Recent advances in the delivery of hydrogen sulfide via a macromolecular approach

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Recent advances in the delivery of hydrogen sulfide via a macromolecular approach

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Engineered macromolecules such as polymer conjugates, micelles, hydrogels and nanofibers continue to attract interest as drug delivery systems due to their potential to improve therapeutic outcomes. One area where engineered macromolecules have considerable potential is in the delivery of endogenous signalling molecules, such as hydrogen sulfide (H_2S). Until recently the application and investigation of H_2S was limited to the use of small molecule donors, although the use of macromolecular systems may confer considerable advantages. This mini review provides a brief overview of recent advances in the use of macromolecules as H_2S donors. In particular, we examine the use of materials that release H_2S both spontaneously and in response to chemical or physical triggers. In many of the highlighted examples macromolecules are reported to provide precise, controlled and sustained production of H_2S, demonstrating their potential as advanced platforms for H_2S delivery.

Introduction

Hydrogen sulfide (H_2S) is an important signalling molecule that elicits effects across a wide range of biological pathways. Abe and Kimura first identified the importance of H_2S as a biological signalling molecule in the nervous system in 1996,[1] with most studies prior to this focusing on its toxic effects[2] and paying little attention to any potential physiological function. The discovery of the role of H_2S in endogenous signalling pathways has led to it joining carbon monoxide (CO) and nitric oxide (NO) in the family of gaseous signalling molecules collectively referred to as the gasotransmitters. Since the initial report, H_2S regulation has become the focus of intense research, and H_2S-mediated signalling has been associated with a wide array of biological events. Moreover, exogenous delivery of H_2S has been demonstrated to provide anti-inflammatory,[3] cardioprotective,[4] vasorelaxant,[5] neuroprotective[6] and anti-oxidant[7] effects. As such, the exogenous delivery of H_2S has been investigated for the treatment of a number of pathologies including Alzheimer's disease[8], Parkinson's disease[9] and diabetes[10].

In biomedical research, a variety of different H_2S donors have been applied.[11] Such agents are not only useful research tools to investigate the biological effects of H_2S in cells and tissues, but also serve as potential therapeutic agents. The simplest, although arguably most impractical means of H_2S administration, is via inhalation. However, this approach is complicated by the toxic and malodorous nature of H_2S, as well as loss of bioactivity due to oxidation. As an alternative, inorganic sulfide salts have been explored, although these release HS^- and H_2S immediately upon solvation, leading to an essentially step-change increase in H_2S concentration. To overcome these limitations, a number of "slow release" small molecule H_2S donors have been developed such as the Lawesson reagent derivative GYY4137[12] (row 1, Table 1) to more closely mimic controlled endogenous production. Some of these donors exhibit suitable H_2S release profiles, although many still suffer from the inherent limitations of small molecule drugs including poor water solubility, high clearance rates and toxicity. For comprehensive reviews on small molecule H_2S donors the reader is referred to previously published works.[11,14]

The use of macromolecular H_2S donors presents an opportunity to overcome or mitigate some of the challenges associated with small molecule and inorganic donors.[15] For example, conjugation of a hydrophobic small molecule to a hydrophilic polymer is a simple way of overcoming limits in water solubility. Likewise, incorporation of a readily cleared small molecule into macromolecular structures can drastically alter its pharmacokinetics.[16] The application of a macromolecular platform has been aided by the development of advanced polymerisation methods such as reversible addition–fragmentation chain transfer (RAFT) which has led to the synthesis of an array of macromolecules with well-defined structure and properties.[17] From the simplest drug-polymer conjugates, to more complex macromolecular architectures, polymeric platforms have emerged as highly promising

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delivery vehicles for drugs. Furthermore, complex architectures which respond to biological cues such as intracellular thiols, find application as smart drug delivery vehicles.[18] In terms of H_2S delivery, the ideal macromolecular donor should deliver a precise amount of H_2S preferably in response to a specific trigger.[14] The donor should also exhibit good pharmacokinetic parameters and degrade into essentially non-toxic products.

