Alpha and Beta human hemoglobins. Hemichromes and their stability according to the proteolysis *in vitro*.

Tatiana Molchanova
Engelgardt Institute Molecular Biology,
Russian Academy of Sciences and
Hematological Scientific Center, Moscow, Russia.
Fragments from Doctoral thesis, 1981.

Key words: metHb, alpha subunit, beta subunit, hemichrome, alpha hemoglobin stabilizing protein (AHSP).

Hemichrome is a collective name for low spin forms of the ferri-hemoglobin (metHb) and its constituent ferri-alpha and ferri-beta subunits.

The origin of hemichromes was studied and classified at the late 60^{th} by E. Rachmilevitz, M. Brunori, M. Wintroube, J. Peisach, W. Blumberg, E. Antonini, E. Bucci, G. Fronticelli and others.

The distinctive feature of the hemichrome is its six liganded state of the heme iron that produced as a result of the discrete reversible and irreversible changes of the globin conformation so that atoms endogenous to protein became bound as a sixth ligand of the heme iron (Rachmilevitz E., 1971). The abundant majority of hemichromes have a proximal (F8) and a distal (E7) His as fifth and sixth ligands consequently.

Hemichrome that could be converted into the native form is called reversible or Hch-1. Some hemichromes could be reduced directly into the high spin deoxy form (Hb, alpha or beta subunit), other being reduced becomes firstly hemochrome and then deoxyHb followed by anaerobic dialysis , or it can be converted quickly to oxyhemoglobin by reacting the hemochrome with CO which is then photolyzed in the presence of oxygen. Other hemichromes could not be converted to the native Hb but only to the low spin hemochrome so they are called irreversible. We found some other types of hemichromes that are presented below.

The tetramer of a low spin Fe $^{2+}$-oxyHb oxidizes to metHb through autoxidation or by oxidizing agents.

Autoxidation of oxyhemoglobin (α-Fe $^{2+}$)$_2$ (β-Fe $^{2+}$)$_2$, isolated oxy-alpha- (α-Fe $^{2+}$) and oxy-beta- (β-Fe $^{2+}$) subunits accompanied with the

release of superoxide – [O^{2-}]. The production of [O^{2-}] could well be one of the important events leading to alteration of the red cells membrane and consequently to reduce life span of thalassemic erythrocytes (1975, Brunori M. et al, Eur. J. Biochem., 53:99).

MetHb is a low spin form at alkaline pH (pK about 8) and is a high spin form at neutral and acid pH. The spontaneous complete conversion of a normal metHb to low spin hemichrome is a slowly process. Isolated alpha- and beta- subunits spontaneously convert into the low spin hemichrome. Hb hemichromes could be observed following by Hb exposure to different conditions (Table 1). There are number of abnormal hemoglobins resulted from a mutation in their alpha- or beta- chain that can produce hemichroms (Table 2.).

Table. 1. Hemoglobin hemichrome transition.

	Effect	Type of hemichrome	Reference
1.	Phenylhydrazine (arylhydrazine)	Irreversible Hch-2	Itano et al, Nature, 1974, 256:665
2.	Sodium dodecyl sulfate	Irreversible Hch-2	Rogers, Enzymologia, 1970, 39:96.
3.	Fatty acids	Reversible Hch-1	Akhrem et al, BBA, 1984, 992:191
4.	4a. Anions: Mg^{2+} Ca^{2+} $Ca^{2+} > Mg^{2+}$ 4b. Anions: ClO_4^- (Na +) Salicylate$^-$ (Na +) I^- (K +) Salicylate$^- > I^- > ClO_4^-$ Anions > Cations	Reversible Hch-1	Molchanova T., Abaturov L.
5.	Aliphatic alcohol (n-butanol)	Reversible Hch-1	Molchanova T.
6.	Dehydration	Reversible Hch-1	Bohm, Abaturov, FEB Letters, 1977, 77:22

7.	Freezing	Reversible Hch-1	Iisuka, BBA, 1969, 194:351
8.	Clycerol (high concentration)	Reversible Hch-1	Anusien et al, Arch Biochem Biophys., 1976, 175:138-43
9.	Polyethylene glycol	Reversible Hch-1	Izv. Tukm. AC. Sciences, 1976, 5:86
10.	Spontaneously	Reversible Hch-1	Rachmilevitz E., Semin. Haemat., 1974, 11:441 Molchanova T.
11.	Thermal Denaturation	Reversible Hch-1	Molchanova T., Abaturov L..
	Effect	Type of hemichrome	Reference
12.	Abnormal hemoglobons	Reversible Hch-1	Rachmilevitz E., Semin. Haemat., 1974, 11:441. Molchanova T., Abaturov L..

Table 2. Spontaneous hemichrome transition of human abnormal hemoglobins.

