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Molecular evolution of myoglobin upon land-to-sea transition of whales traced by the structure factor of small-angle X-ray scattering

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1 Introduction

Extant whales are capable of storing much oxygen in their muscles to dive deeply. The concentration of an oxygen-binding protein, myoglobin (Mb), in aquatic mammals is ~10 times higher than in terrestrial mammals [1]. Aquatic mammal Mb evolved so that it should not aggregate at high concentrations. That lets us ask what kind of molecular mechanisms underlies for that. To address this, we resurrected ancestral whale Mbs based on existing globin sequences (Fig. 1) [1]. Two resurrected Mbs (aMbWb′ and aMbWb) are the common ancestors of the toothed and baleen whale Mbs, which are closely related to Mb of *Basilosaurus* in the sea ~40 million years ago (Mya). The older Mb, aMbWp, is a further common ancestor of whale and hippopotamus Mbs, which would correspond to Mb of a quadruped terrestrial animal (*Pakicetus*) ~50 Mya.

In the present study, to track the molecular evolution of whale Mb, we determined the self-molecular interaction of these ancestral Mbs and the extant ~3000 m-diving sperm whale myoglobin (swMb) by using a small-angle X-ray scattering (SAXS). We analyzed the structure factors $S(q)$, i.e., inter-particle interference, derived from the radial distribution of the protein molecules in solution; the protein-self interaction governs the radial distribution. We determined a second virial coefficient (A_2), an indicator of repulsion ($A_2 > 0$) and attraction ($A_2 < 0$) of molecules in solution.

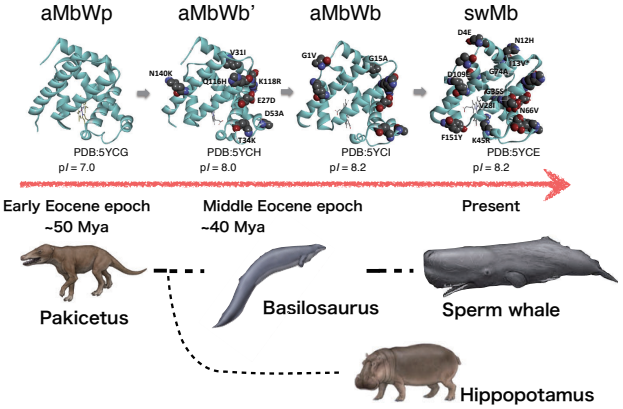

Fig. 1: Molecular evolution of whale Mb. The illustrations of animals are credited to Satoshi Kawasaki (https://paleontology.sakura.ne.jp).

2 Experiment

Mb proteins, aMbWp, aMbWb′, aMbWb, and swMb, were synthesized by using *E. coli* and purified as previously described [1]. The Mb solutions were dialyzed against a 2 mM HEPES-NaOH buffer solution (pH 6.8) at 4 °C for one day. The dialyzed Mb solutions were concentrated to ~3–5 mM, and then centrifuged. The Mb solutions were diluted to the desired concentrations at a pH of 6.9 ± 0.1, and irradiated with X-ray wavelength $\lambda = 0.15$ nm for 2 s in a cell with quartz windows using a sample-flow system (~14.5 μL/min) at 20 ± 0.1 °C. The SAXS experiment was performed at the beamline BL-10C. The camera length of 1 m was calibrated by use of a scattering pattern of silver behenate. X-ray intensities were recorded by a PILATUS3 2 M detector (DECTRIS Ltd., Switzerland). A total of 30 images were collected for each condition, and the circular 1D averaging of the images was performed with the program *Nika* [2]. The scattering parameter $q = |q| = 4\pi\sin\theta/\lambda$, where q is the scattering vector and 2θ is the X-ray scattering angle. The scattering intensity of the protein was converted to the absolute scattering intensity ($I(q)$) by use of water scattering as the standard [3].

3 Results and Discussion

$I(q)$ was extrapolated to the scattering intensity at $q = 0$, $I(0)$, which is related to A_2 as

$$I(0) = kMc_pS(0) \quad (1)$$

and

$$S(0) = 1 / (1 + 2A_2Mc_p) \quad (2)$$

where M is the molecular weight of the protein, c_p is the protein concentration, the k value is equal to $v^2(\varrho_m - \varrho_{solv})^2/N_A$, and $S(0)$ is $S(q)$ at $q = 0$. N_A is Avogadro's number, v is the partial specific volume of the protein (here, 0.7425 cm^3 g^{-1}), and $\varrho_m - \varrho_{solv}$ is the electron density difference between the protein and the solvent (2.8×10^{10} cm^{-2}) [4].

SAXS intensities in the small q region were depressed for all the Mbs by an increase of Mb concentration, indicating repulsive self-interaction between Mb molecules in solution. SAXS profiles of aMbWp in Fig. 2(a) were dependent on the Mb concentration to less extent than those of swMb in Fig. 2(b). Fig. 2(c) shows that A_2 of Mbs, with the oceanic adaptation of whales, increased, i.e., increased repulsion via the molecular evolution. Since the

net charge increase converges in the first half of evolution (aMbWp to aMbWb), the increase of repulsion in the second half of evolution (aMbWb to swMb) would not be satisfactorily explained by the change in the net charge. On the other hand, the structural stability was enhanced in the latter evolution, suggesting a correlation with it (see the reference [1]). For further study, we will progress SAXS studies on the other terrestrial and aquatic mammal Mbs.

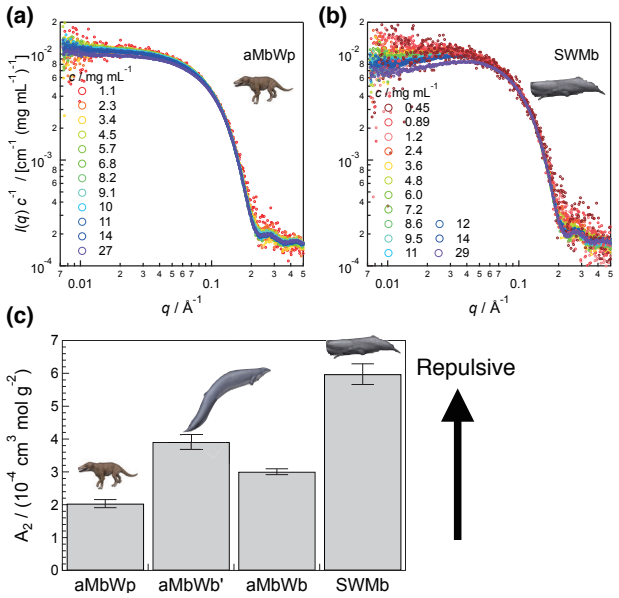

Fig. 2: SAXS analysis of the self-interaction of ancestral and extant Mbs. Concentration-dependence of SAXS profiles of (a) aMbWp and (b) swMb. Change in repulsive interaction between Mb molecules is represented by (c) A_2.

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