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Impaired binding of AHSP to α chain variants: Hb Groene Hart illustrates a mechanism leading to unstable hemoglobins with α thalassemic like syndrome

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Abstract

Alpha hemoglobin stabilizing protein (AHSP) is a small protein of 102 residues induced by GATA-1, Oct-1- and EKLF. It is synthesized at a high level in the red blood cell precursors and acts as a chaperone protecting the alpha hemoglobin (α-Hb) chains against precipitation. AHSP and α-Hb form a heterodimer complex. In the absence of AHSP, α-Hb oxidizes and precipitates within the erythrocyte precursors of the bone marrow leading to apoptosis and defective erythropoiesis. *In vitro* the binding of AHSP to ferrous α-Hb accelerates oxidation of the heme iron in α-Hb, but the complex is more resistant to protein unfolding.

AHSP could act as a modulating factor in beta-thalassemia. Recent studies showed more severe thalassemic syndromes in patients with decreased levels of AHSP and in one patient who carried a structurally abnormal AHSP.

Some α-Hb variants with structural abnormality located in the contact area between α-Hb and AHSP exhibit an instability and a thalassemic like syndrome. We suggest that this could result from a disturbed interaction between α-Hb variants and AHSP. To study this interaction, we constructed the pGEX-α-AHSP vector that co-expressed human α-Hb and AHSP. Using this approach, we investigated the α42 (C7), α104 (G11) and α119 (H2) sites, where variants with some thalassemic features have been described. Results obtained with recombinant Groene Hart α-Hb and Diamant α-Hb, in which proline 119 is replaced by a serine and a leucine, respectively, showed clearly an impaired interaction with AHSP. In contrast, the α mutants at the sites 42 and 104 exhibit a normal interaction with AHSP. The CO rebinding kinetics of the AHSP/α-$Hb^{42mutant}$ complexes were similar to those previously obtained with the AHSP/α-Hb^{WT} complex, which shows a modified rate that is intermediate to the classical Hb allosteric states.

Keywords: AHSP; Thalassemia; Chaperone; Hemoglobin variants; Recombinant hemoglobins

Introduction

Alpha hemoglobin stabilizing protein (AHSP) is a 102 residue-long molecule synthesized at a high concentration in the erythroid precursors [1]. It forms a three-helix bundle [2], which binds to α-hemoglobin (α-Hb), forming a heterodimer, with a Kd of approximately 100 nM, but does not associate with β hemoglobin (β-Hb) or HbA [1–3]. This interaction involves several residues of α-Hb located in, or near to, the α1β1 interface of the tetrameric hemoglobin. AHSP acts as chaperone protecting the free α-Hb chains, which are highly unstable molecular species. In the absence of AHSP, free α-Hb would spontaneously form hemichromes, which precipitate and generate reactive oxygen species (ROS) within the erythrocyte precursors of the bone marrow leading to apoptosis and ineffective erythropoiesis [4].

It has been suggested that AHSP might therefore play a role as a modifier in the clinical expression of β-thalassemia. This was indeed clearly demonstrated in a murine model where

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AHSP−/− mice were generated. When such AHSP knock-out mice were mated with β-thalassemic animals, an exacerbated thalassemic syndrome was observed [1]. It was therefore likely that a structural abnormality of AHSP altering its interaction constant with free α-Hb or a reduced availability of this chaperone molecule in the cell might increase the severity of thalassemic phenotype. Several studies were done in patients to confirm this point. Some were unable to find any association between AHSP haplotypes and thalassemia phenotypes [5]. In contrast, other studies explained a thalassemia intermedia phenotype by a decreased expression of this gene [6,7]. A recent study demonstrated that in healthy individuals AHSP expression varied up to threefold with a significantly higher level in homozygotes for a group of AHSP haplotypes, named clade A, compared with heterozygotes [7]. A reduced level of AHSP might, thus, explain a more severe clinical picture observed in some patients with thalassemia intermedia in which an ααα/αα genotype is involved [7]. In addition, a structurally abnormal AHSP has been recently reported in a thalassemia intermedia patient. In this case the AHSP structural modification was localized away from the interaction area and surface plasmon resonance showed that its binding affinity for α-Hb was normal. Its less effective protection against oxidative-mediated damage in the erythrocyte could perhaps result from some structural, or biochemical, changes of the chaperone protein itself [8].