	Hemoglobin	Substitution	Location in the tetramer	Molecular contacts
1.	Hb J Norfolk	Alpha 57 (E6) Gly -> Asp	External	Alpha1: 52 Ser
2.	Hb Iwate	Alpha 87 (F8) His -> Arg	Internal/ Proximal	Alpha1: heme, 92Arg, 93Val, 98Phe, 136Leu, 140Tyr
3.	Hb Porto Alegre	Beta 9 (A6) Ser ->Cys	External	Beta1: 4Thr (A1, ext)
4.	Hb Freiburg	Beta 23 (B5) Val -> 0	Internal	Beta1:18Val (A15, int.), 68Leu (E8, Int), 110Leu (G12, int.) 113 Val (G15, int. $\alpha 1\beta 1$ cont), 117His (G19, ext., $\alpha 1\beta 1$ cont.)

5.	Hb Reverdale	Beta 24 (B6) Cly->Arg	Internal	Beta1: 42Phe (CD1, ext.), 60Val (E4, int.), 63His (E7, dist.), 64Gly (E8, int.), 106 Leu (G8, heme)
6.	Hb Castilla	Beta 32 (B14) Leu -> Arg	Internal	Beta1 27Ala (B9, int.), 38Thr (C4, int.), 39 Gln (C5, ext.), 42Phe (CD1, ext.), 48Leu (CD7, ext.), 54Val (D5 Int)
	Hemoglobin	Substitution	Location in the tetramer	Molecular contacts
7.	Hb Hammersmith	Beta42 (CD1) Phe -> Ser	External / Surface Crevice	Beta1: Heme (Dist); 28Leu (B10, int.), 32Leu (B14, int.), 48Leu (CD7, ext.), 63His (heme dist.)
8.	Hb Louisville	Beta42 (CD1) Phe -> Leu	External / Surface Crevice	Beta1: Heme (Dist); 28Leu (B10, int.), 32Leu (B14, int.), 48Leu (CD7, ext.), 63His (heme dist.)
9.	Hb Koln	Beta 98 (FG5) Val -> Met	Internal / Heme / $\alpha 1\beta 2$ contact	Beta: heme prox., 41Phe (C7, $\alpha 1\beta 2$), 92His-prox., 103Phe (G5, int., heme), 145Y (HC2, int.,$\alpha 1\beta 2$)

In spite of the fact that the structural characteristics of the hemichromes were studied pretty extensively there are not too much data concerning the conformational state of the globin in this important hemoglobin forms.

The goal of our studies was to evaluate the conformational mobility / stability of the hemoglobin hemichromes.

Reversible and irreversible hemoglobin hemichromes.

I am presenting below few examples of the transition of metHb into the hemichromes. Human MetHb converts into a reversible hemichrome in the solution of 1 M $NaClO_4^-$. This hemichrome can be reduced directly to deoxyHb with sodium dithionite (fig. 1).

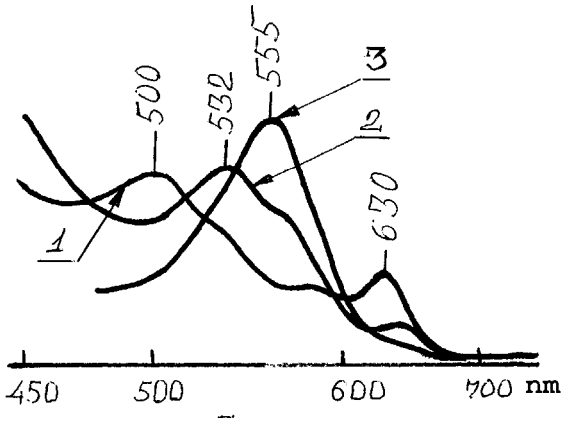


Fig. 1. UV-Vis Spectra: 1- metHb at pH 6.5, 2 - metHb diluted in 1 M $NaClO_4^-$, pH 6.5, 3 - and then sodium dithionite was added.

The typical irreversible hemichrome can be produced from metHb in the presence of sodium dodecyl sulfate (SDS) with the molar ratio SDS: metHb equal 8:1. The transition metHb to hemichrome at pH 8.5 is presented at fig. 2 and fig. 3.

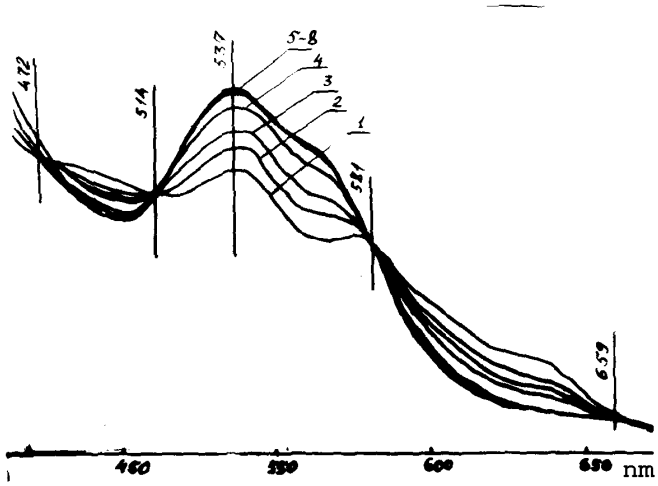


Fig. 2. Spectrophometric changes during the transition of metHb into hemichrome at different molar ratios SDS:metHb: 1-metHb, 2 - SDS:metHb=4:1, 3 - SDS:metHb= 6:1, 4 - SDS:metHb=8:1, 5 - SDS:metHb=10:1, 6 - SDS:metHb=13:1, 7 – 25:1. Ph 8.3, 20oC, 0.1 M Na-phosphate buffer, metHb concentration 0.05 mM per heme.