Herein, we examine recent progress in the development of H_2S donating macromolecules, based on known donors as listed in Table 1. We explore donors that undergo non-specific hydrolysis to release H_2S, such as thiobenzamides and the dithiolethione derivative ADT-OH. We also discuss those triggered by thiols such as S-aroylthiooxime (SATOs), and trisulfides which offer the advantage of stimulated H_2S release in areas of high thiol concentration such as the intracellular environment. An example of nucleophile triggered release of an H_2S precursor from N-thiolcarboxyanhydride is also highlighted. The application of the light and amine triggerable donor, thiobenzaldehyde, is also discussed (not shown in Table 1). Finally, we present examples wherein a non-conjugated small molecule donor is encapsulated in a polymeric matrix. In many of the highlighted examples, conjugation of the H_2S donor to a macromolecular scaffold reduces toxicity compared to the unconjugated donor, provides a more controlled H_2S release profile and alters biological activity compared to the corresponding small molecule.

Hydrolytically Active Macromolecular Donors

(4-Methoxyphenyl)-3*H*-1,2-dithiole-3-thione (also known as anethole dithiolethione, ADT) and 5-(4-hydroxyphenyl)-3*H*-1,2-dithiole-3-thione (ADT-OH) have been applied as donors of H_2S, belonging to the class of compounds incorporating the 3*H*-1,2-dithiole-3-thione (DTT) moiety. DTTs themselves are better known in the pharmaceutical arena as cancer chemopreventive agents and, as such, have been the subject of considerable biochemical investigations.[19] Nonetheless, their extremely complex chemistry is not fully understood. In terms of H_2S donating ability it has been suggested that DTTs are able to do so hydrolytically in buffers such as PBS, whereby the thiocarbonyl is converted to a carbonyl group.[20] Time course analysis of the rate of hydrolysis has shown it to be a slow process, hastened only by temperatures over 100°C.[21] However, the predominant mechanism for the release of H_2S *in vivo* and *in vitro* is thought to involve reducing enzymes, as has been reported to occur in rat liver homogenate, rat plasma as well as isolated mitochondria from human astrocyte U373 cells.[20,22] The 1,2-dithiole-3-thione moiety itself can also react with thiols in a number of ways suggesting possible interactions with target proteins could be via cysteine residues, as well intracellular thiols such as glutathione.[23]

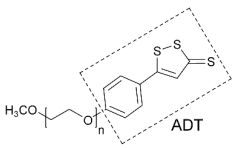
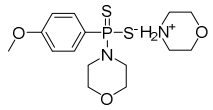
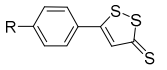
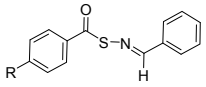
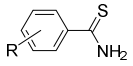
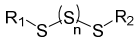
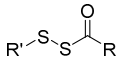
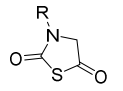
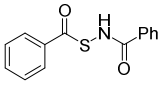
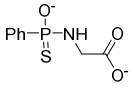


Figure 1. ADT-PEG conjugate[24]

Table 1: Known H_2S donors, some of which have been applied as polymer conjugates

H_2S Donor Moieties	Release Mechanism
 **GYY4137**	Hydrolysis mechanism involving oxidation of donor
 **1,2-Dithiolethiones (DTTs)** R=OH (ADT-OH), H, CH_3, halo, NH_2, OCH_3 or polymer conjugation	Hydrolysis mechanism involving oxidation of thiocarbonyl group is one possibility, although not significant. Reaction with thiol (mechanism unknown).
 **S-Aroylthiooximes (SATOs)** R= H, CH_3, halo, CN, OCH_3 etc. or polymer conjugation	Reaction of thiol with acyl group followed by rapid S→N acyl transfer. Decomposition of products gives rise to H_2S
 **Arylthioamides** R = H, OH, OCH3, halo etc. or polymer conjugation	Hydrolysis mechanism involving oxidation of donor. Reaction with thiol (mechanism unknown)
 n= 1,2 **dialkyl trisulfides, dialkyl tetrasulfides and related polysulfide species.** R_1 and R_2 = various alkyl substitutions (such as in diallyl trisulfide DATS) or conjugated polymer	Exchange reactions with thiols produce persulfides (R-SSH) which release H_2S via further reaction with thiols.
 **Acyl-protected perthiol** R= acyl group, usually phenyl; R'= acyl group or conjugated polymer	Exchange reactions with thiols produces persulfides (R-SSH) which release H_2S via further reaction with thiols.
 ***N*-thiocarboxyanhydrides (NTAs)** R= CH_3 or conjugated polymer	Carbonyl sulfide (COS) released upon ring-opening by a nucleophile. Conversion of COS into H_2S occurs by rapid enzymatic conversion (via carbonic anhydrase) or via a slow hydrolysis mechanism.
 ***N*-(benzoylthio)benzamide (NSHD1)**	Exchange reactions with thiols produces persulfides (R-SSH) which release H_2S via further reaction with thiols.
 **JK1**	pH-dependent (acid) intramolecular cyclization promotes H_2S release.

