structural studies of a number of other RecQ family helicase members demonstrated that DEAH domain are responsible for coupling of ATP hydrolysis to DNA binding, translocation and unwinding (Rigu et al., 2008; Vindignil and Hickson, 2009). A recent NMR structure of the HRDC domain of Sgs1 (the single RecQ homologue in *Saccharomyces cerevisiae*) suggested that this domain might function as an auxiliary DNA binding domain (Bernstein et al., 2009). The role of the RecQ-Ct domain in the helicase remains to be determined (Satoru et al., 2009), however the C-terminal region lacked catalytic activity which was essential for the maintenance of chromosomal stability and nucleolar localization in human cells (Popuri et al., 2009). According to the above reasons, it can be concluded that the DEAH domain was easier to bind with ssDNA than that with dsDNA and coupling of ATP hydrolysis in the ssDNA reaction buffer under the assistance of the HRDC domain. Because the BLM protein is DNA-dependent helicase, that is, the catalyze ATP hydrolysis must be in the presence of the DNA (Yodh et al., 2009). The longer the length of ssDNA cofactors were, the more H^+ or OH^- were exposed to the reaction buffer, the easier the protein bound to the longer ssDNA cofactors, the higher the ATPase activity was.

It is noteworthy that interaction between BLM helicase and DNA plays an important role in regulating functions of cellular and virus in the body. The mechanisms of how BLM helicase and DNA interact with each other to control transcription and replication of somatic cells are gradually being elucidated. At present, there are lots of methods to research on DNA-helicase interaction such as gel-shift assays, foot printing and so on**,** each of which may contribute distinct information about the effect of interaction, but they failed to provide a practically easy way to quantitatively analyze the interaction (Lawrence et al., 2008). However, fluorescence polarization used in present study was proved to be a more rapid, more accurate, and non-radioactive method than other methods to measure directly the interaction between DNA and protein in solution (Guo et al., 2005; Liu et al., 2007;

Yang et al., 2010), revealing that this method may be used to investigate on DNA-helicase interactions. Furthermore, we studied the DNA-binding activity and DNA

helicase activity of $BLM^{642–1290}$ recombinant protein using fluorescence polarization, which may be significant to provide a better understanding on BLM protein and facilitate the elucidation of the mechanism of pathopoiesia in Bloom's Syndrome.

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