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The α-hemoglobin stabilizing protein and expression of unstable α-Hb variants

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Abstract

Objectives: To determine the role of the alpha-hemoglobin stabilizing protein (AHSP) in the clinical expression of α-hemoglobin (α-Hb) variants described as unstable, ten α chain variants have been studied with their chaperone. AHSP specifically binds free α-Hb to form a soluble heterodimer until it is replaced by the ß-Hb partner. In this way, AHSP prevents the precipitation of free α chains which might damage the membrane of erythrocyte. AHSP specifically recognizes the G and H helices of α-Hb that are also involved in the α1ß1 dimer interface. AHSP may act as a modifier in α-thalassemias and lead to the thalassemic phenotypes observed in certain unstable α-Hb variants previously considered unstable.

The different abnormalities of the α chain were located either in the G helix: Hb Bronovo α103(G10)His→Leu, Hb Sallanches α104(G11)Cys→Tyr, Hb Oegstgeest α104(G11)Cys→Ser, Hb Bleuland α108(G15)Thr→Asn, Hb Suan Dok α109(G16)Leu→Arg and as yet undescribed α109(G16)Leu→Gln, in the GH corner: Hb Foggia α117(GH5)Phe→Ser, or in the H helix: Hb Groene Hart α119(H2)Pro→Ser, Hb Diamant α119(H2)Pro→Leu, Hb Utrecht α129(H12)Leu→Pro.

Design and methods: These different mutated α-Hb were co-expressed with their chaperone AHSP as a fusion protein with glutathione S-transferase (GST) and analyzed by SDS-PAGE.

Results: In all cases the proteins were normally synthesized in bacteria as shown by an expression level of mutated GST-α-Hbs similar to that observed for normal GST-α-Hb. In contrast, the recovered quantities of purified mutated GST-α-Hbs associated with AHSP are highly variable. An extreme case is *GST-α-Hb*^{Utrecht} which was only found at trace levels.

Conclusion: One can assume that different mechanisms may be responsible for the amount of abnormal Hb recovered, such as a highly unstable alpha chain or an impaired formation of the complex AHSP/α-Hb or a modification of the αβ dimer formation.

Keywords: Human α-hemoglobin; Molecular chaperone AHSP; Unstable variants α-Hb; α1β1 interface; Alpha-thalassemia; Protein precipitation

Introduction

Molecular chaperones are crucial for the maintenance of the native conformation of their target proteins, and thereby prevent their aggregation. In 2002, Kihm et al. described a protein induced by GATA-1, an essential erythroid transcription factor in the red blood cell precursors, that acts as a chaperone for the α-hemoglobin chain (α-Hb) [1]. This small 102-residue protein, named Alpha Hemoglobin Stabilizing Protein (AHSP) is only expressed in erythroid cells and not in myeloid or megakaryocytic lines. *In vitro* studies showed that AHSP specifically binds free α-Hb to form a stable heterodimeric complex but does not associate with β-hemoglobin (β-Hb) or human adult hemoglobin (Hb A) [1–3]. In the erythrocyte, the AHSP thus protects α-Hb against aggregation and precipitation, which might damage membrane structure and trigger cell death. Further analysis by NMR showed that AHSP adopts a three-helix bundle [4]. The study of the structure crystal of AHSP bound to Fe^{2+}-α-Hb reveals that helices 1 and 2 and the intervening segment of the AHSP molecule recognize specifically the G and H helices of the α-

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Hb. These helices are also involved in the α1β1 interface in Hb dimers [5]. *In vitro* the binding of AHSP to free α-Hb induces a heme pocket reorganization and generates a ferric bis-histidyl structure "His(E7)-Fe^{3+}-His(F8)" in which the capacity to formation of Reactive Oxygen Species (ROS) is decreased [6]. In agreement with these results, analysis of the AHSP/α-Hb complex under high pressure showed a reversible hexacoordination of the α-Hb without protein precipitation [7].

In 2004, dos Santos et al. have shown a coordinated expression of AHSP gene and α-globin gene during maturation of the red blood cell precursors [8]. Furthermore the expression of AHSP was also under the control of transcriptional factors EKLF and Oct-1 for which the interaction sequences were found in the promoter region of the gene [9–10]. More recently, it has been shown that an Iron Responsive Element (IRE)-like stem loop regulates AHSP mRNA [11]. Moreover, Yu et al. have shown *in vitro* that recombinant AHSP promoted folding of newly translated α-globin until its association with partner β chain to form the $\alpha_2\beta_2$ tetramer [12]. These different results demonstrate that AHSP acts as a chaperone for α-Hb and participates in the assembly of normal Hb [13–14].

Because of its role in stabilizing α-globin, it has been suggested that AHSP could act as a modulating factor in the clinical expression of hemoglobinopathies such as β-thalassemia or unstable α chain variants.