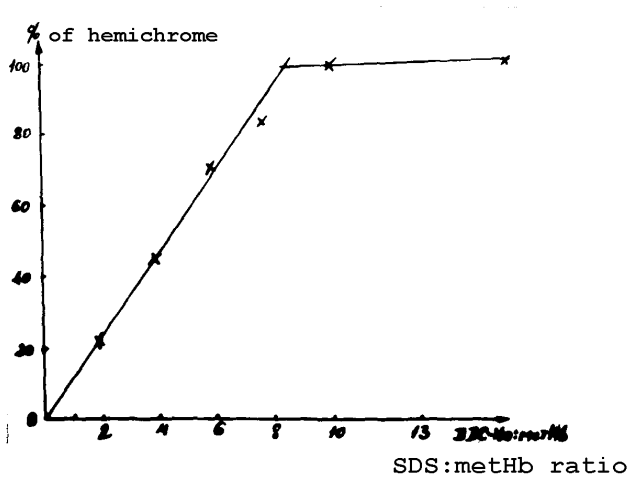


Fig. 3. The titration curve calculated based on the spectrophometric changes in fig. 2.

This hemichrome could not be reduced to high spin desoxyHb but only to a low spin hemochrome (fig. 4).

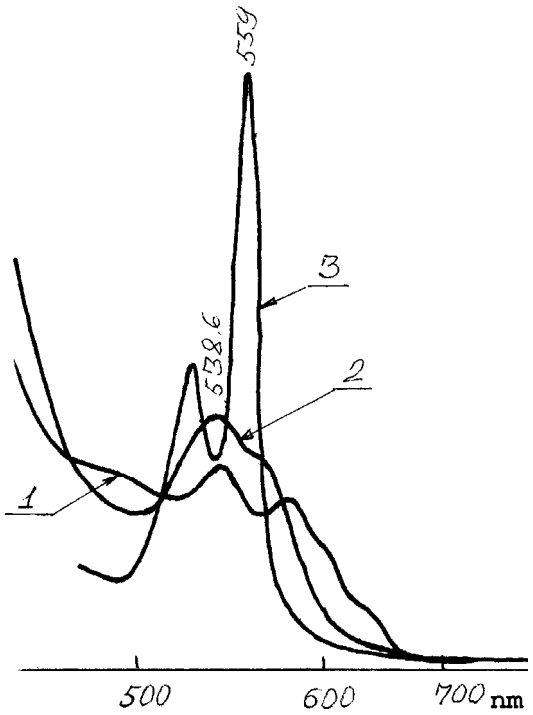
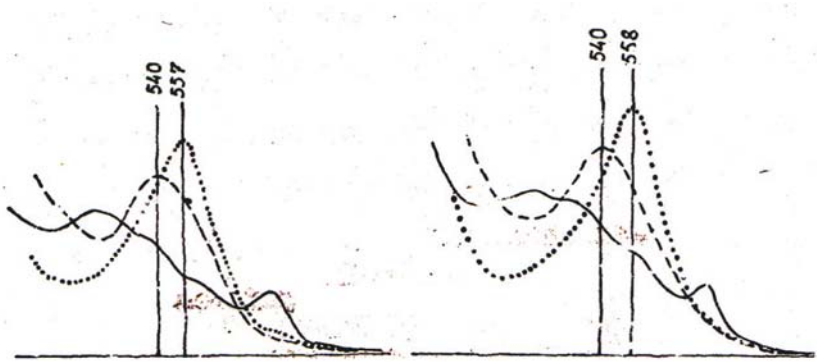


Fig. 4. UV-Vis Spectra of metHb pH 8.5 (1), metHb diluted in SDS-buffer pH 8.5 and ratio metHb:SDS=1:8 (2), and then sodium dithionite was added (3).

Reversible and irreversible hemichromes of the alpha and beta human hemoglobin subunits.

Hemichrome formation of the alpha and beta human hemoglobin subunits was studied in 0.2 M sodium phosphate buffer and protein concentration 0.05 mM. Protein precipitation followed by the higher protein concentration.

Native met alpha- (α-Fe $^{3+}$) and met beta- (β-Fe $^{3+}$)$_2$ subunits at pH 6.6; 20oC has spectra of the high spin metHb that converts into CN-met and deoxy forms following by addition of KCN and sodium dithionite consequently (fig. 5). The signs of the hemichrome could be detected in the solution of a freshly prepared met beta subunit according to the small elevation of the optical density at 535 nm.



Spectra met alpha hemoglobin, pH 6,6; 20oC: solid line; plus KCN: dash line; plus dithionite: point line.

Spectra met beta hemoglobin, pH 6,6; 20oC: solid line; plus KCN: dash line; plus dithionite: point line.

Fig. 5. Spectroscopic data of met alpha and met beta subunits at pH 6.6; 20oC.

