Analytical and Bioanalytical Chemistry
https://doi.org/10.1007/s00216-019-01987-9

RESEARCH PAPER

Surface plasmon resonance based analysis of the binding of LYAR protein to the rs368698783 (G>A) polymorphic Aγ-globin gene sequences mutated in β-thalassemia

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Received: 15 May 2019 / Revised: 12 June 2019 / Accepted: 17 June 2019

Abstract

Recent studies have identified and characterized a novel putative transcriptional repressor site in a 5′ untranslated region of the Aγ-globin gene that interacts with the Ly-1 antibody reactive clone (LYAR) protein. LYAR binds the 5'-GGTTAT-3' site of the Aγ-globin gene, and this molecular interaction causes repression of gene transcription. In β-thalassemia patients, a polymorphism has been demonstrated (the rs368698783 G>A polymorphism) within the 5′-GGTTAT-3′ LYAR-binding site of the Aγ-globin gene. The major results gathered from surface plasmon resonance based biospecific interaction analysis (SPR-BIA) studies (using crude nuclear extracts, LYAR-enriched lysates, and recombinant LYAR) support the concept that the rs368698783 G>A polymorphism of the Aγ-globin gene attenuates the efficiency of LYAR binding to the LYAR-binding site. This conclusion was fully confirmed by a molecular docking analysis. This might lead to a very important difference in erythroid cells from β-thalassemia patients in respect to basal and induced levels of production of fetal hemoglobin. The novelty of the reported SPR-BIA method is that it allows the characterization and validation of the altered binding of a key nuclear factor (LYAR) to mutated LYAR-binding sites. These results, in addition to theoretical implications, should be considered of interest in applied pharmacology studies as a basis for the screening of drugs able to inhibit LYAR–DNA interactions. This might lead to the identification of molecules facilitating induced increase of γ-globin gene expression and fetal hemoglobin production in erythroid cells, which is associated with possible reduction of the clinical severity of the β-thalassemia phenotype.

Keywords β-Thalassemia · Fetal hemoglobin · γ-Globin gene polymorphism · Ly-1 antibody reactive clone · Surface plasmon resonance

Published in the topical collection *New Developments in Biosensors* with guest editors Francesco Baldini and Maria Minunni.

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Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00216-019-01987-9) contains supplementary material, which is available to authorized users.

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Published online: 12 July 2019

Abbreviations

HbF	Fetal hemoglobin
HEPES	*N*-(2-Hydroxyethyl) piperazine-*N*′-ethanesulfonic acid
LYAR	Ly-1 antibody reactive clone
ODN	Oligodeoxyribonucleotide
poly(dI-dC)	Poly(deoxyinosinic-deoxycytidylic) acid
SPR-BIA	Surface plasmon resonance based biospecific interaction analysis
UTR	Untranslated region

Introduction

The β-thalassemias are a large group of hereditary hematological diseases caused by more than 300 mutations of the human β-globin gene, leading to low or absent production of adult β-globin and excess of α-globin content in erythroid cells [1]. This altered globin gene expression causes ineffective erythropoiesis and low or absent production of adult hemoglobin (hemoglobin A) [1–3]. The conventional treatment of patients affected by severe forms of β-thalassemia is based on regular blood transfusions and chelating therapy [4]. At present, the only treatment that can be considered a cure for β-thalassemia is transplantation of hematopoietic stem cells (bone marrow transplantation) [5]. Alternatively, growing evidence supports the concept that induction of γ-globin gene expression and increased production of fetal hemoglobin (HbF) might be of great interest for the development of therapeutic protocols for β-thalassemia [6–9], possibly obviating the need for blood transfusions in β-thalassemia patients [1–3].

In this context, several transcription factors are known that interact with elements of the γ-globin gene promoter, thereby controlling γ-globin gene expression in erythroid cells [10–18]. Several of them (e.g., MYB and BCL11A) are strong repressors of γ-globin gene transcription [19–22]. This issue is of great interest because the possible inhibition of these repressors might lead to the activation of γ-globin gene expression and increased production of HbF in erythroid cells [23, 24]. High HbF levels have been firmly demonstrated to reduce the clinical severity of the β-thalassemia phenotype [17, 25–27]. Several approaches have been used to study protein–DNA interactions, including chromatin immunoprecipitation assay based methods, electrophoretic mobility shift assays, DNAse footprinting, southwestern blotting, yeast one-hybrid assay, circular dichroism analysis, fluorescence resonance energy transfer techniques, atomic force microscopy, NMR analysis, and surface plasmon resonance based biospecific interaction analysis (SPR-BIA) [28]. No data are available on the possible use of SPR-BIA for studying transcription repressors of γ-globin gene expression (most of them working in multiprotein complexes).

