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Journal of Inorganic Biochemistry 99 (2005) 903–911
Journal of Inorganic Biochemistry
www.elsevier.com/locate/jinorgbio

Invertebrate hemoglobins and nitric oxide: How heme pocket structure controls reactivity

Andrew J. Gow [a], Alexander P. Payson [b,*], Joseph Bonaventura [b,c,d,*]

[a] Stokes Research Institute, Children's Hospital of Philadelphia, University of Pennsylvania, Philadelphia, PA 19104, USA
[b] National Institutes of Health, Center of Biomedical Research Excellence (NIH COBRE), Protein Research Center, University of Puerto Rico, Box 9255, Mayagüez, 00681, Puerto Rico
[c] Department of Cell Biology, Duke University Medical Center, Durham, NC 27710, USA
[d] Nicholas School of the Environment, Duke University Marine Laboratory, Beaufort, NC 28516, USA

Received 25 October 2004; received in revised form 29 November 2004; accepted 6 December 2004
Available online 5 February 2005

Abstract

Hemoglobins (Hbs), generally defined as 5 or 6 coordinate heme proteins whose primary function is oxygen transport, are now recognized to occur in virtually all phyla of living organisms. Historically, study of their function focused on oxygen as a reversibly bound ligand of the ferrous form of the protein. Other diatomic ligands like carbon monoxide and nitric oxide were considered "non-physiological" but useful probes of structure–function relationships in Hbs. This investigatory landscape changed dramatically in the 1980s when nitric oxide was discovered to activate a heme protein, cyclic guanylate cyclase. Later, its activation was likened to Perutz' description of Hb's allosteric properties being triggered by a ligand-dependent "out-of-plane/into-plane" movement of the heme iron. In 1996, a *functional role* for nitric oxide in human and mammalian Hbs was demonstrated and since that time, the interest in NO as a physiologically relevant Hb ligand has greatly increased. Concomitantly, non-oxygen binding properties of Hbs have challenged the view that Hbs arose for their oxygen storage and transport properties. In this focused review we discuss *some* invertebrate Hbs' functionally significant reactions with nitric oxide and how strategic positioning of a few residues in the heme pocket plays an large role in the interplay of diatomic ligands to ferrous and ferric heme iron in these proteins.

Keywords: Invertebrates; Nitric oxide; Hemoglobin

1. Introduction

Enormous diversity exists among invertebrate hemoglobins (Hbs) not only in terms of primary sequence but also within secondary, tertiary and quaternary structure. This great range of variability is thought to reflect specialization and adaptation to the wide range of environments in which invertebrates operate [1]. For an extensive review of these structural variations the reader is referred to a recent review by Weber and Vinogradov [1]. Structurally, invertebrate Hbs occur in many types ranging from monomeric globins, through dimeric, tetrameric and polymeric forms to multi-domain subunits that can vary from 17 to 1700 kDa in size. Matching this variety in structure is an extended range of proposed globin functions including sulfide transport, acid–base balance, and a variety of roles in controlling O_2. However, in spite of this enormous variation there are certain features which are consistently maintained and some of which appear as distinctive motifs within subgroups. The unifying feature amongst all of these proteins is the presence of the "globin fold", a "helix on helix" structure which holds the heme prosthetic group in position by means of hydrophobic interaction. The invariant

* Corresponding authors. Tel.: +401 487 9934; fax: +787 833 0565.
E-mail addresses: alexpayson@yahoo.com (A.P. Payson), joeb@duke.edu (J. Bonaventura).

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doi:10.1016/j.jinorgbio.2004.12.001

residues within this fold are the Phenylalanine and Histidine at positions CD1 and F8, respectively [2]. Phylogenetic trees constructed on the basis of these structural similarities point to a single ancestral globin that possibly developed possibly as long as 3500 million years ago [3].

Despite the functional diversity which has been established for invertebrate Hbs, in general the reactivity of these Hbs with NO has not been considered. However, recently the involvement of Hbs in NO metabolism and protection from nitrosative stress has been highlighted [4–9]. These studies have led to the suggestion that these Hbs may be involved in redox-based functions. In this review we will first consider some of the structural features of invertebrate Hbs which may affect their interaction with NO. We will then consider three particular Hbs whose reactions with NO have been considered in detail: (1) The flavohemoglobin of *Escherichia coli* that provides protection from nitrosative stress; (2) Several plant Hbs which control NO levels; (3) The multimeric Hb found in the hemolymph of *Ascaris suum* in which NO regulates O_2 levels.

