Scapharca HbI the direct structural linkage between the hemes therefore results in a tight functional coupling which does not permit regulation of oxygen affinity. Regulation can be achieved only by separating the heme groups via the globin moiety thereby taking advantage of its flexibility. It is no surprise that the mechanism underlying cooperativity in vertebrate tetrameric hemoglobins operates in this way.

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The dimeric and co-operative myoglobin of *Nassa mutabilis*. A peculiar case

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Abstract

The myoglobin present in the radular muscle of the Prosobranchia sea snail *Nassa mutabilis* is a peculiar case among myoglobins. It is a dimer showing co-operative oxygen binding equilibrium curves with $pO_{2_{1/2}} =$ 4.7 mmHg, invariant with pH, and $n = 1.6$. Although the globin is composed of 147 amino acid residues, corresponding to a molecular mass of 15760 D, gel filtration chromatography of the native myoglobin indicate $Mr = 26000 \pm 2000$ D. Similarly, acrylamide electrophoretic analyses in SDS and velocity sedimentation indicate a molecular mass of about 13000 D for the denatured globin. The molecule is highly unstable and forms slowly a chromogen when aged or immediately upon oxidation to the ferric state. The visible region of the absorption spectrum of the O_2 or CO liganded myoglobin derivatives indicate an altered heme environment. Circular dichroism analyses confirm this indication showing negative Cotton effects in all regions of the heme absorption spectra of the MbO_2 and MbCO derivatives. Interestingly, the CD spectrum of the oxidised met-form shows a positive band almost symmetrical with respect to that of the MbO_2 derivative. This is similar to what reported for the monomeric hemoglobin of *Glycera dibranchiata* for which a reversed heme orientation was proposed. Detailed resonance Raman spectroscopic studies have permitted a more direct investigation of the interactions between the heme and the protein. The proximal Fe-Im bond shows a stretching mode frequency down shifted by 5 cm^{-1} with respect to the corresponding band of horse heart myoglobin, in good correlation with the much higher instability of *Nassa m.* myoglobin and its much lower oxygen affinity. The unusual bond instability finds additional support in a kinetic study in which the myoglobin is mixed with CO in buffered solutions at different pH values. This approach gives evidence that the Fe-Im bond is broken upon lowering the pH, with a pK of 4.0 ± 0.2, the highest among those of deoxy hemoproteins. The rupture of the proximal bond appears to occur with a proton-linked transition showing $n = 1.8 \pm 0.1$, again indicating cooperativity between the two subunits. The vinyl and propionate heme substituents show resonance Raman spectroscopic bands indicating different modes of interaction with their environment with respect to other myoglobins. Most interestingly, the vinyl stretching mode frequency, typically a single band, appears split in two bands in *Nassa m.* myoglobin. This splitting is evident in all the investigated derivatives of the myoglobin, indicating that vinyl 2 and 4 are not equivalent in this molecule. A similar splitting has been found so far only in *Chironomus t.t.* hemoglobin.

The appearance of a chromogen electronic spectrum upon myoglobin oxidation finds a counterpart in the resonance Raman spectrum of the met-derivative of *Nassa m.* myoglobin that shows the properties of a six coordinated low spin heme, indicating the formation of a bond between the hem-iron and an endogenous distal ligand upon oxidation of the metal. The heme environment of *Nassa m.* myoglobin has been studied also by EPR spectroscopy of the NO derivative in phosphate buffer at different pH values. The results show a spectrum that is similar to that of monomeric mammalian myoglobins at pH 8.7, indicating an axial symmetry of the heme pocket. Upon lowering the pH, the g_x component of the spectrum changes and at pH 7 the pattern is similar to that

found in *Scapharca i.* hemoglobin. Further lowering of the pH produces changes in the g_z region and the spectrum acquires the typical features described for hemoproteins in which the Fe-Im bond is strongly weakened or broken. The pK of this transition is 6.7. No obvious kinetic counterpart of the co-operative oxygen equilibrium binding curves is found in *Nassa m.* myoglobin. No co-operativity and no quickly reacting species are observed in CO association or dissociation kinetic experiments. Oxygen dissociation displays biphasic time courses. The pattern is not affected by the initial pO_2 and is observed also in oxygen pulse experiments. This shows that two sites, with different oxygen affinity, are operative in the molecule. In contrast with what observed in other hemoproteins, the kinetic control of co-operativity in oxygen binding appears to depend on an increase in the 'on' rate of the high affinity site, rather than on the usual decrease of the 'off' constant. This is another peculiarity of this dimeric myoglobin. The pH dependence of the two rates is characterised by two distinct pK values: 6.7 for the fast and 8.5 for the slow phase. Arrhenius plots show the same activation energy for the two kinetic components, indicating that the difference in their rate constants is of entropic origin.

As a final point, the amino acid sequence of *Nassa m.* globin shows 63% identity with the globin of *Busycon c.* and 46% with that of *Cerithidea r.*, both Prosobranchia molluscs like *Nassa m.* In contrast, sequence conservation is less than 20% when the comparison is done with the myoglobins of the sea snails belonging to the Opistobranchia sub-class, like *Aplysia l.*, while molluscs of this sub-class have myoglobins with a very high sequence homology. A comparison of *Aplysia l.* myoglobin aminoacid sequence with the primary structure of *Scapharca i.* dimeric hemoglobin and with that of human myoglobin shows percent conserved residues similar to what observed comparing *Aplysia l.* myoglobin and *Nassa m.* myoglobin sequences, as if the separation between two sub-classes of sea-snails and between these and obviously more distant organisms, such as the Arcid clam *Scapharca i.* and the mammal human, was about the same.

Acknowledgements. The results discussed here have been obtained in collaboration with: Massimo Coletta, University of Camerino; Giulietta Smulevich, Anna Rita Mantini and Mauro Poli, University of Florence; Marina Piscopo and Rosanna del Gaudio, University of Naples; Alessandro Desideri, University of Messina; Francesca Polizio, Second University of Rome 'Tor Vergata'; Paolo Ascenzi, University of Turin.

Proteins - Paradigms of complex systems

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Introduction

The past decade has seen an emerging interest of physicists in complexity. Complex systems range from glasses and spin glasses to the brain. To study the physics of complexity calls for a system that is large enough to be truly complex, yet small enough so that it can be understood and managed experimentally. Proteins satisfy these conditions and their exploration, over wide ranges in time, temperature, and external conditions provide an insight into some of the salient features of complexity. The most conspicuous aspect is the existence of a rough energy landscape; proteins exist in a dynamic equilibrium among a very large number of slightly different structures. This characteristic appears to be the dominant property that marks all complex systems. The experiments with proteins are beginning to expose some crucial attributes of the energy landscape and they may form the beginning of a quantitative physics of complexity.

Complex systems

Complexity, in one form or another, is all around us. Music is complex and so are languages, economies, societies, and the brain. For many years, physical scientists shied away from complex systems and were content or even smug in concentrating their work on 'simple' systems. Within the last few decades, even physicists have started looking at complex systems and have begun to search for unifying concepts and laws. Such a search can start with two questions:

1) which are the best systems in which to study complexity? and
2) how do we study complexity?

The 'simplest' complex systems are probably glasses and spin glasses. Glasses, of course, have been known for a few thousand years and have been studied in great detail[1]. Despite the large number of investigations, many problems remain unsolved. Spin glasses,