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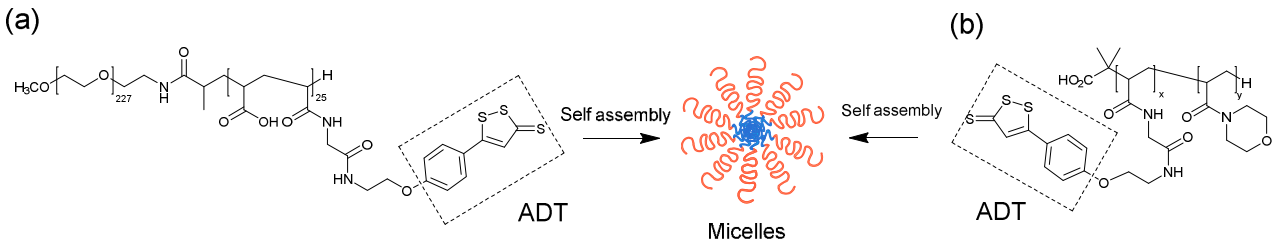

Figure 2. a. PEG-PADT block copolymer b. PAM-PADT block copolymer.[25,26]

In 2014, Hasegawa and van der Vlies reported the conjugation of ADT-OH to poly(ethylene glycol) (PEG).[24] The conjugated ADT was constructed using a non-cleavable ether bond linking ADT and PEG (Figure 1). This was favoured over an ester bond due to hydrolytic stability of the resulting construct. ^{1}H NMR and high resolution mass spectrometry were used to confirm successful nucleophilic substitution of PEG mesylate with ADT–OH, affording the desired PEG–ADT. The synthesised donor conjugate was observed to have lower H2S generating capacity than its parent compound, ADT-OH. However, encouragingly, the authors also reported PEG-ADT as having a reduced toxicity relative to ADT–OH. This observation was attributed to localisation of PEG-ADT within endosomes compared to the freely diffusing ADT-OH leading to a reduced propensity to react with intracellular proteins. This early study exemplifies the potential utility of using macromolecular H_2S to offset the toxicity which can be associated with small molecule analogues of the same donor.

In subsequent studies, the same group prepared ADT-functionalised micelles using various methodologies (Figure 2).[25,26] In the first instance, RAFT polymerisation was used to prepare copolymers incorporating hydrophilic polyethylene glycol (PEG) and hydrophobic dithiolethione-bearing (PEG-PADT) segments which formed micelles with an average hydrodynamic diameter of 36 nm, as measured by dynamic light scattering (DLS) (Figure 2a). The authors reported that the donors were stable in cell culture medium and only released H_2S in the presence of the cell lysate of rat cardiomyocytes, suggesting that reaction with intracellular components was a requirement for H_2S release. Interestingly, the authors also reported that in contrast to the possibility of cellular damage induced by NF-kB activation by ADT-OH, no such effect was induced by ADT micelles. This highlights the capacity to reduce the cytotoxicity of ADT-OH by incorporation into micellar structures. In the second example, copolymers of hydrophilic poly (*N*-acryloyl morpholine) and hydrophobic dithiolethione-bearing monomer (PAM-PADT) were synthesised (Figure 2b). These copolymers also formed micelles with an average hydrodynamic diameter of 34 nm. The authors reported that these ADT micelles were effective at protecting cells from apoptosis under ischemic conditions at ADT concentrations as low as 0.1 µM, a result attributed to sustained H_2S release.

Another class of donors which contain a thiocarbonyl group and release H_2S via an as-yet-unknown mechanism are thiobenzamides (also known as aryl thioamides).[27] The H_2S release profiles of a library of small molecule arylthioamides was tested in buffer, using an amperometric sensor, both in the presence and absence of L-cysteine. Interestingly, a majority of the structures tested produced significant, yet sustained levels of H_2S in the presence of thiol and gave reduced levels in buffer alone. However, this was found to be highly dependent on electronic substitution on the aromatic ring. One of the lead compounds of the study was found to strongly abolish noradrenaline-induced vasoconstriction in isolated rat aortic rings and hyperpolarized the membranes of human vascular smooth muscle cells in a concentration-dependent fashion, an anticipated physiological effect of H_2S signalling. This was achieved without any added thiols indicating the applicability of these class of compounds as potent H_2S donors in a biological setting.

