The integrative and evolutionary biology of gas-binding copper proteins: an introduction

Heinz Decker,1,* Nora Terwilliger,† and Hans-Otto Pörtner§

*Johannes Gutenberg University Mainz, Germany; †University of Oregon, OR, USA; §Alfred Wegner Institut, Bremerhaven, Germany

Synopsis This article summarizes the contributions given at the symposium “The Benefits of Gas-binding Proteins. Integrative and Evolutionary Physiology of Copper Proteins: Molecules to Organisms and their Environment,” presented at the First International Congress of Respiratory Biology, August 14–16, at Bad Honnef/Bonn, Germany.

There are two major respiratory proteins, hemoglobin and hemocyanin. At the first glance they are different in color; upon oxygenation hemoglobin changes from dark red to bright red and hemocyanin changes from transparent to blue. The reason for this color difference is the metal to which a dioxygen molecule is reversibly bound in the active site of each protein. In the case of hemoglobin, oxygen binds to an iron atom sitting in the middle of a porphyrin ring—the heme group, while for hemocyanin, a molecule of oxygen is bound between two copper atoms. Hemoglobin molecules of vertebrates and of some invertebrates are transported within cells (erythrocytes) as small proteins with four subunits. In contrast, extracellular hemoglobins of invertebrates, with up to 144 oxygen-binding sites, and extracellular hemocyanins, with up to 160 oxygen-binding sites, bind oxygen with the highest Hill coefficients observed in nature. The molecular basis for this high cooperativity is still debated due to lack of detailed quaternary structures. This session of the ICRB meeting dealt only with hemocyanins.

In nine contributions the integrative and evolutionary physiology of copper proteins were highlighted, ranging from molecules to organisms in their environment. All contributions were summarized in three papers (Decker et al. 2007a; Terwilliger 2007; Melzner et al. 2007). Within the past 10 years great progress in hemocyanin research has been made, including the phenoloxidase activity of hemocyanin and its activation, the molecular basis of the catalysis of phenoloxidases, the homotropic and heterotropic interaction in a Nesting model, the reconstruction of hemocyanins with high resolution, and the importance of hemocyanins for the organism under specific environmental conditions.

Phenoloxidases comprise tyrosinases that catalyze monophenols to o-quinons in two reactions (cresolase and catecholoxidase activity) via diphenolic intermediates and catecholoxidases that catalyze only the second step as atmospheric oxygen is incorporated. In successive noncatalytical steps, melanin, a chemically resistant polymer net, is synthesized. Phenoloxidases occur in all organisms and are involved in essential biological functions such as immunological processes (strong antioxidative protection; attacking invaders with reactive quinones; encapsulation of invaders; protection against energy-rich UV light), wound healing and molting by crosslinking proteins with quinons in the sclerotization process. Although hemocyanins serve as oxygen carriers in both molluscs and arthropods, their structures, especially on the quaternary level, are different in the two phyla. Phenoloxidases and hemocyanins belong to the same copper type-3 family. They are characterized by a very similar active site (van Holde et al. 2001) in which one molecule of oxygen is bound in a side on coordination between two copper atoms.

In the first paper, the keynote speaker Nora Terwilliger postulated in her talk “Defense against the Dark Arts: Hemocyanins and the Immune Response” that recent outbreaks of infections including black-shell disease in crustaceans emphasize the need to better understand how arthropods cope with immunochallenges (Terwilliger 2007). Hemocyanins as well as phenoloxidases seem to be involved in crustaceans. In contrast, hemocyanin
appears to be the primary source of phenoloxidase activity in chelicerates and peracaridan crustaceans whose hemocytes show no phenoloxidase activity. There is hope for understanding these different behaviors through analysis of the evolution of these proteins (Terwilliger and Ryan 2006).

In a second session, concentrated research on hemocyanin in Mainz was presented (Decker et al. 2007). The conversion of an oxygen carrier to an enzyme exhibiting phenoloxidase activity was explained on a molecular basis (Elmar Jaenicke, speaker). In collaboration with Felix Tuczek (speaker, Kiel) the activation of phenoloxidases and hemocyanins as well as the molecular basis of the catalysis was presented, based on the important newly resolved crystal structure (Matoba et al. 2006), which had been thoroughly summarized recently (Decker et al. 2006, 2007a). Nadja Hellmann (speaker) explained the analysis of the complex oxygen-binding curves of hemocyanins on the basis of a Nested MWC model and described how allosteric interactions must be considered in this model. The introduction of the expanded Nesting theory and applications had recently been published with our collaborators Manfred Grishaber (Menze et al. 2005). New results were presented for the question of how two parameters, temperature and pH, and the interplay between them influence the oxygen-binding behavior of hemocyanins from animals occurring in different biotopes. It seems that maximum cooperativity is found under conditions specific for particular biotopes.

Results of a collaboration among research groups in Mainz and Padova, Italy (Mariano Beltramini, speaker), were presented. The question was addressed as to whether the large variety of structural architecture and functional plasticity can be simply interpreted in a context of phylogenetic relationships. The structural features and the physiological properties of the 4 × 6-meric hemocyanin isolated from Upogebia pusilla (Decapoda: Thalassinidea) were characterized (for review, see was Paoli et al. 2007).

Essential for the molecular interpretation of the cooperative interactions and conversion of hemocyanins in enzymes is the knowledge of the hemocyanin structures at a high resolution. Since it is very difficult to obtain crystals and to resolve crystals from very large proteins, cryo-electron microscopy has become an increasingly important technique (Ulrich Meissner, speaker). New results were presented for the largest arthropod hemocyanin (8 × 6-meric Hc from Limulus polyphemus) (Meissner et al. 2007) and the decameric hemocyanin from the ancient mollusc Nautilus (Martin 2007). The analysis unravels, in great detail, the architecture of the whole molecules and the contact regions between the components. Further study on both reconstructions under oxygenated and deoxygenated conditions may help to understand conformational transition at a better resolution. Another ongoing project in Mainz is the investigation of the respiratory protein hemocyanin from molluscs using cDNA and genomic DNA sequencing and phylogenetic tree reconstruction (Bernd Lieb, speaker). The data have provided strong support for the view that, during the late Precambrian, mollusc hemocyanin evolved by three subsequent gene duplications of a functional-unit (FU) precursor, leading to a subunit containing eight FUs. This probably was facilitated by two bordering introns still present as “linker” introns in all hemocyanin genes studied.

The third paper dealt with the common cuttlefish (Sepia officinalis) from the English Channel. This species has been shown, through noninvasive studies by nuclear magnetic resonance, to switch to an anaerobic mode of energy production when acutely exposed to critically low or critically high temperatures (Melzner et al. 2007). The data are in line with the recent concept of oxygen-limited and capacity-limited thermal tolerance, postulated to be unifying for water-breathing ectotherms if not for all metazoans (Pörtner 2001). The concept implies that upper and lower limits of thermal tolerance are set by limitations in aerobic scope, due to onset of a mismatch between supply and demand of oxygen and a reduced capacity to supply oxygen. These limitations bear consequences for the ecosystem, as the reduction in aerobic scope lowers functional capacity and fitness and leads to reduced survival in the field during thermal extremes (Pörtner and Knust 2007). In S. officinalis, the physiological data from the whole animal and the functional properties of hemocyanin indicate that blood-oxygen transport is set to be optimal within the thermal window and contributes to thermal limitation at temperatures beyond that level. The physiological and ecological relevance of the existence of hemocyanin isoforms within a panmictic population was discussed within the context of thermal adaption in S. officinalis (Frank Melzner and Felix Mark, speakers).

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