Ju et al. [29] demonstrated that the Ly-1 antibody reactive clone (LYAR) protein is a novel putative repressor of γ-globin gene transcription [29]. This zinc-finger transcription factor binds to the 5'-GGTTAT-3' LYAR-binding site of the 5′ untranslated region (UTR) of the human Aγ-globin gene and exhibits a repressor function in K562 cells.

One of the possible effects of the Aγ-globin gene G>A mutation (corresponding to the rs368698783 G>A polymorphism) is a decrease of the efficiency of LYAR binding to the 5′-GGTTAT-3′ binding site. In this respect, we found that the rs368698783 G>A polymorphism is present in β-thalassemia patients [30, 31]. Moreover, Chen et al. [9] further characterized the role of LYAR in Aγ-globin gene transcription. Chromatin immunoprecipitation assays and methylation pattern studies resulted in the proposal that the rs368698783 G>A polymorphism triggers the attenuation of binding of LYAR to the Aγ-globin gene, as well as of other two repressive epigenetic regulators, DNA methyltransferase 3α and protein arginine methyltransferase 5. This mediates increase of γ-globin gene transcription by facilitating demethylation of CpG sites in erythroid progenitor cells from β-thalassemia patients [9]. The repressor function of LYAR with respect to γ-globin gene transcription might therefore explain the increased basal and induced levels of HbF in erythroid cells [9, 29–31].

The present study, using SPR-BIA and a Biacore biosensor, was undertaken to determine whether the rs368698783 G>A polymorphism present in the 5′ UTR of the Aγ-globin gene and in β-thalassemia patients alters the binding of LYAR and LYAR complexes to the target DNA sequences. To this aim, double-stranded oligodeoxyribonucleotides (ODNs) containing the LYAR-binding sites were immobilized on the surface of a sensor chip of a Biacore biosensor, and crude K562 nuclear extracts, lysates from LYAR-overexpressing HEK293T cells, and recombinant LYAR were injected.

Experimental

K562 nuclear extracts and western blotting

K562 cells were purchased from the American Tissue Culture Collection (ATCC CCL-243). Nuclear extracts were prepared as described elsewhere [32]. K562 cells that had been washed with phosphate-buffered saline were lysed and nuclear proteins were extracted by on-ice incubation of the nuclei with 20 mM *N*-(2-hydroxyethyl)piperazine-*N*′-ethanesulfonic acid (HEPES)–KOH, pH 7.9, 25% glycerol, 420 mM NaCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 0.5 mM dithiothreitol, 0.2 mM phenylmethanesulfonyl fluoride, aprotinin (1 μg/ml), leupeptin (1 μg/ml), 2 mM Na_3VO_4, and 10 mM NaF (Sigma, St Louis, MO, USA) [32]. Western blotting was performed as described in [32] with 2 μg of rabbit

polyclonal anti-LYAR antibody (PA5-14213, Thermo Fisher, Rockford, IL, USA) (see the Electronic Supplementary Material (ESM) for an in-depth description).

SPR-BIA technology

Interactions of crude K562 nuclear extracts, lysates from LYAR-overexpressing HEK293T cells (LC428988, OriGene Technologies, Rockville, MD, USA), and recombinant LYAR (H00055646, Novus Biologicals, Littleton, CO, USA) with double-stranded DNA sequences were studied by SPR-BIA with a Biacore X100 system (Biacore, GE Healthcare) [33]. SA sensor chips (Biacore, GE Healthcare) precoated with streptavidin were used. SA sensor chips containing single-stranded normal or mutated LYAR ODNs were obtained following injection of 1 μM biotinylated LYAR ODNs in HBS-EP+ buffer (10 mM HEPES, 150 mM NaCl, 3 mM EDTA, 0.05% v/v surfactant P20, pH 7.4) (Biacore, GE Healthcare) onto the sensor chip surface to reach an immobilization level of about 700–800 resonance units (RU). The sequences of the biotinylated ODNs immobilized on the sensor chip were (+25)-Aγ-globin 5′-AAC GTC TGA GGT TAT CAA TAA GCT-3′ (nonmutated sequence), and MUT(+25)-Aγ-globin 5’-AAC GTC TGA GAT TAT CAA TAA GCT-3’ (mutated sequence, mutation underlined). Double-stranded target ODNs immobilized on the sensor chips were obtained by a 4-min injection of 2.5 μM complementary ODNs diluted in HBS-EP+ buffer into the flow cell containing the biotinylated single-stranded LYAR ODNs. HBS-EP+ buffer was used as a running buffer as well. To study protein interactions with the immobilized double-stranded LYAR ODNs, K562 nuclear extracts and lysates from LYAR-overexpressing HEK293T cells were used. In this case poly(deoxyinosinic-deoxycytidylic) acid [poly(dI-dC)] was used to decrease unspecific binding, as reported elsewhere [34]. We compared the effects of poly(dI-dC) at different concentrations (1.5, 3, 6, 12, and 18 ng/μl) in SPR-BIA and electrophoretic mobility shift assay experiments and found that 6 ng/μl was able to strongly inhibit nonspecific binding in the SPR-BIA settings (data not shown). K562 nuclear extracts and LYAR-overexpressing HEK293T lysates were incubated with poly(dI-dC) for 5 min at room temperature before injection into TFB binding buffer [50 mM KCl, 20 mM tris(hydroxymethyl)aminomethane hydrochloride, pH 7.5, 1 mM $MgCl_2$, 0.2 mM EDTA, 0.01% Triton X-100]. To study LYAR interactions with the immobilized double-stranded LYAR ODNs, recombinant LYAR was injected (70 μl LYAR, 10 μg/ml) into binding buffer. To determine the stability of protein–DNA complexes, binding buffer was injected. The chips were regenerated with a 30-s injection of 1 M NaOH. All procedures were performed at 25 °C and a flow rate of 5 μl/min. The differences between the final resonance units after the injection binding phase (RUfin) and the initial resonance units before the injection binding phase (RUi) were taken as an indication of the overall binding of proteins to the immobilized double-stranded LYAR ODNs. The differences between the final resonance units after the injection binding phase and the residual resonance units after the washing step were taken as an indication of the stability of the protein–DNA complexes.

