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Review Article

Pseudo-Enzymatic Properties of Haptoglobin:Hemoglobin Complexes

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Abstract

Hemoglobin (Hb) and free heme are physiologically released into human plasma upon hemolysis of senescent erythrocytes and erythroblasts enucleation, and pathologically because of severe hematologic diseases and blood transfusion. To inhibit the heme-based formation of free radicals, several plasma proteins have been evolved. In particular, haptoglobin (Hp) binds αβ dimers of Hb allowing the Hb removal via the reticuloendothelial system and the CD163 receptor-mediated endocytosis in hepatocytes, Kupffer cells, and tissue macrophages. In turn, the Hp:Hb complexes facilitate heme-based detoxification of NO and peroxynitrite and display NO_2^- reductase activity opening new avenues in removing reactive nitrogen species in plasma. The correlation between Hp glycosylation, the stability of the Hp:Hb complexes and pathological conditions (i.e., cancer) has been observed.

Keywords: haptoglobin 1-1:hemoglobin complex; haptoglobin 2-2:hemoglobin complex; pseudo-enzymatic properties.

Abbreviations: CCP: Complement control protein domain; Hb: Human hemoglobin; Hb(II): Ferrous Hb; Hb(II)-NO: Nitrosylated Hb(II); Hb(II)-O_2: Ferrous oxygenated Hb; Hb(III): Ferric Hb; Hp: Human haptoglobin; Hp:Hb: Hp:Hb complex; Hp:Hb(II): Ferrous Hp:Hb complex; Hp:Hb(II)-NO: Nitrosylated Hp:Hb(II)complex; Hp:Hb(II)-O_2: Ferrous oxygenated Hp:Hb complex; Hp:Hb(III): Ferric Hp:Hb complex; Hp:Hb(III)-NO: Nitrosylated Hp:Hb(III) complex; Hp:Hb(III)-$OONO^-$: Peroxynitrite-bound Hp:Hb(III); Hp1-1: Phenotype 1-1 of Hp; Hp1-1:Hb: Hp1-1:Hb complex; Hp1-1:Hb(II): Ferrous Hp1-1:Hb complex; Hp1-1:Hb(III): Ferric Hp1-1:Hb complex; Hp2-2: Phenotype 2-2 of Hp; Hp2-2:Hb: Hp2-2:Hb complex; Hp2-2:Hb(II): Ferrous Hp2-2:Hb complex; Hp2-2:Hb(III): Ferric Hp2-2:Hb complex; RBC: Red blood cell; SP: Serine protease-like domain

Hemoglobin

The deleterious effects of oxidative and nitrosative stress, such as damage to cellular proteins, DNA, and lipids, are well characterized. Red blood cell (RBC) homeostasis is an excellent example of redox balance: erythroid progenitors accumulate hemoglobin (Hb) during development and continuously transport large amounts of oxygen over the course of their lifespan. Moreover, RBCs represent an important inter-organ communication system with a key role in the control of systemic nitric oxide metabolism. To inhibit the heme-based formation of free radicals, several plasma proteins have been evolved [1,2].

Hb is physiologically released into human plasma upon hemolysis of senescent RBCs and erythroblasts enucleation, and pathologically because of severe hematologic diseases and blood transfusion [3-7]. Since plasmatic Hb brings about the formation of free radicals, living organisms have evolved efficient mechanisms to trap extra-erythrocytic Hb scavenging [5,6,8-10]. Haptoglobin (Hp) traps αβ dimers of Hb leading to very stable non-covalent complexes, which allow the removal of Hb via the reticuloendothelial system and the CD163 receptor-mediated endocytosis in hepatocytes, Kupffer cells, and tissue macrophages [11-14]. Hp binding to Hb (i) prevents Hb entry into the endothelium, (ii) affects heme redox properties, (iii) inhibits the heme dissociation, and (iv) protects several Hb residues at the Hp:Hb interface from oxidative modifications [15-18].