In 2015 Ercole *et al.* employed thiobenzamides to produce H_2S donating macromolecules.[28] In this case the thiobenzamide H_2S donor groups were introduced via thionation of benzonitrile containing polymeric precursors. A post-polymerisation approach was chosen over directly polymerising thioamide functionalised monomers to avoid potential interference of the thiocarbonyl groups with radical reactions which are essential to the polymerization reaction. Specifically, 3-(4-cyanophenoxy)propyl methacrylate (CPPMA), incorporated into either the hydrophilic or hydrophobic domain of a block copolymer, was subjected to thionation. Incorporation of the H_2S donor moieties into either the hydrophilic or hydrophobic segments enabled preparation of micelles wherein the H_2S releasing moiety was resident in either the core or corona (Figure 3). The resulting copolymers were shown to exhibit slow H_2S release upon hydrolysis and a more rapid release when triggered by cysteine, as measured using an amperometric sensor.

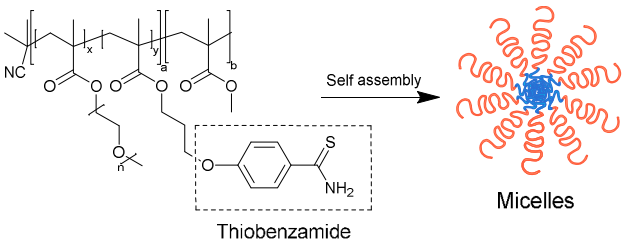

Figure 3 Aryl thioamide-containing block copolymer[28]

Notably, this study also examined the propensity of the macromolecular H_2S donors to trigger subcellular signalling events. Macromolecular H_2S donors with the thiobenzamide localised to the micelle corona elicited a slow and sustained increase in cytosolic ERK signalling and a small, fast and sustained increase in plasma membrane-localized PKC activity immediately following addition to HEK293 cells. Studies using a H_2S-selective fluorescent probe in live cells also confirmed release of H_2S over physiologically relevant time scales.

Also in 2015, Bowden and co-workers examined the incorporation of thiobenzamide H_2S donors into macromolecular structures.[29] This was achieved by copolymerising L-lactide with a 4-alkoxythiobenzamide-functionalised lactide derivative. To yield the desired monomer, thiobenzamide was conjugated to lactide by employing a mild reaction of propanedithiol with catalytic I_2. The copolymerisation was then optimised in CH_2Cl_2 achieving a 96% conversion with dimethylaminopyridine (DMAP) catalyst. The polymer formed microparticles, although attempts to detect H_2S liberated from them were unsuccessful. The authors suggested this was due to the low loading and slow release of donor as well as loss of H_2S either to the atmosphere or via oxidation. It should be noted that no thiol was added during their testing which may account for the low levels of H_2S released. Nevertheless, this constitutes the first attempt to incorporate H_2S donor moieties into biocompatible polyesters via ring opening polymerization.

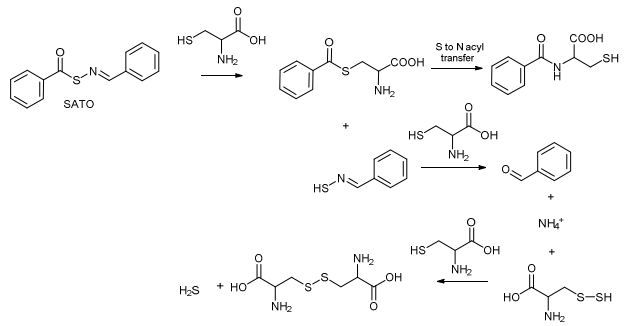

Figure 4. Proposed route of H_2S generation from SATOs.[31]

Macromolecular H_2S Donors triggered by Thiols

In 2014, Foster and Matson reported the first H_2S-releasing polymer for biological applications which could only be triggered by thiols (*i.e.* no hydrolytic pathway for H_2S release occurs).[30] These polymers contained *S*-aroylthiooxime (SATO) functionality which had previously been shown to exhibit H_2S release upon exposure to the thiol (L-cysteine).[31] The release mechanism is thought to occur by the pathway as shown in Figure 4 as elucidated by analysis of degradation products. Notably, the reversible thiol exchange between the SATO and cysteine gives the arylidenethiooxime along with *N*-benzoylated cysteine via an S- to N-acyl transfer step, similar to native chemical ligation. The subsequent reaction of a second equivalent of cysteine with the thiooxime generates cysteine perthiol, ammonia, and the original aldehyde. The perthiol intermediate then reacts with another equivalent of cysteine to generate cystine and H_2S.