The AHSP/α-Hb interaction could be altered by a structural abnormality located in either of the two partners. This interaction area involves helices 1 and 2 and the intervening segment in the AHSP molecule, and the G and H helices in α-Hb [9]. Abnormalities of AHSP should have an effect similar to that of an α-gene triplication. Some abnormalities of α-Hb may lead to the phenotypes observed in non-deletional α-thalassemias. In these cases the defect is not due to a decreased amount of available normal α-Hb but to the presence of an α-Hb variant displaying an impaired interaction with the partner β chain, hampering formation of a α1β1 dimer. This has been recently shown for two elongated α hemoglobin variants, Hb Constant Spring and Pakse [10]. The elongated region of these molecules lies in a region close to the α1β1 interface impairing therefore binding to AHSP.

In this paper we studied the AHSP/α-Hb interaction for several α-Hb variants for which a mechanism altering this function could be hypothesized. In a recent study, we have shown that soluble recombinant α-Hb could be produced at high yield as a complex with its chaperone [11]. To demonstrate the impaired AHSP/α-Hb we expressed together in *E. coli* the recombinant α-abnormal Hbs and AHSP and analyzed the consequence of the α mutations on the chaperone–target protein interaction. Using this approach, the α42 (C7), α104 (G11) and α119 (H2) sites were investigated. The carrier of Hb Barika [α42Tyr→His] presented with an unexplained α+-thalassemia phenotype [12]. Since binding of AHSP to α-Hb modifies the neighborhood of the heme, we looked for a possible altered function of this moderately increased oxygen affinity Hb variant. Patients with Hb Groene Hart (α119 Pro→Ser) presented an α-thalassemic phenotype [13,14], but the carrier of Hb Diamant (α119 Pro→Leu) was normal [15]. Patients with Hb Sallanches (α104Cys→Tyr) [16,17] and Hb Oegstgeest (α104 Cys→Ser), also display an α-thalassemic phenotype [18]. Among these structural modifications, we found that only those at position α119 Pro led clearly to an impaired AHSP/α Hb interaction.

Material and methods

Expression of mutant α-Hb with the chaperone

Previous work by our laboratory has detailed the construction of pGEX-α-AHSP for the expression of the AHSP/α-Hb wild type (α-Hb^{WT}) complex [11].

Plasmids which express the α119 Pro→Leu (Hb Diamant), α119 Pro→Ser (Hb Groene Hart), α104 Cys→Tyr (Hb Sallanches), α104 Cys→Ser (Hb Oegstgeest), α42 Tyr→His (Hb Barika) or α42 Tyr→Phe mutations were derived from pGEX-α^{WT}-AHSP with the QuickChange site-directed mutagenesis kit (Stratagene Europe, Amsterdam, the Netherland) and with appropriate oligonucleotides containing the expected codons. The different plasmids were checked by DNA sequencing (MWG Biotech, Roissy-en-France, France).

Expression and solubilization of different fusion proteins

The different pGEX-α-AHSP constructs were expressed in *E. coli* BL21 cells. Different glutathione *S*-transferase (GST) fusion proteins were expressed after induction by isopropyl β-thiogalactipyranoside (IPTG) at 0.2 mM at 37°C and supplemented with hemin (30 μg/ml) as described previously [11]. The bacterial cells were harvested by centrifugation. The pellets were resuspended in PBS (150 mM NaCl, 10 mM Na_2HPO_4, pH 7.4) containing 5 mM dithiothreitol and 1 g/l lysozyme. After 45-min incubation on ice, the cells were disrupted in a Sonifier II disrupter (Branson Ultrasonic, Carouge-Geneva, Switzerland). The sonicated solution was incubated in the presence of 1% Triton X-100 for 1 h at 4°C. The homogenate was then centrifuged at 14,000 rpm for 30 min at 4°C and the supernatant containing the complex of GST fusion proteins was withdrawn.

Purification of different fusion proteins

The different soluble fractions containing the complex of GST fusion proteins were mixed with Glutathione Sepharose 4B beads (Amersham Bioscience part of GE Healthcare, Uppsala, Sweden) for 1 h with a volume ratio of 4:1 (supernatant:bead) as previously described [11]. Briefly, the beads were then washed by 10 (bead) volumes of PBS. The different GST fusion proteins were eluted by the addition of 2 bead volumes of elution buffer (50 mM Tris–HCl, 20 mM reduced glutathione, pH 8.0).

Characterization of different mutated fusion proteins

Different aliquots were collected during expression, solubilization and after purification of recombinant proteins and