Bioinformatic and molecular docking analyses

The computational studies were performed with a four-CPU (Intel Core 2 quad-core CPU Q9550, 2.83 GHz) ACPI x64 Linux workstation with the Ubuntu 12.04 operating system [35] as reported in detail by Bianchi et al. [30]. The DNA sequences with LYAR-binding features (5′-ACGT CTGAGGTTATCAATAAGC-3′, 5′-GTCTGAGGTTATCA ATAA-3′, and 5′-GAGGTTATCA-3′) were built with the 3D-DART modeling server [35, 36]. The solution structure of the N-terminal zinc-finger domain of LYAR (NMR spectroscopy; Protein Data Bank ID 1WJV) was downloaded from the Protein Data Bank. The protein was derived from *Homo sapiens* (sequence MVFFTCNAC GESVKKIQVE KRVSVCRNCE CLSCIDCGKD FWGDDYKNHV KCISEDQKYG GKGYEGK). The docking procedures have been reported elsewhere [37–39]. Gromacs was used for energy minimization of the selected binding poses [36], and PyMOL was used for analysis of the binding mode [38] (see the ESM for an in-depth-description of these procedures).

Results

In β-thalassemia patients the LYAR-binding site is G>A mutated at nucleotide +25 of the Aγ-globin gene, and corresponds to the rs368698783 polymorphic site.

The Aγ-globin gene was sequenced with use of genomic DNA from β-thalassemia patients, and only one mutation was found at the level of LYAR-binding sites (i.e., a G>A mutation located at +25 [30, 31] and corresponding to the known rs368698783 G>A polymorphism). This mutation was not present in the Gγ-globin genes [30]. In addition, our data excluded additional mutations in the LYAR-binding sites [30]. The frequency of this mutation was higher in patients carrying a β^0-thalassemia genotype (β^0-39) with respect to the β^+-thalassemia genotype (e.g., β^+-IVSI-110) in a cohort of patients recruited at S. Anna Hospital (Ferrara, Italy) [31]. With respect to the overall frequency in β-thalassemia patients, although most of them carry a G/G genotype (64.5%), 22.6% are heterozygous (G/A) and 12.9% are homozygous (A/A) for this G>A mutation. The location of the G>A (+25) Aγ-globin gene sequence and a representative sequencing of a β-thalassemia A/A homozygous patient are depicted in Fig. 1.

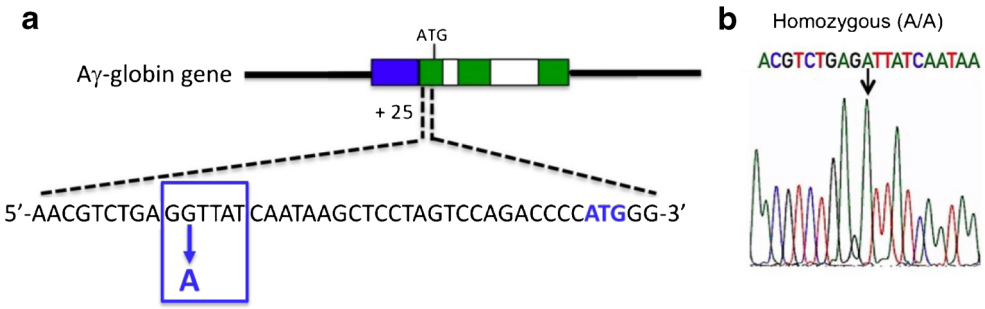

Fig. 1 **a** Location within the human Aγ-globin gene and nucleotide sequence of the rs368698783 G>A polymorphism. **b** Representative sequence of a β-thalassemia rs368698783 A/A patient