Both α and β met chains converted spontaneously but slowly into hemichrome at pH 6.6 as at 20oC so at 4oC. The rate of the transition into a hemichrome was much higher at 4oC than at 20oC. Moreover the hemichrome of beta chain received by incubation at 4oC could spontaneously convert into met form at 20oC (fig. 6) so it is a reversible hemichrome. Met beta chain converted into the hemichrome faster than met alpha chains at the same conditions.

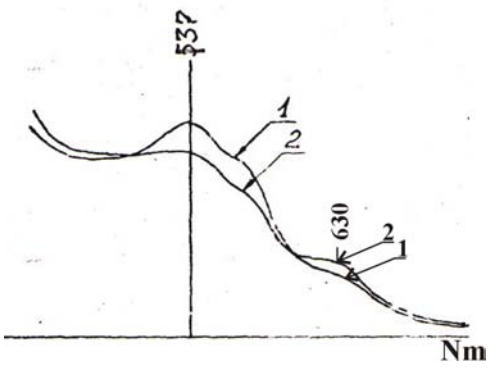


Fig. 6. Spectroscopic changes isolated met beta subunits at pH 6.6.
1 – met beta chain was incubated at 4oC during 12 hrs; 2 – after first incubation the same sample was incubated at 20oC during 12 hrs.

Both isolated met alpha and met beta subunits converted spontaneously and rapidly into the hemichrome at pH 8.5; 4oC (less than 1 hr) and also at 20oC. The rate of this transition was much higher at 4oC. Met beta chains converted into the hemichrome faster than met alpha chains at the same conditions. The type of a hemichrome (alpha and beta chains) was reversible after 1-3 hrs incubation at 4oC as it can be reduced to deoxy form with the sodium dithionite (fig. 7).

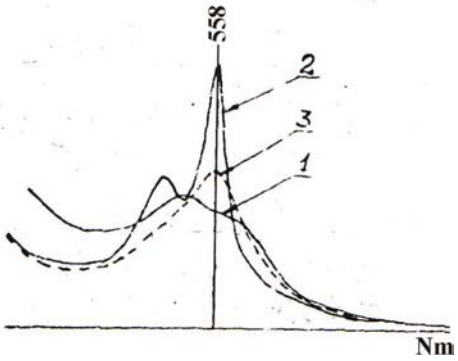


Fig. 7. 1- transition of a met alpha chain into a hemichrome at pH 8.5 after incubation of the isolated met alpha chain during 1 hr at 4oC;
2 – sodium dithionate was added to sample 1 at 4oC;
3 – sample 2 was incubated 1 hr at 20oC.

The longer incubation as 72 hrs leads to transition both alpha and beta subunits into irreversible hemichrome (fig. 8) that did not differ spectroscopically from the hemichrome of a metHb received in the presence of 8 M SDS.

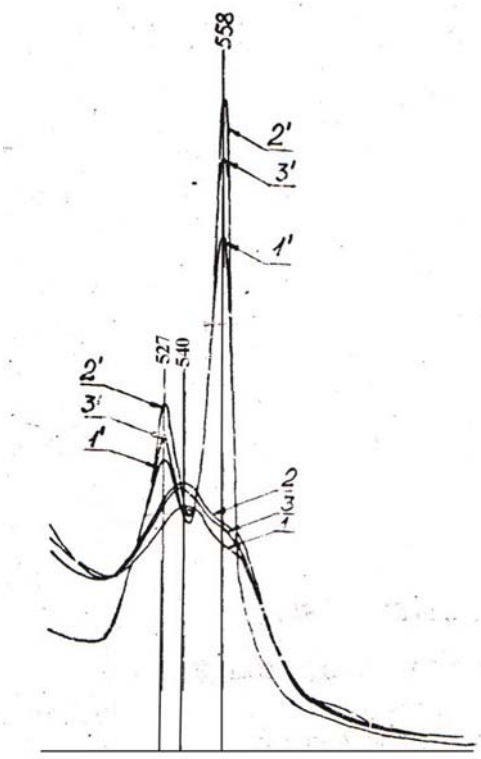


Fig. 8. 1- transition of a met beta chain into a hemichrome at pH 8.5 after incubation of the isolated met alpha chain during 72 hr at 4oC; 2 – sample 1 with 8M SDS per heme; 3 – metHb with 8 M SDS per heme. 1', 2', 3' - the same samples reduced with sodium dithionite.

Data presented above demonstrate that we observed new conditions suitable for the transition of isolated alpha and beta subunits into hemichromes. The reversible and then irreversible hemichromes are resulted from a long-term cooling at 4oC of isolated met alpha and met beta subunits rather than its incubation at room temperature. This phenomenon reminds the cold inactivation of some enzymes. The rate of this transition increases with the increase of pH. Met beta chains are more sensitive for the cold dependent transition into hemichrome than alpha one at pH 8.5 so at pH 6.6.

The conformational stability of hemichromes derived from the isolated met alpha- and met beta subunits.

The protein conformational stability of the hemichromes was studied by the proteolytic degradation. The proteolytic degradation is an adequate method for the evaluation of the protein conformational stability. The method and mechanism of the proteolytic degradation of the native forms Hb are described in our publications:

1. Molchanova T. P. et al. – Hemoglobin M Saskatoon ($\alpha_2\beta_2$ 63His (E7) -> Tyr). Structural identification, formation of hemichrom, and

proteolytic degradation" - Molecular Biology, 14 (6):989-1000, 1980 (translated from Russian).