Haptoglobin

The human Hp gene consists of three structural alleles: *Hp1F, Hp1S*, and *Hp2*. The products of the *Hp1F* and *Hp1S* alleles differ by only one amino acid, whereas the *Hp2* allele is the result of a fusion of the *Hp1F* and *Hp1S* alleles giving rise to a longer chain. Human Hp is a 90 kDa single polypeptide chain constituted by a complement control protein (CCP) domain and a serine protease- (SP-) like domain. The SP-like domain is proteolytically cleaved into an α and a β chain covalently linked by a disulfide bond. *Hp1* consists of a single CCP domain while *Hp2* contains two CCP domains [19]. The CCP domains are responsible for a head-to-head dimerization of Hp through an unusual β strands swap and intermolecular disulfide bonds. The occurrence of the *Hp1* and *Hp2* alleles in humans gives rise to Hp1-1 dimers (covalently linked by Cys15 residues), Hp1-2 hetero-oligomers and Hp2-2 oligomers (covalently linked by Cys15 and Cys74 residues) [19]. Each Hp β chain of the SP-like domain

binds the αβ dimer of Hb in a 1:1 stoichiometry making extensive contacts that mask the Hb dimer-dimer interface. This is at the roots of the high binding affinity between the two proteins and of the ability of Hp to bind only the dimeric form of Hb (Figure 1) [20-22].

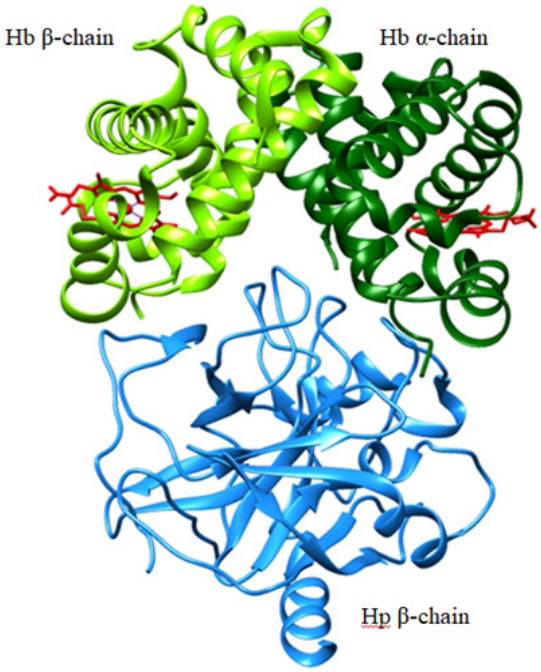

Figure 1: Three-dimensional structure of the human Hp:Hb (PDB ID: 4X0L) [20]. The β chain of Hp is rendered in blue, the α and β chains of Hb are rendered in dark and light green, and the hemes are rendered in red. The picture has been drawn with the UCSF-ChimeraX package [21].

Haptoglobin:hemoglobin complexes

Hp:Hb complexes (i.e., Hp1-1:Hb and Hp2-2:Hb) (i) react with O_2, CO, NO, cyanide, fluoride, azide, thiocyanate, and imidazole [23-29], (ii) undergo reductive nitrosylation [26], (iii) facilitate the heme-based detoxification of NO and peroxynitrite [15,30], and (iv) catalyze the NO_2^- conversion to NO (Table 1) [31-37]. Noteworthy, the ligand binding and reactivity properties Hp1-1:Hb and Hp2-2:Hb are reminiscent those of the R quaternary state of the Hb tetramer [26-31,38]. The inspection of the global pathway of Hp:Hb reactivity (Fig. 2) allows the following considerations.

Table 1: Ligand binding and reactivity properties of Hb, Hp1-1: Hb and Hp2-2: Hb.

