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

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1 Introduction

Myoglobin (Mb) is more concentrated in the tissues of aquatic mammals than in those of terrestrial mammals [1]. Highly concentrated Mb with its ligand O_2 enables the aquatic mammals to dive into the deep-sea. Tracing molecular evolution of Mb upon land-to-sea transition could provide molecular mechanisms of how proteins make them concentrated and escape from their self-aggregation.

A structure factor $S(q)$ in a small-angle X-ray scattering (SAXS) gives protein-self interaction such as a second virial coefficient (A_2). We previously found A_2 increased, i.e., being repulsive between Mbs, upon whale Mb evolution. In the present study, we measured SAXS of ancestral seal Mbs and extant ~1500 m-diving elephant seal Mb (Fig. 1) [2].

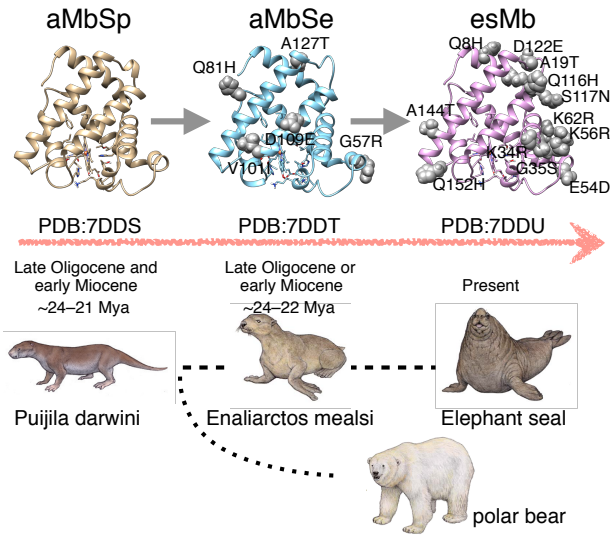

Fig. 1: Molecular evolution of seal Mb. The illustrations of animals are credited to Satoshi Kawasaki (https://paleontology.sakura.ne.jp).

2 Experiment

Mb proteins (aMbSp, aMbSe, and esMb; Fig.1) were synthesized using *E. coli* and purified as previously described [1,2]. The Mb solutions were dialyzed against a 2 mM HEPES-NaOH buffer solution (pH 6.8) at 4 °C for one day and diluted to the desired concentrations at a pH of 6.8 ± 0.2. The samples were irradiated with X-ray wavelength λ = 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 with a camera length of 1 m. X-ray intensities were recorded by a PILATUS3 2 M detector (DECTRIS Ltd., Switzerland). Circular 1D averaging of the images was performed with the program *Nika* [3]. 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 [4].

3 Results and Discussion

$S(0)$ was determined by extrapolating $I(q)$ and the equation, $I(0) = kMc_pS(0)$, where M is the molecular weight of the protein, c_p is the protein concentration, and the k value is equal to $v^2(\varrho_m - \varrho_{solv})^2/N_A$. 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}) [5]. The parameter A_2 was given by fitting analysis of $S(0)$ vs. c_p using $S(0) = 1 / (1 + 2A_2Mc_p)$.

Fig. 2(a), (b), and (c) show the Mb-concentration dependence of the SAXS profiles of aMbSp, aMbSe, and esMb, respectively. Repulsive Mb-self interaction for all

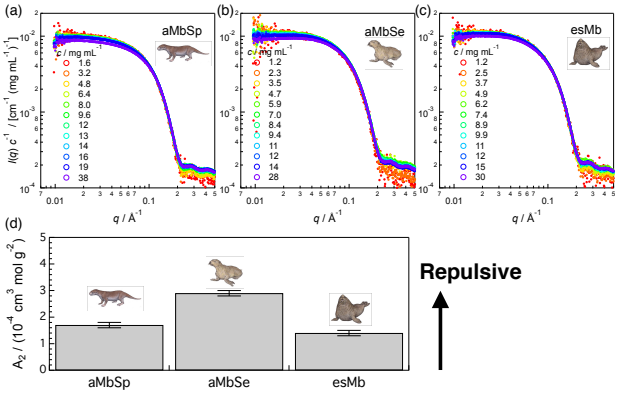

Fig. 2: SAXS analysis of the self-interaction of ancestral and extant seal Mbs. Concentration-dependence of SAXS profiles of (a) aMbSp, (b) aMbSe, and (c) esMb. Change in repulsive interaction between Mb molecules, which is represented by (d) A_2.

the Mbs is identified by a decrease in SAXS in the small q region by increasing Mb-concentration. Early Mb evolution upon the land-to-sea transition (aMbSp to aMbSe) indicates an increase in A_2, i.e., gaining the repulsive character. This is similar to whale Mb evolution [1]. On the other hand, against expectations late Mb evolution upon diving to deep-sea (aMbSe to esMb) does not show the A_2 increase.

Acknowledgement

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