2. Molchanova T.P., Malozemova N.G., and Abaturov L.V. - "Proteolitic degradation of native hemoglobin and its constituent parts – isolated subunits and globin. I. Kinetic data and nature of the process of breakdown of native forms" - Molecular Biology, 16 (6):897-910, 1982 (translated from Russia).

3. Abaturov L.V. and Molchanova T.P. - "Proteolitic degradation of native hemoglobin and its constituent parts – isolated subunits and globin. II. The mechanism of burst-like proteolytic breakdown of native proteins" - Molecular Biology, 16 (6):911-927, 1982 (translated from Russian).

4. Molchanova T.P. et al. "Localization of amino acid substitutions in human hemoglobin. Mass-spectral analysis of tryptic peptides" - Molecular Biology, 23 (1):169-180, 1989 (translated from Russian).

Method (short description): proteins were characterized with spectra data. Protein concentration was 0.1- 0.05 mM calculated per 1 heme. The protein:enzyme ration was 1:10 by weigh (trypsin, pronase VI, and pepsin). The fewer ratios were used if the rate of the degradation was too high. The rate of the proteolytic degradation was registered by three independent methods: a/the precipitation of indigested protein in the 7% HClO4 and detection of the soluble peptide; b/ the solubilization of the native hemoglobin forms in the 12 % Na2SO4, 1 M K-phosphate buffer pH 7.0; c/ ninhydrin staining of the N-end amino acids. The proteolytic degradation of hemichromes was recorded at 280 nm following by the precipitation of indigested protein in the 7% HClO4 and detection of an absorbance of the soluble peptides.

The mechanism of the proteolytic degradation of the native form of the human hemoglobin at the conditions described above is a burst-like and realized through the local conformation motility of the protein globular. The native undigested Hb (Hb, alpha or beta subunits) and short proteolytic peptides are present in the solution at each moment of the proteolysis. The same mechanism is true for the proteolysis of the isolated met alpha and met beta subunits (fig. 9). For example the addition of KCN that converted met alpha subunit into much more stable CN-met form decreases the rate of the proteolysis dramatically (fig. 9; curves: 2 and 3). Met alpha and met beta subunits are the most unstable heme containing native forms of the human hemoglobin according to the proteolytic degradation with all three enzymes (fig. 10).

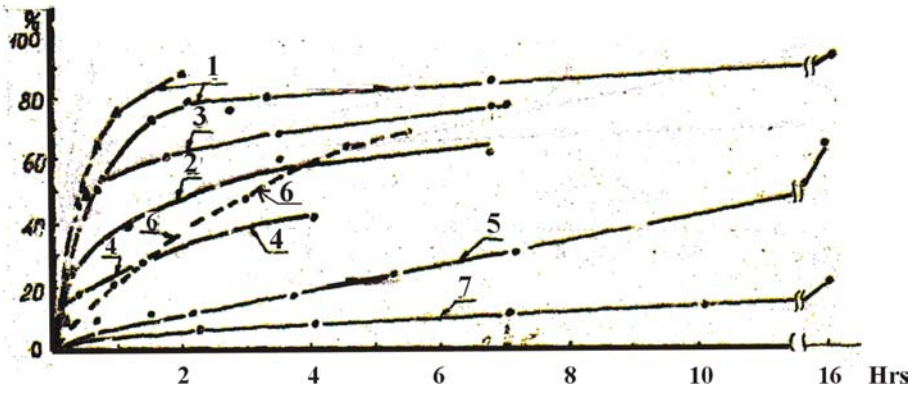


Fig. 9. Proteolytic degradation of the freshly prepared met alpha subunits
with pronase VI at pH 6.6; 20oC.
1 – proteolysis of met alpha chain; 2 – KCN was added (10:1 ratio) after 11 min of the proteolysis of the met alpha chain; 3 – KCN was added (1:10 ratio) after 40 min of the proteolysis of the met alpha chain; 4 – proteolysis of the metHb reconstituted from the met alpha and met beta chains at the ratio 1.1. Met beta chain was freshly prepared, and met alpha chain was incubated 9 hrs at 20oC; 5 – proteolysis of MetHb; 6 – proteolysis met alpha chain at 4oC; 7 – proteolysis metHb at 4oC.
X axis – time in hrs; Y axis – percentage of the depredated protein.