Reaction	Heme-protein	Kinetic and Thermodynamic Parameters		
O_2 binding[a]	Hb (II) T-state	$k_{on1} = 1.1\times10^{7}$ M^{-1} s^{-1}	$k_{off1} = 3.7\times10^{3}$ s^{-1}	$K_1 = 3.4\times10^{-4}$ M
		$k_{on2} = 5.0\times10^{6}$ M^{-1} s^{-1}	$k_{off2} = 1.8\times10^{3}$ s^{-1}	$K_2 = 3.6\times10^{-4}$ M
	Hb (II) R-state	$k_{on1} = 7.6\times10^{7}$ M^{-1} s^{-1}	$k_{off1} = 3.2\times10^{1}$ s^{-1}	$K_1 = 4.2\times10^{-7}$ M
		$k_{on2} = 3.6\times10^{7}$ M^{-1} s^{-1}	$k_{off2} = 1.6\times10^{1}$ s^{-1}	$K_2 = 4.4\times10^{-7}$ M
	Hp1-1: Hb (II)	$k_{on1} = 4.5\times10^{7}$ M^{-1} s^{-1}	$k_{off1} = 2.8\times10^{1}$ s^{-1}	$K_1 = 6.2\times10^{-7}$ M
		$k_{on2} = 4.5\times10^{7}$ M^{-1} s^{-1}	$k_{off2} = 1.6\times10^{1}$ s^{-1}	$K_2 = 3.6\times10^{-7}$ M
	Hp2-2: Hb (II)		$k_{off1} = 2.7\times10^{1}$ s^{-1}	
			$k_{off2} = 1.4\times10^{1}$ s^{-1}	
NO binding[b]	Hb (II) T-state	$k_{on} = 2.4\times10^{7}$ M^{-1} s^{-1}	$k_{off} \sim 1\times10^{-3}$ s^{-1}	$K \sim 4\times10^{-11}$ M
	Hb (II) R-state	$k_{on} = 2.4\times10^{7}$ M^{-1} s^{-1}	$k_{off} \sim 1\times10^{-5}$ s^{-1}	$K \sim 4\times10^{-13}$ M
	Hp1-1: Hb (II)	$k_{on} = 1.1\times10^{7}$ M^{-1} s^{-1}	$k_{off} < 2.0\times10^{-4}$ s^{-1}	$K < 2\times10^{-11}$ M
	Hp2-2: Hb (II)	$k_{on} = 9.3\times10^{6}$ M^{-1} s^{-1}	$k_{off} < 2.0\times10^{-4}$ s^{-1}	$K < 2\times10^{-11}$ M
NO binding[c]	Hb (III)			$K = 8.3\times10^{-5}$ M
	Hp1-1: Hb (III)			$K = 1.4\times10^{-4}$ M
	Hp2-2: Hb (III)			$K = 2.1\times10^{-4}$ M

NO detoxification[d]	Hb (II)-O_2	k_{on} = 6.0×10^6 M^{-1} s^{-1}		
	Hp1-1: Hb (II)-O_2	k_{on} = 6.1×10^6 M^{-1} s^{-1}		
	Hp2-2: Hb (II)-O_2	k_{on} = 6.0×10^6 M^{-1} s^{-1}		
Peroxynitrite detoxification[e]	Hb (III)	k_{on} = 1.2×10^4 M^{-1} s^{-1}		
	Hp1-1: Hb (III)	k_{on} = 1.7×10^4 M^{-1} s^{-1}		
	Hp2-2: Hb (III)	k_{on} = 1.6×10^4 M^{-1} s^{-1}		
Nitrite reduction[f]	Hb (II) T-state	k_{on} = 1.2×10^{-1} M^{-1} s^{-1}		
	Hb (II) R-state	k_{on} = 6.0 M^{-1} s^{-1}		
	Hp1-1: Hb (II)	k_{on} = 7.3 M^{-1} s^{-1}		
	Hp2-2: Hb (II)	k_{on} = 1.2×10^1 M^{-1} s^{-1}		
Reductive nitrosylation[c]	Hb (III)	k_{OH^-} = 3.2×10^3 M^{-1} s^{-1}	k_{H2O} = 1.1×10^{-3} s^{-1}	
	Hp1-1: Hb (III)	k_{OH^-} = 4.9×10^3 M^{-1} s^{-1}	k_{H2O} = 2.6×10^{-3} s^{-1}	
	Hp2-2: Hb (III)	k_{OH^-} = 6.7×10^3 M^{-1} s^{-1}	k_{H2O} = 2.6×10^{-3} s^{-1}	