Functional effects of the Aγ-globin gene G>A mutation

The rs368698783 G>A Aγ-globin gene polymorphism belongs to the 5′-GGTTAT-3′ binding site of the zinc-finger transcription factor LYAR. Relevant to possible application in therapy for β-thalassemia, LYAR has been proposed as a repressor of γ-globin gene transcription in K562 cells [29]. In consideration of the transcription inhibitor function of LYAR, one of the possible effects of the Aγ-globin gene G>A mutation is a decrease of the efficiency of LYAR binding to the target 5′-GGTTAT-3′ binding site, as first hypothesized by Ju et al. [29], who demonstrated, using the CASTing (cyclic amplification and selection of targets) method, that LYAR directly binds to DNA and this binding activity is lower in the presence of point mutations of the consensus sequence. With the aim of verifying this possibility, two complementary approaches were followed: SPR-BIA and molecular docking of LYAR to LYAR-binding sites.

SPR-BIA study suggests a less efficient binding of unfractionated K562 nuclear extracts and of LYAR-enriched lysates to the MUT(+25) -Aγ-globin ODN

In the SPR-BIA experiments the binding of K562 nuclear extracts to sensor chips containing immobilized (+25)-Aγ-globin and MUT(+25)-Aγ-globin ODNs was studied without or with preincubation with poly(dI-dC) to compare overall binding and sequence-specific binding. Immobilization of biotin-labeled (+25)-Aγ-globin and MUT(+25)-Aγ-globin ODN probes in two flow cells of two different sensor chips was followed by a 4-min injection of 2.5 μM complementary ODNs. When K562 nuclear extracts were injected into sensor chip flow cells containing double-stranded (+25)-Aγ-globin and MUT(+25)-Aγ-globin ODNs without preincubation with poly(dI-dC), no major differences were, as expected, observed (see the representative experiment shown in Fig. 2a, left). In contrast, when preincubation with poly(dI-dC) was performed, a significant difference was found in the binding of K562 nuclear extracts to (+25)-Aγ-globin (solid lines) and MUT(+25)-Aγ-globin (dotted lines) sequences (Fig. 2a, right).

These results are compatible with the presence in the K562 nuclear extracts of proteins able to nonspecifically interact with the target immobilized DNA sequence. On the other hand, the differential binding of K562 nuclear proteins to (+25)-Aγ-globin (solid lines) and MUT(+25)-Aγ-globin (dotted lines) sequences might indicate that LYAR or LYAR-containing protein complexes might differentially bind to the rs368698783 G>A polymorphism of the Aγ-globin gene. This is a relevant result because practical procedures for measurement of the protein–DNA association in crude nuclear extracts are yet to be defined in several experimental systems because of the tendency of the analyzed DNA-binding protein(s) to generate multicomponent complexes with other proteins present in the extracts [40, 41]. Differential binding was confirmed also when LYAR-overexpressing HEK293T cell lysates were used (Fig. 2b). Therefore, to conclusively demonstrate the very interesting hypothesis of differential LYAR binding to the rs368698783 G>A polymorphism of the Aγ-globin gene, recombinant LYAR and an anti-LYAR antibody were used.

Less efficient binding of LYAR to the MUT(+25) -Aγ-globin ODN

When recombinant LYAR was injected into Biacore sensor chip flow cells containing double-stranded (+25)-Aγ-globin and MUT(+25)-Aγ-globin ODNs, a significant difference was found in the binding (Fig. 3).

We first demonstrated that the monoclonal antibody PA5-14213 was able to bind to LYAR by performing western blotting analysis. This is shown in Fig. 3a, which also gives a clear indication of the quality of the commercially available purified recombinant LYAR. Second, we demonstrated that preincubation of LYAR with PA5-14213 strongly inhibited the LYAR–DNA interactions with the immobilized double-stranded (+25)-Aγ-globin ODN (Fig. 3b, dotted line).

With regard to efficiency of binding, the results in Fig. 3b–d clearly demonstrate that the binding to the MUT(+25)-Aγ-globin ODN (Fig. 3c, dotted line) was much less efficient than the binding to the (+25)-Aγ-globin ODN (Fig. 3b, solid line). A summary of the results of the experiments performed with purified LYAR is reported in Fig. 3d.

