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Genetic diversity of coastal bottlenose dolphins revealed by structurally and functionally diverse hemoglobins

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Abstract

Studies of structure–function relationships in the respiratory proteins of marine mammals revealed unexpected variations in the number and types of hemoglobins (Hbs) present in coastal bottlenose dolphins, *Tursiops truncatus*. We obtained blood samples from free-ranging coastal bottlenose dolphins as a component of capture–release studies. We found that the oxygen-binding functions of bottlenose dolphin blood are poised between effector-saturated and unsaturated levels, enabling exercise-dependent shifts in oxygen transfer functions. Isolated bottlenose dolphin Hbs showed elevated pH sensitivities (Bohr effects) and appreciably lower oxygen affinities than adult human Hb in the absence of allosteric effectors. These properties may be an adaptive modification that enhances oxygen delivery during diving episodes when oxygen tensions and effector levels are low. The Hbs of individual dolphins showed similar oxygen affinities, responses to effectors, and expression of heme–heme interaction in oxygen binding, but differed in their redox potentials and rates of autoxidation. The heterogeneity suggested by these functional variations in Hbs of individual dolphins was born out by variations in the molecular weights and numbers of their α and β globin chains. Although coastal bottlenose dolphins were expected to have a single type of Hb, the mass differences observed revealed considerable genetic diversity. There were multiple Hb forms in some individuals and differences in Hb patterns among individuals within the same community.

Keywords: Population diversity; Alleles; Oxygen binding; Allostery; Redox chemistry

1. Introduction

Bottlenose dolphins are widely distributed in both coastal and offshore waters throughout the world. Their relatively easy accessibility by researchers has made them one of the most frequently studied marine mammals (Leatherwood and Reeves, 1990). Despite this, few studies have sought to describe the characteristics of bottlenose dolphin hemoglobin (Hb). Our studies add to knowledge of these diving mammals by documenting exercise-dependent oxygen-binding functions of whole blood, by determining oxygen-binding and oxidative functions of bottlenose dolphin Hb in the presence and absence of allosteric effectors, and by showing an unexpected diversity in coastal bottlenose dolphins Hbs.

Hb is found in the erythrocytes of dolphins and other vertebrates and is responsible for the transport of oxygen from the lungs to the other tissues of the body. Increases in animal body

Abbreviations: Hb A_0; adult human hemoglobin; DPG; 2,3-diphosphoglycerate; IHP; inositol hexaphosphate; EDTA; ethylenediaminetetracetic acid.

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sizes and metabolic rates required the development of an efficient method of oxygen transport (Burggren et al., 1991). Hb has evolved to meet this need, with elegant molecular adaptations to serve diverse physiological and environmental challenges. Our studies of structure–function relationships of bottlenose dolphin Hbs show that genetic alterations have led to adaptive modifications in the Hb's intrinsic oxygen affinity that can enhance oxygen delivery to the tissues of these marine mammals during diving episodes. Other genetic alterations affecting Hb gene expression were found that are not uniformly distributed among individuals of the coastal dolphin communities examined. These variations are apparent in mass differences and significantly different redox potentials for bottlenose dolphin Hbs. These differences in the Hbs of individual dolphins do not appear to be adaptive, but can be understood in light of the theory of neutral evolution (Kimura, 1968).

Molecular adaptations of Hbs have been accomplished during evolution by genetic alterations of the protein's primary structure and its allosteric control mechanisms. Hb's intrinsic oxygen affinity is largely dictated by the local heme environments of the α and β globin chains that make up $\alpha_2\beta_2$ Hb tetramers. Allosteric control mechanisms are dictated by structural features that allow for interactions between the Hb subunits and the ability of effector molecules to modify oxygen affinity. Our study of the Hbs of the bottlenose dolphin, *Tursiops truncatus*, showed alterations of both intrinsic oxygen affinity and responses to allosteric effectors relative to adult human Hb.