[a]From Olson et al. [32]; Chiancone et al. [24]; Sawicki & Gibson [33]; Unzai et al. [38]; Ascenzi et al. [28].
[b]From Moore and Gibson [34]; Ascenzi et al. [26].
[c]From Hoshino et al. [35]; Ascenzi et al. [26].
[d]From Azarof et al. [15].
[e]From Herold and Shivashankar, [36]; Ascenzi & Coletta [30].
[f] From Huang et al. [37]; Ascenzi et al. [31].

1) Ferrous oxygenated Hp:Hb (Hp:Hb(II)-O_2) catalyzes NO detoxification reacting very rapidly with NO; this reaction leads to the formation of the ferric derivative Hp:Hb(III) by way of the transient peroxynitrite-bound species (Hp:Hb(III)-OONO$^-$) which release vary rapidly NO_3^- (~ 90%) and NO_2^- (~ 10%) [15].

2) (ii) O_2 may facilitate also the detoxification of ferrous nitrosylated Hp:Hb (Hp:Hb(II)-NO) as reported for ferrous nitrosylated Hb (Hb(II)-NO) [39]. The reaction of Hb(II)-NO with O_2 is limited from the NO dissociation and the transient formation of the Hb(II)-O_2 species. In turn, Hb(II)-O_2 reacts with NO leading to the formation of ferric Hb (Hb(III)), NO_3^- and NO_2^- by way of the Hb(III)-OONO$^-$ derivative.

3) (iii) The Hp:Hb(III) species facilitate peroxynitrite detoxification leading to the formation of NO_3^- (~90%) and NO_2^- (~10%) by way of the transient Hp:Hb(III)-OONO$^-$ species.

4) (iv) The Hp:Hb(III) complexes react with NO leading to the formation of the very stable Hp:Hb(II)-NO complex. This process is characterized by the formation of the nitrosylated Hp:Hb(III) derivative (Hp:Hb(III)-NO) that is in equilibrium with the Hp:Hb(II)-NO$^+$ species; under alkaline conditions, Hp:Hb(II)-NO$^+$ converts to Hp:Hb(II)-NO [26].

5) Under reductive conditions, ferrous Hp:Hb (Hp:Hb(II)) traps NO_2^- leading to the formation of the highly stable Hp:Hb(II)-NO derivative. This reaction is characterized from the transient formation of Hp:Hb(III) and NO species [30].

Clinical implications

Considering that about 10% of senescent RBCs undergo hemolysis in plasma, the Hp:Hb-based reactions shown in Figure 2 may participate to the detoxification of reactive nitrogen species present in plasma that are implicated in atherosclerosis, inflammation, and neurodegenerative disorders [40-53]. Indeed, the ligand binding and reactivity properties of extra-erythrocytic Hp:Hb are not limited from membrane diffusion of NO, peroxynitrite, and NO_2^- in RBCs. Indeed, this represents the pre-requisite for intra-erythrocytic Hb-based catalysis. Glycoslation has been reported to play a clinical role on Hp actions [54]. In fact, the Hp β chain contains four N-glycosylable sites (i.e., Asn184, Asn207, Asn211, and Asn241) that can be linked to either sialylated or fucosylated glycans [55,56]. However, in the Hp2-2 complex the steric hindrance of the neighboring monomers prevents or reduces the normal glycan incorporations [57]. The affinity of Hb for Hp is affected by Hp glycosylation, and the Hb:Hp glycosylated complex is more thermostable compared to either the Hb:Hp deglycosylated complex or to the free Hb and Hp molecules [56].