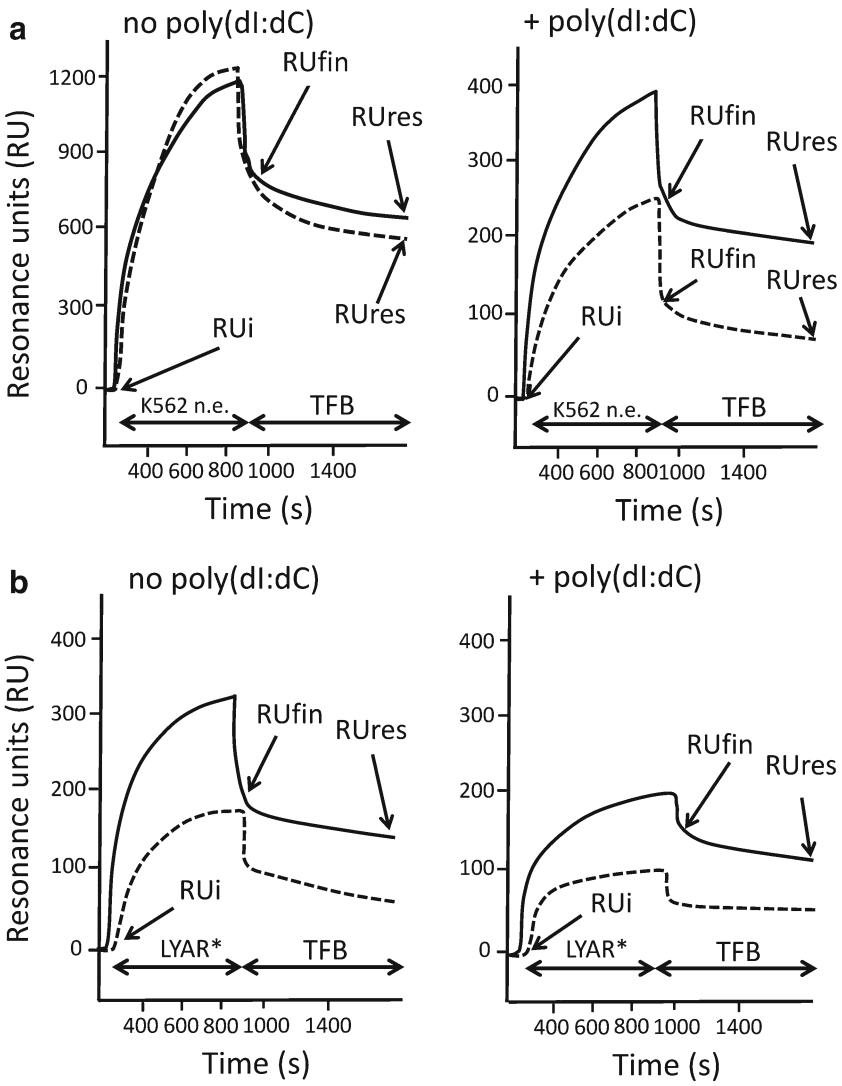

Fig. 2 Surface plasmon resonance based biospecific interaction analysis of the binding of **a** K562 nuclear extracts (n.e.) and **b** lysates from Ly-1 antibody reactive clone (LYAR)-overexpressing HEK293T cells (LYAR*) to (+25)-Aγ-globin ODN (solid lines) and MUT(+25)-Aγ-globin ODN (dotted lines) immobilized on the sensor chip. The binding experiments were conducted in the absence (left) or in the presence (right) of poly(dI-dC) (6 ng/μl). RUfin final resonance units, RUi initial resonance units, RUres residual resonance units, TFB binding buffer

To confirm these results and to obtain information on the biochemical basis of the different interaction efficiencies (Fig. 3b,c) docking analyses were performed.

Molecular docking studies confirm the less efficient binding of LYAR to the MUT(+25)-Aγ-globin ODN

Molecular docking simulations confirmed marked electrostatic and shape complementarities between LYAR and the DNA consensus sequence 5′-GGTTAT-3′ as previously reported by Bianchi et al. [30]. The following double-stranded sequences were used (only sense strands are indicated): 5′-ACGT CTGAGGTTATCAATAAGC-3′ (Fig. 4a), 5′-GTCT GAGGTTATCAATAA-3′ (Fig. 4b), and 5′-GAGGTTATCA-3′ (Fig. 4c). The LYAR conformations were confirmed for all the target oligonucleotide sequences used, suggesting that the 5′-GAGGTTATCA-3′ oligonucleotide sequence is sufficient to allow efficient LYAR–DNA recognition.

The docking simulations (Fig. 4c) suggested the following amino acid residues (Fig. 4d) are key players in LYAR–DNA interaction: (a) Met1, Lys15, and Ile16, which have hydrophobic interactions with the methyl group of the thymine in the complementary sequence of the GGTTAT LYAR-binding site; (b) Lys14, Ile16, and Gln17, which through their side chains generate a cleft accommodating the methyl functions of the GGTTAT sequence; (c) the ammonium ion of the Lys20 side chain, which interacts with N7 and with the carbonyl of the GGTTAT sequence and the carbonyl of the GGTTAT sequence. These amino acid residues are shown in Fig. 4d and are conserved throughout evolution (unpublished results).