2. Structural features of the invertebrate Hbs

A recurring feature of many invertebrate Hbs is a hydrogen bonding network within the distal pocket (Fig. 1). Such a network, characterized by the presence of a glutamine at the E7 position and a tyrosine at the B10 position as in *Ascaris* Hb [10], results in a high O_2 affinity as a result of a low O_2 off rate. Furthermore, this network is thought to stabilize bound O_2 in a superoxide-like form and to inhibit its release thus making it resistant to autoxidation. However, this structural interaction hints at enzymatic functions rather than oxygen transport [11–13]. This motif has been observed in a number of invertebrate Hbs all of which possess a high affinity for O_2, including the truncated monomeric Hb of *Paramecium* [14]. Another feature of this motif is that the E7 glutamine residue has been proposed to facilitate the binding of NO to ferric heme [7].

Within the invertebrate Hbs there are three other basic classes of distal pocket architecture: (1) a tyrosine residue at both the E7 and B10 positions as demonstrated by the cytoplasmic Hb of the trematode *Paramphistomum epiclitum* [15]; (2) a glutamine in the E7 position with a non-polar residue in the B10 position, as seen in the dimeric Hb of the crustacean *Daphnia magna* [16], cytoplasmic HbI of the insect *Gastrophilus intestinalis* [17], and HbI of the mollusc *Calyptogena magnifica* [18]; (3) non-polar residues at both the E7 and B10 positions as exemplified by the monomeric Hb of the polychaete *Glycera dibranchiata* [19], which possesses a leucine in the E7 position, and the Hb of the mollusc *Aplysia*, which has an E7 valine (although there does appear to be a hydrogen bond from the flexible E10 arginine) [20,21].

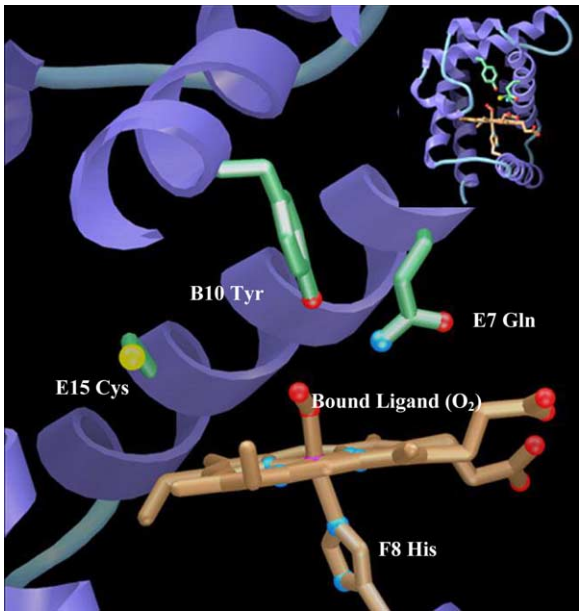

Fig. 1. The heme pocket structure as exemplified by *Ascaris* Hb A molecular model of the D1 subunit of *Ascaris* Hb is shown to highlight the residues of interest in invertebrate Hb reactivity (inset shows the heme pocket within the context of the globin domain). The F8 His, the so-called proximal his, is relatively invariant although can be substituted for by Cys. It is coordinated to the heme iron and provides much of the basis for controlling heme reactivity [34]. The residues B10 and E7 are manipulated throughout the invertebrate Hbs to alter the degree of interaction which occurs with bound ligand. When these residues are polar there is the ability to produce a hydrogen-bonding network that allows for electron drift between the heme iron and the ligand and stabilizes binding [61]. The E7 Gln allows for stabilization of the ferricnitrosyl intermediate [7,10]. When His is in the E7 position then there is the possibility for the formation of hexacoordinated Hb [27,28]. The presence of redox active Cys within the heme pocket allows for the generation of SNO and promotes redox activity [7,37].

From the evidence of prior studies one would predict that *Paramphistomum* Hb would have a high affinity for O_2 and a low rate of autoxidation [22], furthermore one would suggest that in contrast to *Ascaris* Hb Fe(III)NO complexes would not be stable in this protein. Interestingly, recent research has shown that the tyrosine residue at E7 is oriented out of the heme pocket, and that the B10 tyrosine alone is responsible for the stabilizing hydrogen bond in the heme pocket of *Paramphistomum* [23,24]. While *D. magna* is well known for its ability to increase concentrations of various Hbs at low O_2 levels [25], the possibility that NO plays a role in this response has not been examined yet. The HbI of Gastrophilus is particularly interesting as it does possess a high affinity for O_2 as a result of a reduced off rate but this must be through some other mechanism than an extended hydrogen bonding network as the B10 residue is non-polar. This is further demonstrated by its high rate of