This suggests a potential effect of Hp glycosylation in the intrinsic physical and redox properties of Hp, with possible systemic effects on the circulation and the clearance times [54,56]. Several clinical studies hypothesized a correlation of Hp glycosylation with different diseases. Glycosylation of Hp have been associated with infectious sera of patients [58] but also with many types of cancers, including pancreatic [59-61], hepatic [62,63], prostate [64], lung [65,66], ovarian [67], colon [68], and gastric cancer [69]. Although differences in Hp glycosylation have been reported, methodological hitches in characterizing glycosylated complexes preclude interpretations. Overall, based on the reported aberrant glycan patterns, Hp could possibly become a potential biomarker for risk prediction and identification of some disorders, eventually being combined with other biomarkers in order to increase the diagnostic efficiency of specific diseases [Ratanasopa et al., 2013; Zhang et al., 2016]. Indeed, it has been reported that

the fucosylated Hp in combination with the CA125 biomarker (also known as mucin 16, the only clinically reliable diagnostic marker for ovarian cancer [70]) could be used to discriminate between healthy condition, early-stage ovarian cancer, and stage III ovarian cancer [54,63,65,71].

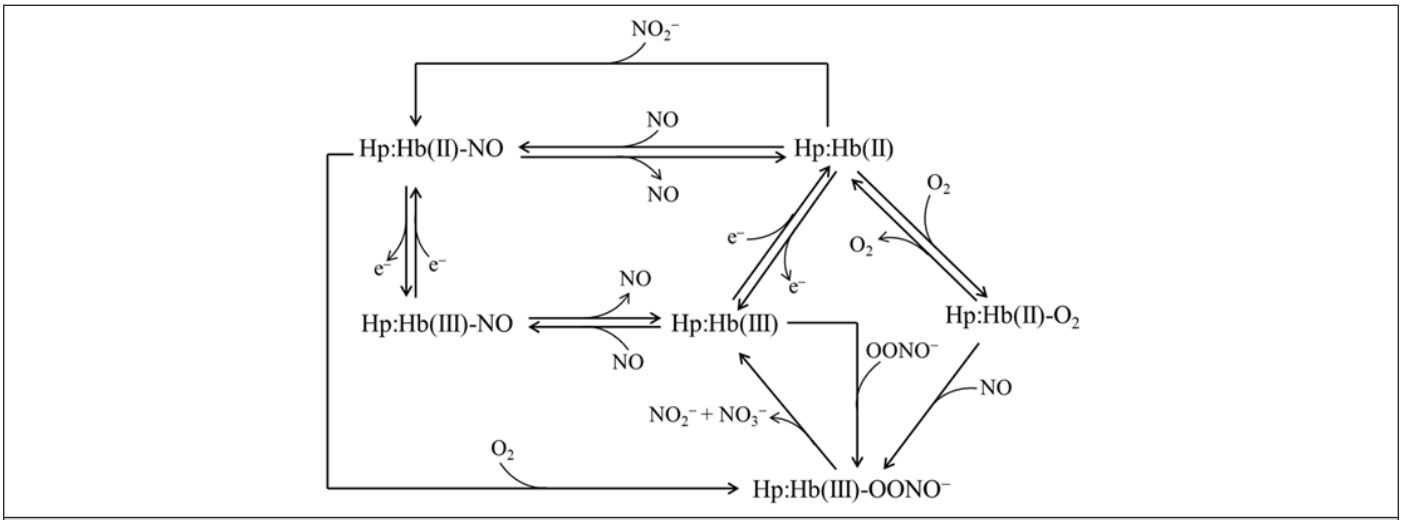

Figure 2: Global pathway of human Hp: Hb reactivity.

It remains to be determined if variations in the glycan composition of Hp play a role also in the onset of some diseases, possibly perturbing the protective role of Hp or reflecting a variation of the glycosyl transferease expression systems. The development of novel diagnostic technologies will allow to better determine Hp glycosylation status and the specific glycosylated sites; in turn, this information will be fundamental for an unequivocal correlation between Hp glycosylation and a pathological condition and for their accurate diagnosis.

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