The G>A mutation, generating a GATTAT sequence (mutation underlined), does not support the generation of one of the hydrogen bonds (Fig. 4e), suggesting that the rs368698783 G>A Aγ-globin gene polymorphism might be associated with a decreased efficiency of LYAR–DNA

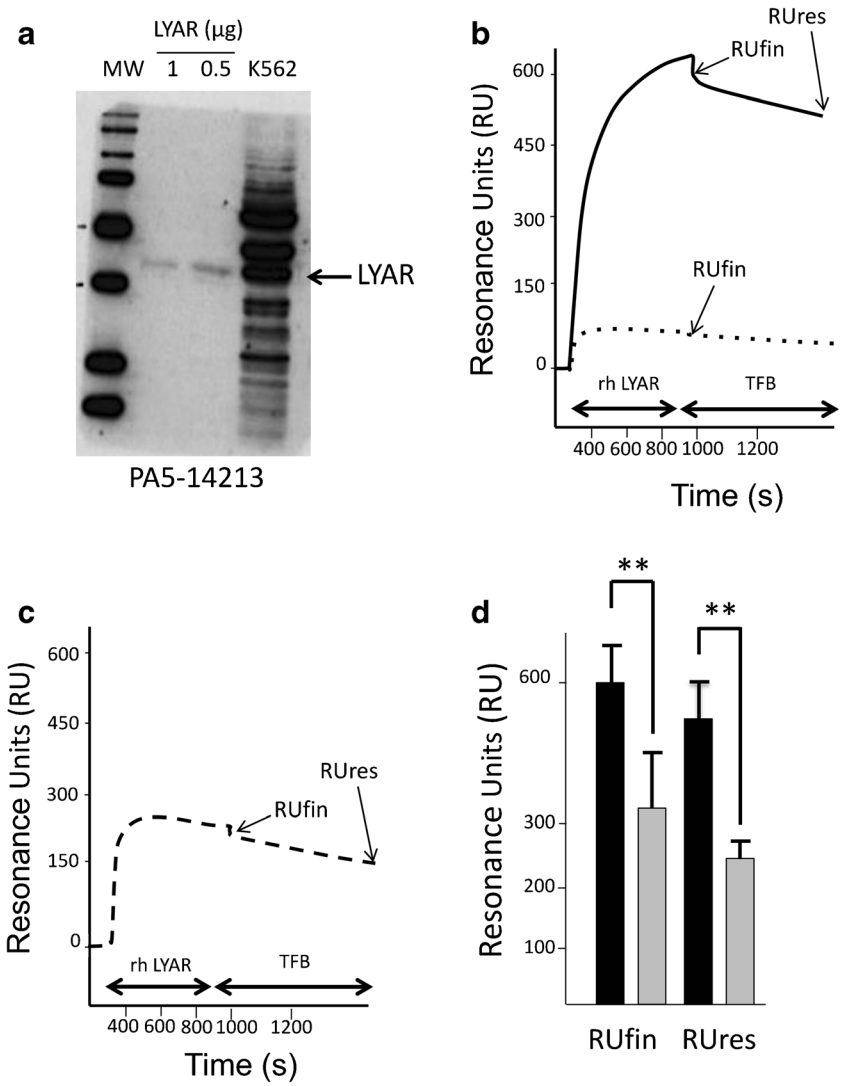

Fig. 3 **a** Western blotting using purified Ly-1 antibody reactive clone (LYAR) and K562 nuclear extracts and the PA5-14213 anti-LYAR monoclonal antibody. **b–d** Surface plasmon resonance based biospecific interaction analysis of the binding of purified LYAR (10 ng/μl) to (+25)-Aγ-globin ODN (solid lines, **b**; black histogram, **d**) and MUT(+25)-Aγ-globin ODN (dotted lines, **c**; gray histogram, **d**). In **b** the effect of pretreatment with PA5-14213 monoclonal antibody on LYAR–DNA interactions is also shown (dotted line). In **d** a quantitative summary of the effects of the G>A mutations is reported. The data represent the average ± standard deviation of three independent binding experiments. MW molecular weight, rh recombinant human, RUfin final resonance units, RUres residual resonance units, TFB binding buffer. ** $p < 0.01$, highly significant statistic difference

recognition. Figure 4f reports the docking pattern obtained with the human LYAR sequence and the mutated 5′-GAGATTATCA-3′ double-stranded sequence (mutation underlined). The mode of binding of human LYAR with 5′-GAGATTATCA-3′ DNA indicates low distortion of the DNA geometry as a consequence of the unspecific (mostly electrostatic) interaction with LYAR.