As expected, AHSP knockout mice exhibit reticulocytosis, abnormal erythrocyte morphology with intracellular inclusion bodies, and increased formation of reactive oxygen species (ROS) with subsequent cellular oxidative damage as observed in β-thalassemia [1]. When such AHSP knockout mice were mated with β-thalassemic animals, an exacerbated thalassemic syndrome was observed [15]. In this disease, less β-chains are produced resulting in an excess of α-chains in the red cells which overloads the AHSP capacity. The α-chain monomers precipitate, damaging the cell and lead to a shortened life-span of circulating erythrocytes [16].

Several studies were performed to determine if AHSP is a potential modifier of β-thalassemia [17–20]. The AHSP gene analysis of selected β-thalassemia patients and control populations brought conflicting results. A preliminary study of 120 Thai patients with HbE/β-thalassemia showed no relationship between the clinical severity and haplotypes or mutations found in the gene of the AHSP [17]. In contrast, other studies explained a thalassemia intermedia phenotype by a decreased expression of this gene [18–19]. A reduced level of AHSP might, thus, explain the marked clinical severity observed in some patients with thalassemia intermedia in which only an ααα/αα genotype is involved. A recent multicenter study, involving 366 subjects [21] from five different regions of the world has been done to determine the presence of mutations leading to qualitative or quantitative defects of AHSP. Missense mutations are rare and, up to now, only one case (N75L) has been reported where the function of the protein is altered. This mutation does not prevent the stable binding of AHSP to α-Hb, but the mutated AHSP has impaired ability to inhibit reactive oxygen species production by α-Hb. Futhermore a high-frequency polymorphism in intron 1 of the AHSP gene has also found that alters the sequence of Oct-1 transcription factor binding site important for the gene expression.

It was therefore likely that a structural abnormality of AHSP altering its interaction with α-Hb or a reduced availability of this chaperone molecule in the cell might increase the severity of the thalassemic phenotype.

The AHSP/α-Hb interaction could be altered by a structural abnormality located in either of the two partners. Some structural abnormalities of α-Hb located in the contact area with AHSP may lead to the instability of Hb and an α^+-thalassemic like syndrome. Hb Constant Spring and Hb Pakse which have an elongated α-chain are marked reduced in the red cell and behave as α^+-thalassemias. It has been explained by the inherent instability of the abnormal mRNA [22]. Recently in a first study, it has been shown that Hb Constant Spring and Hb Pakse failed to interact properly with AHSP [23]. The elongated region of these molecules lies in a region close to the α1β1 interface impairing therefore binding to AHSP. More recently, we have shown that a variant of Hb frequent in North Africa and southern Italy, Hb Groene Hart [α119(H2)Pro → Ser], behaves as an α^+-thalassemia due to the modification of interaction between α-$Hb^{Groene\ Hart}$ and AHSP [24]. We suggest that a similar defect of interaction with AHSP may be involved in the stability and solubility of several other variants.

In this paper we have studied different AHSP/α-Hb complexes using the pGEX-α-AHSP vector that co-expressed human α-Hb and AHSP, as we have previously described [25]. The different recombinant α-Hbs, reported in this work, have their mutations located in the G helix (Hb Bronovo α103(G10)

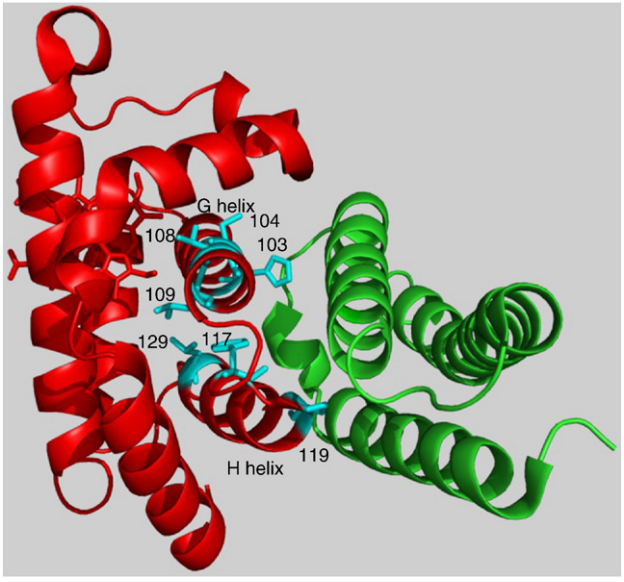

Fig. 1. Three dimensional view of the complex between AHSP (green) and α-Hb (red). The residues investigated in this work are represented in blue: α103(G10) His, α104(G11)Cys, α108(G15)Thr, α109(G16)Leu, α117(GH5)Phe, α119(H2) Pro, α129(H12)Leu. From crystallographic structure of AHSP/α-Hb complex (1Z8U), the figure was obtained using Pymol software (http://www.pymol.org.) (DeLano, W.L. The PyMOL Molecular Graphics System. DeLano Scientific LLC, Palo Alto, CA, USA. 2008).