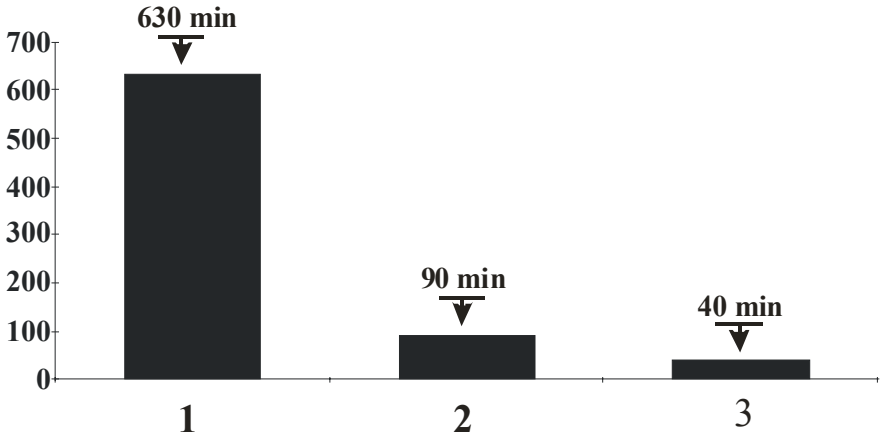


Fig. 10. The half time of the proteolytic degradation of the freshly prepared met alpha subunits in comparison with metHb.
Degradation with pronase VI at pH 6.6; 20oC
1 – metHb; 2 – met beta subunit; 3 – met alpha subunit.
Y axis – time in min.

The rate of the proteolytic degradation of hemichromes that are derived from met alpha and met beta chains through the spontaneous transition is 5-10 folds higher than corresponding native met forms and 10-200 folds higher than their native oxy forms (fig. 11).

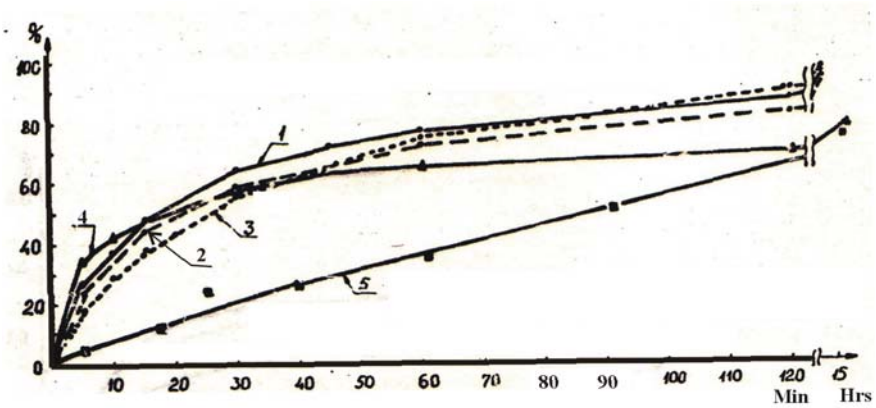


Fig. 11. Proteolytic degradation of the spontaneous hemichromes derived from met alpha and met subunits after 72 hrs incubation at pH 8.5; 4oC. Conditions: pronase VI, pH 8.5, 4oC. 1 – hemichrome of beta subunit; 2 – sample 1 in the presence CN^- at molar ration 1:10; 3 – metHb and met beta chain in the presence of 8M SDS (rates of proteolysis are consistent); 4- hemichrome of alpha subunit; 5 – native met alpha subunit. X axis – time in min (hrs); Y axis – percentage of the depredated protein.

The rate of the proteolitic degradation of the spontaneous hemichromes derived from met alpha and met beta subunits after long-term (72 hrs) incubation at ph 8.5; 4oC is the same as the rate of the proteolitic degradation of irreversible hemichromes derived from metHb and met form of subunits in the presence of 8 M SDS. Addition of KCN did not influence the rate of the proteolysis of hemichromes significantly. So spontaneous hemichromes derived from met alpha and met beta subunits after long-term (72 hrs) incubation at ph 8.5; 4oC are relevant to the irreversible type according to the spectral data and the proteolysis. As a result it can be concluded that both types of hemichrome: induced with cold and SDS, have similar conformational mobility of their protein structure and are not different from the point of view of the stability of their protein structures.

The proteolytic degradation of the reversible hemichromes derived from metHb and from isolated met alpha and met beta subunits have shown no differences in their conformational stability. There are no practically differences in the conformational stability of the reversible and irreversible hemichromes derived from hemoglobin or its isolated subunits (fig. 12).

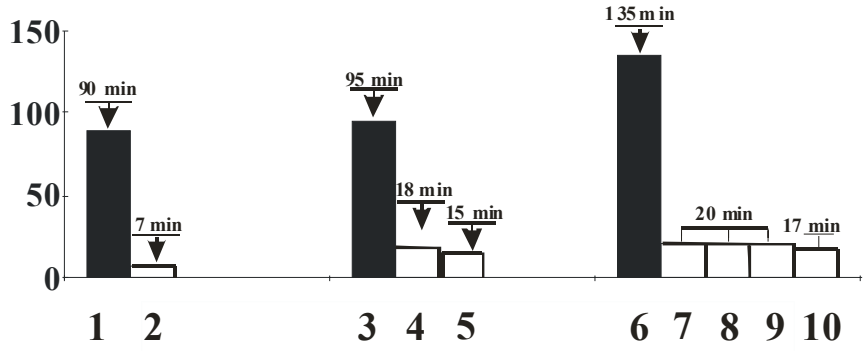


Fig. 12. The half time of the proteolytic degradation (pronase VI) of different hemichromes in comparison with the native forms.
1 and 2 – pH 6.6; 20oC: 1 – met beta subunit; 2 – spontaneous reversible hemichrome beta subunit. 3, 4, 5 – pH 8.5; 4oC: 3 – met alpha subunit; 4 – spontaneous hemichrome alpha subunit; 5 - spontaneous hemichrome beta subunit. 6, 7, 8, 9, 10 – pH 6.6; 4oC: 6 - met alpha subunit; 7 - spontaneous hemichrome beta subunit; 8 – reversible hemichrome of metHb in 1 M NaClO4 (fig. 1);
9 - reversible hemichrome of metHb in 1 M KI;
10 – irreversible hemichrome of met Hb in 1 M sodium salicylate.
Y axis – time in min.