To prepare the SATO-functionalised polymers, the authors employed a post-polymerisation modification of pendant aldehyde functionalised polymers which were prepared by RAFT polymerisation. The desired material was generated in three steps (Figure 5). First, the aldehyde containing monomer 2-(4-formylbenzoyloxy)ethyl methacrylate (FBEMA) was copolymerised with 2-(2-methoxyethoxy) ethyl methacrylate (MEO_2MA). Secondly, the thiocarbonyl-thio end-groups were removed using an excess of AIBN to exclude the possibility of end-group reactions with thiol triggers. Finally, the copolymers were treated with three different *S*-benzoylthiohydroxylamines (SATHAs) in the presence of catalytic trifluoroacetic acid (TFA) to form the thiooximes. The resulting polymers were shown to undergo thiol-triggered decomposition to release H_2S by reaction with cysteine and glutathione. Moreover, the kinetics of H_2S release, as measured with an amperometric microelectrode, were shown to be affected by the electronics of the thiooxime ring, consistent with observations from the initial study on small molecule donors. Specifically, electron-donating groups at the para- position of the SATHA-derived ring were shown to slow the rate of H_2S release.

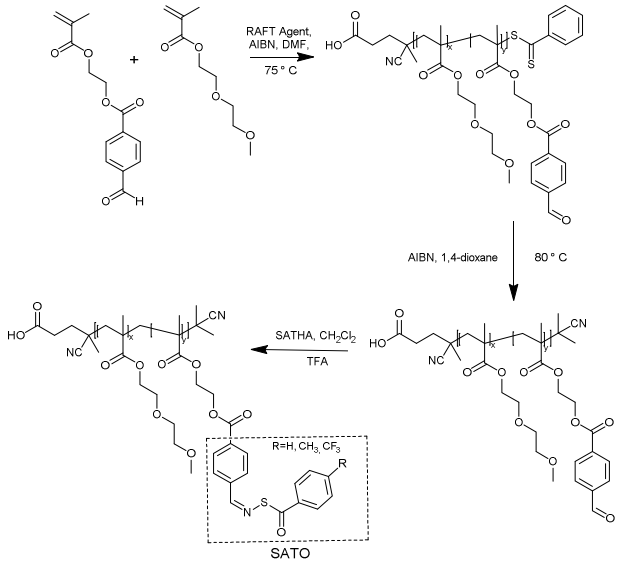

Figure 5. Synthesis of *S*-aroylthiooxime (SATO) functionalised polymers.[30]

In a subsequent study, the same group examined SATO functionalised amphiphilic block copolymer micelles as an advanced H_2S delivery vehicle to overcome some of the limitations of small linear polymers (i.e. clearance).[32] Polymeric micelles in general, especially those with a PEG coating, exhibit prolonged circulation time in blood compared to small molecules.[33] This material was prepared using the same general approach outlined above. However, in this case, FBEMA was polymerised using a PEGylated chain transfer agent to produce a block copolymer, PEG-*b*-poly(FBEMA). The pendant aldehydes in the resulting polymer were then reacted

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to yield thiooxime functionalised amphiphilic block copolymers which, due to the hydrophobicity of the SATHA pendants, formed micelles. The kinetics of H_2S release was measured using a methylene blue assay in which the rate of formation of the blue-coloured dye is monitored spectrophotometrically (UV-Vis) and correlates to the rate of H_2S release. Upon reaction with thiols, the polymer micelles showed slower and more sustained H_2S release compared to a related small molecule SATO. A pseudo-first-order kinetic plot of H_2S release, as generated using a methylene blue assay, gave a 9-fold increase in the half-life of H_2S release for the micelles compared to the corresponding small molecule (i.e., 3.3 ± 0.4 h *vs.* 22 minutes for the SATO molecules). The authors attributed this observation to the slower diffusion of thiols into the hydrophobic cores of the polymer micelles.