The electrostatic contributions to the binding were comparable for both 5′-GAGGTTATCA-3′ and mutated G>A 5′-GAGATTATCA-3′ sequences (-1225 kcal/mol for DNA–LYAR; -1350 kcal/mol for G>A DNA–LYAR). Conversely, the van der Waals energies were very different (-320 kcal/mol for DNA–LYAR; -16 kcal/mol for G>A DNA–LYAR), further confirming that the nonmutated DNA sequence showed a more favorable and stable interaction with LYAR. Together with the analysis depicted in Fig. 4e, these in silico analyses strongly support the concept that LYAR might interact with lower efficiency with the mutated Aγ-globin gene LYAR-binding sequences.

Discussion

Recent studies have identified and characterized a novel putative repressor site of Aγ-globin gene transcription in a region corresponding to the 5′ UTR, recognized by LYAR [29]. The conclusion of these studies was that LYAR binds to the 5′-GGTTAT-3′ binding site of the Aγ-globin gene promoter and that this molecular interaction causes a transcriptional repression of γ-globin expression. In β-thalassemia patients, a polymorphism has been demonstrated (rs368698783 G>A polymorphism) within the 5′-GGTTAT-3′ LYAR-binding site of the Aγ-globin gene [30, 31]. One of the possible effects of

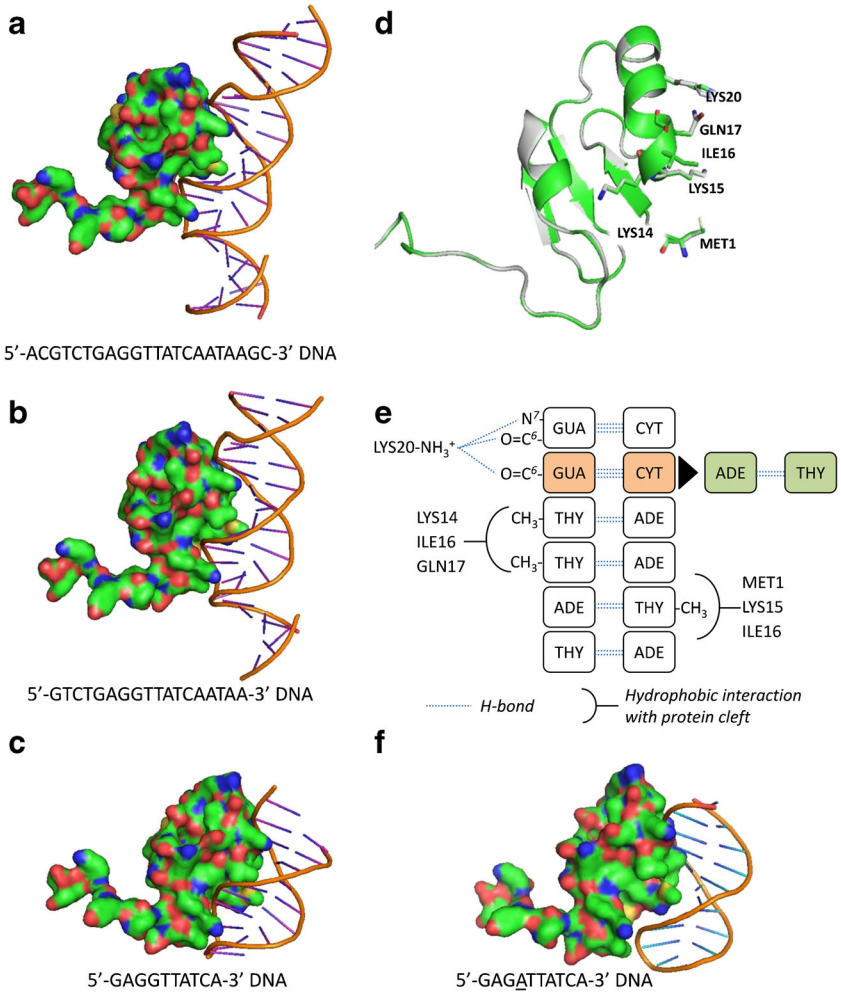

Fig. 4 **a**–**c** Molecular docking simulations using the following double-stranded sequences (only sense strands are indicated): 5′-ACGTCTGAGGTTATCAATAAGC-3′ (**a**), 5′-GTCTGAGGTTATCAATAA-3′ (**b**), and 5′-GAGGTTATCA-3′ (**c**). The Ly-1 antibody reactive clone (LYAR) conformations were confirmed for all the target oligonucleotide sequences used. **d** Identification of the amino acid sequences involved in the protein–DNA interactions shown in **c**. **e** Interactions of the amino acid residues with the 5′-GGTTAT-3′ core sequences of the LYAR-binding sites present in the 5′ region of the human Aγ-globin gene. **f** Molecular docking simulations using the mutated 5′-GAGATTATCA-3′ double-stranded sequences (only sense strands are indicated; G>A mutation is underlined). ADE adenine, CYT cytosine, GUA guanine, THY thymine

the Aγ-globin gene G>A mutation is a decrease of the efficiency of LYAR binding to the 5′-GGTTAT-3′ binding site.