Our data revealed that the stability of the hemichrome protein structure evaluated with the proteolytic degradation is quite decreased as compared with the native forms. The native metHb is about 7 fold more stable than met beta subunit and 16 fold is more stable than met alpha subunit (fig. 10). But their hemichromes of the different origin are 5-10 folds less stable. Moreover there are no differences in the stability of hemichromes derived from MetHb or from the isolated subunits. All hemichromes have similar rate of their proteolitic degradation (fig. 12). The stability of the hemichrome is similar to the stability of the isolated globin ($\alpha\beta$) in the presence of the SDS, and much less stable than the native apo-Hb.

Hemoglobin hemichromes including spontaneous α and β hemichromes have slightly diminished percentage of alpha helixes according to the circular dichroism and fluorescent studies. However their protein structure is still compact but it is not as tight as the native one. As a result the conformational mobility (stability) of the hemichrome protein structure is increased 5-50 fold in comparison with the native protein structure (Hb, α and β subunits) according to the proteolysis in vitro and the deuterium hydrogen exchange methods. The reversible character of the hemichrome derived from hemoglobin (Hb, isolated α and β subunits) assumes that conformation of the hemichrome corresponds with the molten globular protein structure.

Alpha Hemoglobin Stabilizing Protein (AHSP).

Many papers were published during last six years that discussed the role and properties of the alpha hemoglobin stabilizing protein (AHSP) (M. Weiss et al.). AHSP (102 kDa protein) is considered as the specific chaperone that binds any form of alpha chain hemoglobin. According to these publications AHSP promotes alpha globin chain folding, prevent alpha subunit (heme-alpha chain) from aggregation, and decreases the level of the reactive oxygen spices (ROS) produced during the oxidation of the excess of alpha subunits for example at beta-thalassemia carriers.

Here I would like to raise few questions that are base on our experimental and also some literature data.

Oxidation of α-Hb and hemichrome transition.

The oxy-α subunit is rearranged considerably in the [oxy- α -AHSP] complex according to the X-ray data (Liang Feng et al., Cell, 119:629, 2004). As a result the heme group is coordinated by the distal His (58His (E7)) in the [oxy-alphaHb-AHSP] complex but not by the proximal His (87His (F8)) as it is coordinated in the native tetramer oxyHb. AHSP binds oxy-α subunit (ratio 1:1) and spontaneously converts [Fe^{2+}oxy-α–subunit] into the hemichrome during few minutes according to the biochemical data. The distal and proximal histidines become the fifth and sixth ligands of the [Fe^{3+} heme]-α-subunit during this transition.

The transition of the oxy-α subunit into the hemichrome in the complex with AHSP reminds the interaction of SDS with human native oxyHb. It was shown the disappearance of the bands of oxyHb at 541 and 576 nm and the appearance at 537 nm. The resultant spectra were characteristic of low spin (Fe3+) hemichrome. The interaction of SDS with oxyHb suggests a conformational change of the protein in the heme pocket, which may induce the binding of distal histidine to iron leading to the formation of superoxide radical (Kumar Sau A, Currell D, Mazumdar S, Mitra S., Biophys Chem. 98(3):267-73, 2002).

So the appearance of the superoxide radicals during the transition of a [AHSP - oxy- α Hb] into hemichrome is quite obvious. If AHSP decreases ROS formation in vivo so are there other mechanisms that promote the decrease of superoxide radicals when there is an excess of [Fe^{2+}oxy-α–] subunits?

Hemichrome transition stabilizes α-Hb (?).

The transition of oxy- α Hb into hemichrome following binding AHSP induces structural disarrangements of the native α-subunit. The transition into hemichrome assumes the increase of the conformational mobility of the alpha globin structure in the [(hemichrome α Hb) – AHSP] complex. As a result one can assume the increase of the susceptibility of [(hemichrome α Hb) – AHSP] to the proteolysis in comparison with isolated oxy- α Hb (please see our data presented above).

It is curious to know does AHSP protect the alpha hemichrome against proteolysis and decrease the rate of its proteolytic degradation in comparison with the hemichrome derived for example spontaneously from the met alpha subunit? Or is the [(hemichrome α Hb) – AHSP] complex a transient state on the way of its proteolitic degradation in vivo?

Hemichrome was considered so far as Hb-form that is transient on the way to the hemoglobin denaturation and precipitation. We observed the hemichrome formation during the thermal denaturation of the normal and abnormal hemoglobins.