Very recently, Lu *et al*. reported the first self-fluorescent polymeric H_2S donor (Figure 6).[34] Similar to the previous study the authors employed a post-polymerisation modification to install a SATO donor group into the macromolecular structure. However, in this example, a salicylaldazine aggregation induced emission (AIE) fluorophore was also incorporated to facilitate intracellular tracking of the donor. Specifically, RAFT polymerization was used to polymerise well defined poly (3-formyl-4-hydroxybenzyl methacrylate) FHMA. The salicylaldehyde groups in the synthesised polymer served as handles for the sequential attachment of aminooxy-terminated PEG (PEG-ONH_2), hydrazine and *S*-benzoylthiohydroxylamine (SBTHA) to introduce water-solubility and biocompatibility, AIE self- fluorescence and H_2S donating capability, respectively.

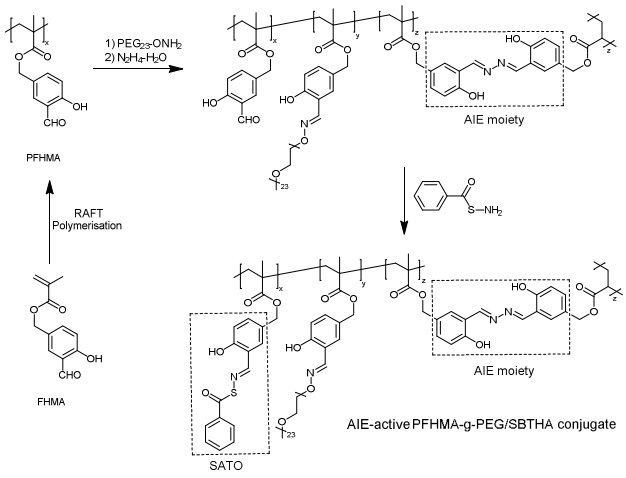

Figure 6. Synthesis of self-fluorescent S-aroylthiooxime (SATO) functionalised polymers.[34]

The resulting polymer formed nanoscale aggregates when dispersed in water, as confirmed by both scanning electron microscopy (SEM) and dynamic light scattering (DLS) (which gave an average hydrodynamic diameter of ~141 nm). The PFHMA-*g*-PEG/SBTHA conjugate also showed good biocompatibility to L929 cells, which the authors attributed to the PEG side chains. Upon the addition of a thiol the nanoparticles were reported to disassemble, increasing access of cysteine to the SATO groups, which the authors suggest is responsible for the relatively high release rate observed. Polymer solutions with an equivalent SATO functional group concentration of 100μM produced a peaking H_2S concentration value of 80 μM after 55 min in the presence of 1 mM cysteine, as determined using a methylene blue assay. Finally, the authors demonstrated the internalization of the donor polymers by exploiting the AIE behaviour using fluorescence imaging.

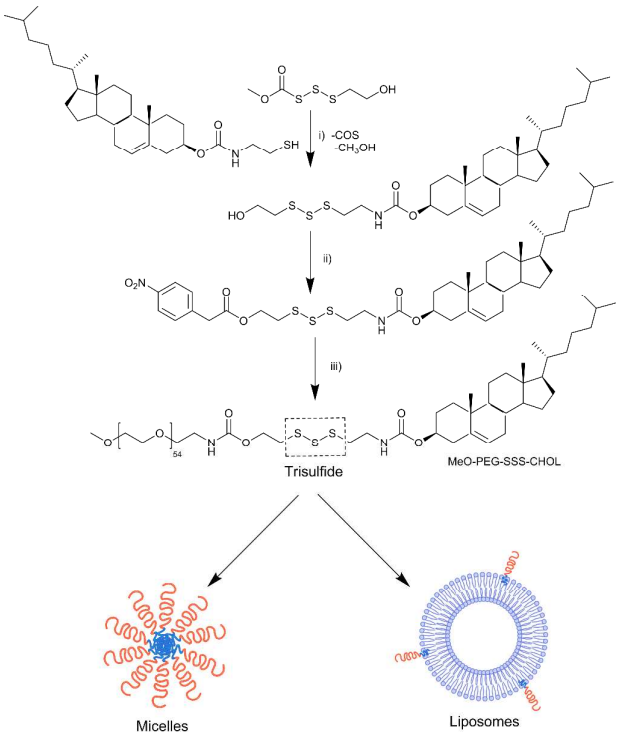

Figure 7. Synthesis of trisulfide conjugate. i) $CHCl_3$, N-methylmorpholine ii) 4-nitrophenyl chloroformate, TEA, CH_2Cl_2 iii) PEG(2K)-NH_2, DIPEA, DMAP, CH_2Cl_2.[35]