The present study was undertaken to determine whether the mutations in this Aγ-globin gene region and elsewhere reported to be present in β-thalassemia alter the binding of LYAR and LYAR complexes to the target DNA sequences by a Biacore analysis of these molecular interactions. The major result obtained from the SPR-BIA studies fully supports the hypothesis that the rs368698783 G>A polymorphism of the Aγ-globin gene attenuates the efficiency of LYAR binding to the LYAR-binding site. This might lead to a very important difference among erythroid cells from β-thalassemia patients with respect to basal and induced levels of HbF production. Molecular docking simulations of the interaction between LYAR, the DNA target sequence, and the mutated (G>A) sequence further supported the SPR-BIA data.

These results are of interest because they support the hypothesis that LYAR is a major player in γ-globin gene regulation in β-thalassemia. Recently published studies have demonstrated that several transcription factors interact with several elements of the γ-globin gene promoter to control γ-globin gene expression in erythroid cells [9–12]. For instance, several of them (e.g., BCL11A) are (as LYAR) strong repressors of γ-globin gene transcription [17–22]. This issue is of interest for the development of possible therapeutic protocols because the conclusions reached predict that the possible inhibition of these repressors might lead to the activation of γ-globin gene expression and production of HbF in erythroid cells [6].

With regard to the novelty of our work, although SPR-BIA has been extensively used to study interactions between transcription factors and DNA [42–45], no reports are available on SPR-BIA studies on transcriptional repressors (BCL11A, Krüppel-like factor 1, MYB) of γ-globin gene expression. To the best of our knowledge, this is the first report showing the usefulness of SPR-BIA to study the effects of gene mutations (such as the rs368698783 G>A polymorphism of the Aγ-globin gene) on the binding efficiency of specific nuclear proteins (in our case LYAR) relevant to human genetic diseases (in our case β-thalassemia). Our data show that these effects can be studied by SPR-BIA even with crude nuclear extracts, after the reduction of the nonspecific protein–DNA

interactions by incubation of the proteins to be analyzed with poly(dI-dC) before injection onto the sensor chip functionalized with immobilized ODNs carrying the LYAR-binding sites, either nonmutated or mutated.

The results presented in this study are relevant not only from the theoretical viewpoint but might also be of interest in applied pharmacology. The method developed and validated here might be used for the screening of drugs able to inhibit LYAR–DNA interactions, thereby activating γ-globin gene transcription, as done with SPR-BIA in different experimental systems [46–49]. In this context, SPR-BIA has been extensively used in studies focused on the effects of low molecular weight drugs able to interfere with protein–DNA interaction. These studies would be relevant for the design of therapeutic protocols for β-thalassemia, since increase of HbF production has been firmly demonstrated to reduce the clinical severity of β-thalassemia [7, 8]. Furthermore, our study strongly supports the interest in determining the rs368698783 G>A polymorphism in β-thalassemia patients and in verifying possible use to predict HbF production as well as response to HbF inducers. This might have a clear impact in the recruitment of β-thalassemia patients in clinical trials.

Acknowledgements RG is funded by Fondazione Cassa di Risparmio di Padova e Rovigo, Consorzio Interuniversitario per le Biotecnologie, the UE THALAMOSS Project (Thalassemia Modular Stratification System for Personalized Therapy of Beta-Thalassemia; no. 306201-FP7-HEALTH-2012-INNOVATION-1), the Wellcome Trust (innovator award 208872/Z/17/Z) and AIFA (AIFA-2016-02364887). This research was also supported by Associazione Veneta per la Lotta alla Talassemia, Rovigo.

Compliance with ethical standards

No violation of human rights occurred during this investigation. The collection and processing of the human biological samples for this research were approved by the Ethics Committee of Ferrara District, number 06/2013 (approved on June 20, 2013). The study complies with the Declaration of Helsinki, the principles of good clinical practice, and all further applicable regulations. All samples of peripheral blood were obtained after written documentation of informed consent from patients or their legal representatives. Copies of the written consent were collected for archiving by "Day Hospital for Thalassemia and Hemoglobinopathies, S. Anna Hospital, (Ferrara, Italy). Consent to submit this article was received from all coauthors.

Conflict of interest The authors declare that they have no competing interests.

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