Hemichrome was also considered as a high mobile (type of molten globular) reversible Hb form that can transiently appeared during the natural hemoglobin conformational transitions (L. Abaturov et al.).

More studies have to be done to understand the unusual observation that AHSP stabilizes the low spin (Fe3+) α–Hb hemichrome.

There was shown that AHSP decreases the rate of limited proteolysis with trypsin of the nascent alpha globin (probably 2-5 folds). CN-heme decreases proteolysis of nascent alpha globin by approximately similar way in the TNT reaction (Xiang Yu et al, J. Clin.Invest., 117 (7):1856, 2007).

Alpha chain - AHSP binding versus beta chain binding.

AHSP binds α chain through the contacts that are located in the α1β1 interface in the Hb tetramer. The experiments in the solution have shown that CO-β-subunit competes well for binding CO-α-subunit in the presence of AHSP and displace CO-α-subunits from (CO-α-subunits – AHSP) complex. The same is true for α- and β-globins interactions with AHSP (David Gell et al., JBC, 277 (43):40602, 2002).

So if there are available free β -chains then will α–chain bind directly to β -chain but not to AHSP in vivo?

Some conclusions.

A brief review of the literature data reveals that AHSP: a/ binds unfolded a α -globin (Xiang Yu et al., J. Clin. Invest., 117 (7):1856, 2007); b/ increases the solubilization of the lyophilized α -globin (unstructured) (David Gell et al., JBC, 277 (43):40602, 2002); c/ rearranges dramatically the conformation of oxy-α-subunit in the AHSP-complex (Liang Feng et al., Cell, 119:629, 2004); d/ facilitates transition of oxy-α-subunit into hemichrome in the AHSP-complex (Suiping Zhou et al., JBC, 281(43):32611-8, 2006) so actually increases the rate of autoxydation of oxy-α-subunit; e/ assists disaggregation α -chains (investigated at low protein concentration in vitro).

As a conclusion it looks like that AHSP has tendency to associate with less structured α-chains and even facilitates the transition of the relatively stable and compact oxy-α-subunit into less structured hemichrome. If AHSP is a specific α -chain chaperon so its function would be more consistent with the point of view that this molecular chaperones are involved in macromolecular disassembly processes, such as the partial unfolding of subunits when some proteins carry out their normal functions, and the re-solubilisation and/or degradation of proteins partially denatured and/or aggregated by mutation or by exposure to environmental stresses, such as high temperatures and oxidative conditions" (R. John Ellis "Chaperone Function: The Orthodox View" in "Molecular Chaperones and Cell Signalling" Edited by Brian Henderson, 2005).

Natural α-mutant hemoglobins with the mutation of amino acids that are crusual for AHSP interaction.

The interesting observation was done by group of authors from Prof Henry Wajsman's Laboratory, France: "In the three-dimensional structure of the complex formed by oxidized α-Hb bound to AHSP, Feng et al. identified several residues interacting at the interface between these two molecules and they replaced them by an Ala both in AHSP and in the α-Hb. Using a yeast two-hybrid assay, they showed that when Lys 99 (G6), His 103 (G10) or Phe 117 (GH5) was replaced by an Ala the formation of the complex was impaired. No general conclusion could be obtained from the hematological presentation of the various natural variants observed at these positions" (Corinne Vasseur-Godbillon etal., Blood Cells, Molecules, and Diseases, 37:173, 2006). I added few newly discovered mutant Hbs to their data that did not change their general conclusion.

	Alpha 99 (G6) Lys	Alpha 103 (G10) His	Alpha 117 (GH5) Tyr	Ref.
Hb Beziers (Hb Harlow)	**Lys -> Asn (alpha1) 15.8 % of total alpha Normal stability Hematologically normal**			
Hb Turriff	**alpha2 or alpha1 Lys>Glu 22 % of total alpha Hematologically normal (10.5% unclear etymology)**			
Hb Bronovo		**alpha2 His>Leu alpha thal-like syndrome, no mutant Hb found;mild anemia; mild microcytosis Mild clinical symptoms**		**1**
Hb Lombard		**alpha2 His>Tyr 8.4 % of total Hb No clinical effect Heterozygote: normal Relative stability: Normal**		
Hb Charolles		**alpha1 His>Tyr 11% of the total hemoglobin microcytosis and hypochromia.**		
Hb Contaldo		**alpha2 or alpha1 His>Arg 15 - 20 % of total Hb Heterozygote: hemolytic, moderate anemia. Unstable**		
Hb Ambroise			**Phe>Ile 27% of total alpha Heterozygote Hb 13.4 g/dL; MCH 32.5 pg MCV 96.0 fL; RBC 4.14 10^12/L sarcoidosis-like syndrome**	**2**

1. Harteveld C. L. et al, Haematologica 2006; 91:570-571
2. A database of Human Hemoglobin Variants and Thalassemias (on line).

The future studies should be very interesting in the understanding of the role of an alpha hemoglobin stabilizing protein as an alpha chain chaperon.

Tatiana Molchanova, PhD in Biological Sciences,
Former senior scientist in the Prof. Titus Huisman ‘s
Sickle Cell Center, Augusta, Georgia, USA.
May 8, 2008.
WEB Site: http://molchanova.com