Inspired by the potent H_2S donor found in garlic, diallyl trisulfide (DATS), Ercole *et al*. developed a PEG–cholesterol conjugate linked by a trisulfide bridge (MeO–PEG–SSS–CHOL, Figure 7).[35] Such trisulfide species are known to produce H_2S via exchange reactions with thiols (e.g. GSH) via perthiol intermediates.[36] The trisulfide moeity was yielded via the fragmentation reaction of a thiol with methoxycarbonyl 3-(2-hydroxyethyl)trisulfane to form trisulfide-functionalised cholesterol (HO–SSS–CHOL). The final MeO–PEG–SSS–CHOL conjugate was then achieved by coupling PEG-NH_2 with the nitrophenyl carbonate derivative of HO–SSS–CHOL. The trisulfide linkage was shown to be an efficient and degradable H_2S donor compared to non-H_2S releasing controls: (i) a disulfide linked PEG-cholesterol conjugate which, although capable of exchanging with thiols, does not release H_2S; and (ii) a non-cleavable, non-H_2S releasing conjugate. The hydrophobic and hydrophilic domains in the synthesised material facilitated preparation of both micelles and the first

literature report of H_2S donating liposomes. Such structures effectively overcome the solubility limitation of DATS which is sparingly soluble in aqueous buffers. These results demonstrate the potential of donor moieties identified from natural sources as inspiration for H_2S releasing macromolecules. Moreover, the capacity to employ donor moieties as functional linkers that can trigger concomitant disassembly of nanoscale assemblies was also demonstrated for the first time in this work.

Yu *et al*.[37] recently reported the synthesis of polymers with an H_2S donating, acyl-protected perthiol chain terminus inspired by the small molecule donors of Xian and coworkers.[38] In the initial study, Xian *et al* reported that the corresponding small molecule compound (at an initial concentration of 100 μM), with the same electronic structure as the polymer-conjugated form, produced a H_2S peaking concentration of ~80 μM at 39 minutes. For the corresponding macromolecular donor, the authors directly modified the thiocarbonylthio end-group formed during RAFT polymerization as a convenient means to incorporate the acyl-protected perthiol moiety. The resulting acyl-protected perthiol end-groups are thought to release H_2S upon exposure to endogenous thiols via perthiol intermediates as in Figure 8.

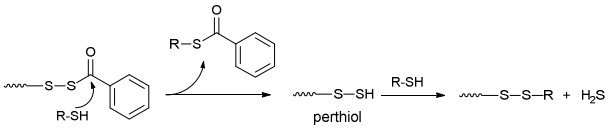

Figure 8. Production of H_2S from polymers with acyl-protected perthiol chain termini.

Specifically, the authors first converted the benzodithioate end-group of a RAFT polymer into a pyridyl disulfide, and then reacted with thiobenzoic acid to yield a benzoyl-capped perthiol (Figure 9). This approach was used to modify a series of structures including a homopolymer of (oligoethylene glycol methyl ether) methacrylate (POEGMA), a hydrophilic–hydrophobic block polymer, P[OEGMA]-*b*-[n-butyl methacrylate], and a pH-responsive block copolymer, P[OEGMA-*co*-*N,N*-(dimethylamino) ethyl methacrylate]-*b*-[*N,N*-(diisopropylamino)ethyl methacrylate].

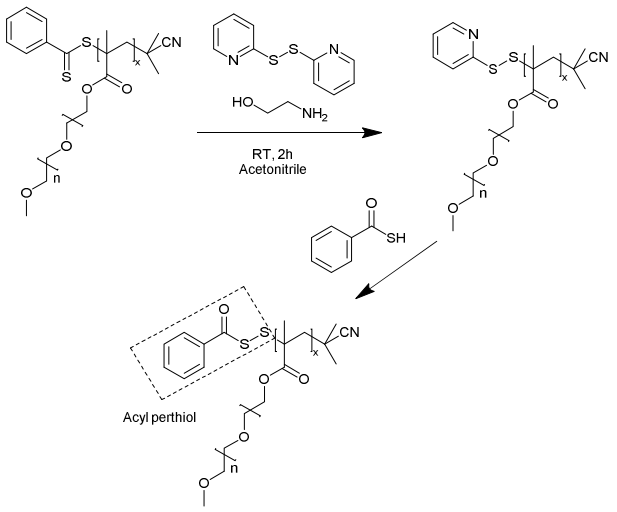

Figure 9. End-group modification of benzodithioate to acyl protected perthiol.[37]