We observed multiple numbers and types of Hbs in the coastal bottlenose dolphins studied, contrary to previous reports. Duffield and co-workers reported that coastal bottlenose dolphins, unlike offshore ecotypes, have a single type of Hb (Duffield et al., 1983; Duffield, 1986; Hersh and Duffield, 1990). Dolphin blood containing a single Hb type was used in characterizing the amino acid sequences of the α and β chains of bottlenose dolphin Hb (Kleinschmidt and Braunitzer, 1983). A single electrophoretic band was also found in a recent study of Hb isolated from an aquarium-bred coastal dolphin (Tellone et al., 2000). In surprising contrast to these prior reports, we observed considerable structural and functional heterogeneity among the Hbs isolated from individuals of two coastal bottlenose dolphin communities. Variations were observed in the number and type of α and β globin chains present in the blood of the individual dolphins sampled. The Hbs of this study also showed remarkable functional variations in their rates of autoxidation and the redox potentials that determine their thermodynamic propensity for oxidation. The Hb variations observed are indicative of considerable genetic diversity among the individual dolphins sampled in these two coastal dolphin communities. Further work will be required to determine if this diversity exists in other coastal bottlenose dolphin communities.

2. Materials and methods

2.1. Blood samples

A blood sample from a stranded bottlenose dolphin ("Buster") and blood from 9 coastal bottlenose dolphins sampled in Sarasota Bay in 1998 were used in initial stages of this investigation. Although Buster was stranded on a Gulf of Mexico Beach off Sarasota, FL, he was atypical of the long-term resident bottlenose dolphin community that primarily inhabits the bays, sounds, and estuaries near Sarasota. Notably, his treatment was complicated by his long inter-breath intervals while out of water, which is a characteristic of dolphins more accustomed to deep and long dive periods than dolphins of the coastal dolphin community that is resident in Sarasota Bay. The small samples from the 9 coastal dolphins were pooled and purified by FPLC chromatography prior to study. Three peaks were eluted (Peaks I–III) and found to be heterogeneous with regard to the number and types of molecular weights of globin chains they contained. Other bottlenose dolphin blood samples used in subsequent stages of this study were collected as part of a capture–release project undertaken by scientists working with the Chicago Zoological Society's Sarasota Dolphin Research Program, in Sarasota Bay, FL. The project's purpose is to assess the health of the resident bottlenose dolphin community (Wells and Scott, 2002; Wells et al., 2004). Thirteen animals were captured, sampled, and released during a 2 week period in June of 2003. An additional sample from a single individual was collected in early February of 2004. All animals were determined by morphological characteristics to be of the coastal ecotype. Blood samples were taken from each individual animal immediately after they were encircled by a seine net and placed on a research vessel (Pre-Rest samples). A second blood sample was drawn prior to the dolphin's release, roughly an hour and a half later (Post-Rest samples). The samples were collected in 10 mL sodium heparin tubes and shipped on ice at the end of each collection day for next-day evaluation of their blood oxygen-binding characteristics.

Blood samples were also obtained from five coastal bottlenose dolphins caught in coastal waters of New Jersey. Samples from these dolphins were collected and analyzed by scientists of NOAA's Southeast Fisheries Science Center, Beaufort, NC, as part of an evaluation of the stock structure of bottlenose dolphin populations on the Atlantic coast.

2.2. Sample treatment

Small samples of whole dolphin blood were analyzed for their oxygen-binding properties. The remainder was used for preparation of isolated Hbs. For this purpose the blood was centrifuged at 4000 rpm to separate the buffy coat from the red blood cells. The red blood cells were then washed repeatedly with 0.9% sodium chloride and re-centrifuged. The cells were then lysed and the Hb released was precipitated with ammonium sulfate and purified following standard procedures (Benesch et al., 1968). Anionic effectors were removed by putting hemolysates through an amberlite MB-3 column. Hb concentrations were determined spectrophotometrically and samples were placed in 0.001 M Tris–HCl buffer (pH = 8.3) for storage in liquid N_2. A small aliquot of the Hb isolated from individual dolphins was also subjected to structural analysis by electrospray ionization mass spectrometry.