Therapeutic Hydrogen Sulfide Delivery System based on Polymeric Micelles

André J. van der Vlies1,2 and Urara Hasegawa2,3

1Frontier Research Center, Graduate School of Engineering, Osaka University, 2Department of Applied Chemistry, Graduate School of Engineering, Osaka University, 3Frontier Research Base for Global Young Researchers, Graduate School of Engineering, Osaka University
2-1 Yamadaoka, Suita, Osaka 565-0871, Japan
urara.hasegawa@chem.eng.osaka-u.ac.jp

ABSTRACT SUMMARY
Hydrogen sulfide (H2S) has been recently identified as the third gas transmitter which controls the immunological, cardiovascular and nervous systems in mammals. This study aims to develop novel polymeric micelles for H2S delivery. Well-defined amphiphilic diblock copolymers (PEG-b-PADT) composed of a hydrophilic poly (ethylene glycol) block and a hydrophobic H2S-releasing block bearing anethole dithiolethione (ADT) moieties were synthesized. The polymers formed micelles of 30 nm in diameter with a narrow size distribution. In murine macrophages, the micelles enhanced the lipopolysaccharide (LPS)-induced proinflammatory responses without obvious toxicity.

INTRODUCTION
With the discovery of important biological roles of endogenously-produced gases, their use as therapeutic agents has attracted growing attention. Among them, hydrogen sulfide (H2S) has recently emerged as the third gaseous signaling molecule in mammals. H2S has been shown to regulate inflammation, relax vascular smooth muscles, promote angiogenesis and mediate neurotransmission.1 Although the precise mechanisms of its actions are not fully understood, the accumulating evidence that H2S regulates the immunological, cardiovascular and nervous systems clearly shows the high potential of H2S-based therapy. An increasing number of H2S donors, inorganic/organic sulfur compounds that release H2S under physiological conditions, have been developed to investigate the H2S biology and evaluate its therapeutic potential.2 However, poorly-controlled pharmacokinetic profiles of these donors often result in adverse effects and low therapeutic efficiency which limits their practical applications. In addition, it seems that the effects of H2S are not only dependent on H2S concentration but also on the duration of exposure to H2S. Therefore, it is of importance to develop novel systems for H2S delivery to the specific sites in the body over a certain period of time at the right concentrations.

In the field of drug delivery, polymeric nanoparticles, generally in the 10-100 nm range, are often used to deliver a wide variety of drugs including anti-cancer drugs, nucleic acids and proteins. They have been shown to be powerful in overcoming many intrinsic problems of small drugs including rapid diffusion throughout the body, short circulation time in the blood stream and low bioavailability. Among known nanoparticles, polymeric micelles, spherical supramolecular assemblies from amphiphilic block copolymers, have been recognized as one of the most promising drug carriers due to their unique characteristics such as easy formulation and functionalization, high colloidal stability, high drug loading capacity and low toxicity.

Here we report a novel H2S delivery system based on polymeric micelles carrying anethole dithiolethione (ADT) moieties which release H2S (Figure 1). The proinflammatory effects in macrophages were also examined.

Figure 1 H2S-releasing micelles.
EXPERIMENTAL METHODS

Diblock copolymers composed of poly(ethylene glycol) (PEG) and poly(glycine acrylamide tert-butyl ester) (PEG-b-P(Gly-OtBu)) were synthesized via reversible addition-fragmentation transfer (RAFT) polymerization of glycine acrylamide tert-butyl ester using PEG-pyrorlecarbodithioate as a chain transfer agent and 2,2'-azobis(isobutyronitrile) (AIBN) as an initiator. The tert-butyl ester protecting groups were removed in TFA/H₂O (9:1). The resulting polymers were further modified with ADT derivatives to yield amphiphilic diblock copolymers bearing H₂S-releasing ADT moieties (PEG-b-PADT). PEG-b-PADT block copolymers were characterized by ¹H NMR and gel permeation chromatography (GPC). The H₂S-releasing micelles were prepared by adding a PEG-b-PADT/DMF solution dropwise to milliQ water followed by dialysis and characterized by dynamic light scattering (DLS) and transmission electron microscopy (TEM). The effects on lipopolysaccharide (LPS)-induced nuclear factor-κB (NF-κB) activation and proinflammatory cytokine production in murine macrophages, RAW Blue cells, were assessed by Quanti Blue and ELISA assays. Cytotoxicity of the micelles was analyzed by MTT cell viability assay.

RESULTS AND DISCUSSION

We first prepared PEG-b-P(Gly-OtBu) block copolymers by RAFT polymerization. The polymerization proceeded quantitatively in well-controlled manner as shown by ¹H NMR and GPC. PEG-b-P(Gly-OtBu) were treated with TFA and further modified with ADT derivatives. The degree of modification was about 70% by ¹H NMR.

PEG-b-PADT was dispersed in water to form H₂S-releasing micelles. DLS showed that the micelles were monodisperse with a Z-average diameter of 30 nm (Figure 2 (a)). Further characterization by transmission electron microscopy (TEM) confirmed that the polymers formed spherical micelles with a narrow size distribution (Figure 2 (b)).

CONCLUSION

We developed a micellar form of H₂S delivery system. The H₂S-releasing micelles regulated the immunological responses and showed low cytotoxicity in murine macrophages. This H₂S delivery system may offer a safe and efficient delivery of H₂S in immunotherapy.

REFERENCES

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