Entropy-driven ring-opening polymerization of the cyclic hemoglobin monomer containing a high molecular weight PEG

(*Department of Chemistry, Nara Medical University*) ○Takashi Matsuhira, Hiromi Sakai
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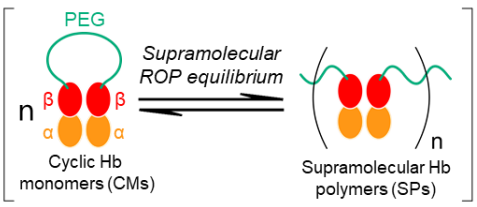
A hemoglobin (Hb) molecule consists of an $\alpha_2\beta_2$ tetramer which dissociates reversibly into two αβ dimers ($2\alpha\beta \leftrightarrows \alpha_2\beta_2$). We previously reported that an exchange reaction of dimeric αβ subunits proceeds between native Hb and Cys-93(β) PEGylated Hb at a sub-millimolar concentration although they fundamentally favor stable $\alpha_2\beta_2$ tetrameric structures.[1] Moreover, we revealed that a cyclic Hb monomer (CM), in which two Cys-93(β) residues in an $\alpha_2\beta_2$ tetramer were connected through a flexible PEG chain, undergoes ring-opening polymerization (ROP) to form a linear supramolecular polymer (SP) with Hb−PEG alternating structure through the reversible inter-molecular exchange of αβ subunits (Fig. 1).[2] In the present work, thermodynamics of supramolecular ROP was evaluated for four CMs containing high molecular weight PEGs (2, 5, 10, or 20 kDa).

The equilibrated mixture of the produced SPs and the remained CMs were quantified by covalent fixing method[1,2] using site-specific ββ-cross-linker, bis(3,5-dibromosalicyl) fumarate (DBBF). The ββ-cross-linking was conducted at different monomer concentrations $[M]_0$ and at different temperatures. Then, the cross-linked products were quantified using size exclusion chromatography. When DBBF was reacted at a lower $[M]_0$, a fixed CM was obtained mainly. In contrast, a reaction with DBBF at a higher $[M]_0$ generated dominantly a fixed SP. The results indicate the existence of ROP equilibrium depending on $[M]_0$. We found that the critical monomer concentration $[M]_{crit}$, a threshold for starting ROP, decreased with increasing of the ring size. Additionally, Van't Hoff plots for ROP equilibrium constant at $[M]_0$ = 1 mM revealed negligibly small enthalpy changes (ΔH_p < 1 kJ·mol^{-1}) and considerably positive entropy changes that increased with the ring size (ΔS_p = 26.8−33.2 J·mol^{-1}·K^{-1}) (Fig. 1). These results indicate that the entropy-driven mechanism governs supramolecular ROP, and a CM with a larger ring size prefers to convert to a SP.



Cyclic Hb monomers (CMs)	$[M]_{crit}$ (mM)	ΔH_p (kJ·mol^{-1})	ΔS_p (J·mol^{-1}·K^{-1})
Hb-PEG2k	0.86	< 1	26.8
Hb-PEG5k	0.33	< 1	27.3
Hb-PEG10k	0.13	< 1	28.5
Hb-PEG20k	0.063	< 1	33.2

Fig. 1 Supramolecular ring-opening polymerization (ROP) equilibrium of cyclic Hb monomers (CMs) and supramolecular Hb polymers (SPs), and summary of their thermodynamic parameters.

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(2) Matsuhira, T.; Yamamoto, K.; Sakai, H. *Biomacromolecules* **2019**, *20(4)*, 1592–1602.