The H_2S flux from the donors in the presence of cysteine, as measured using an amperometric sensor, was shown to be dependent on the polymer structure. Consistent with earlier studies the authors reported that H_2S release from block copolymer micelles was reduced compared to the homopolymer. This was attributed to substantial shielding of the donor end-group in the core of the micelle. The ability to modulate the H_2S release as a function of pH was demonstrated by using micelles assembled with block copolymer having a pH-responsive poly(*N,N*-(diisopropylamino)ethyl methacrylate) domain. At pH 5 the tertiary amines in the pH-responsive segment become protonated, which promotes micelle disassembly, revealing the end-group and enabling reaction with the trigger (L-cysteine). In contrast, reaction with the trigger is effectively prevented at pH 7.4 where the micelle remains intact. Importantly, the authors also demonstrated that these materials were capable of releasing H_2S when exposed to endogenous concentrations of thiol in HEK cells, as shown using the fluorescent H_2S-selective chemosensor SF4.

Matson and co-workers recently developed an alternative macromolecular platform for H_2S delivery using *N*-thiocarboxyanhydride (NTA) containing polymers capable of releasing carbonyl sulfide (COS), a potential H_2S precursor.[39] In this case the liberated COS from the NTA moieties was converted to H_2S by the enzyme carbonic anhydrase (CA). To prepare the materials, a norbornene-NTA monomer was first synthesised and then copolymerised with poly(ethylene glycol) (PEG) functionalised norbornene using ring opening metathesis polymerisation (ROMP), thereby providing a water soluble COS-releasing copolymer. The copolymer was then shown to generate H_2S in the presence of glycine and CA, as confirmed using both electrochemical sensing and the methylene blue assay. A 3-fold increase in H_2S release half-life (280 min) was observed when the polymer was employed compared to the corresponding small molecule NTA, as measured by methylene blue assay. Interestingly, while the small molecule NTA was shown to increase proliferation of

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brain-derived endothelial cells to a similar extent to sodium sulfide, the copolymer had no impact on proliferation. The authors tentatively attribute this discrepancy to the different release rates associated with the small molecule and copolymer, indicating that the prolonged yet low levels of H_2S release from macromolecules may not always lead to a favourable outcome when compared to the more rapid release from small molecules. Nonetheless, this report demonstrates an elegant, alternative approach to H_2S delivery that exploits cellular machinery to convert a released intermediate into the final target molecule.

Macromolecular H_2S Donors triggered by light

Xiao *et al.* have recently proposed the use of a multimodal trigger to release H_2S.[40] In this system H_2S production was facilitated by first irradiating the material to liberate a reactive intermediate, which then reacted with an amine to liberate H_2S. Specifically, a stable (β-carbonyl)thioether precursor underwent light-triggered conversion to a thiobenzaldehyde, and the subsequent reaction with an amine formed an imide yielding H_2S as a by-product (Figure 10).

Interestingly, the authors found the photoactivation to be the rate-determining step compared to the relatively fast imide formation. They also demonstrated the ability to incorporate these donors into water-soluble polymers, hydrogels and polystyrene films by copolymerising a thiobenzaldehyde-containing monomer with appropriate comonomers. Overall this study demonstrated an interesting alternative method for controlled H_2S delivery based on thioaldehyde chemistry. The non-reliance on a thiol trigger sets this approach apart from many of those previously mentioned. However, the required light irradiation and presence of an amine may itself present challenges for clinical translation of this approach.

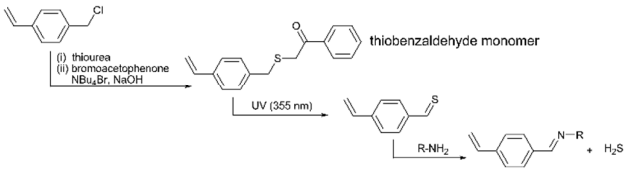

Figure 10. Thiobenzaldehyde monomer synthesis and H_2S release mechanism.[40]

Polymer-encapsulated H_2S Donors- Nanofibers

In addition to the direct chemical modification of polymers with H_2S releasing moieties, it is also possible to physically entrap small molecule donors within a polymer matrix. For example, in 2015, Wang and colleagues fabricated H_2S donating microfibers and evaluated their potential for application in wound dressings.[41] In this case the authors utilised electrospinning of polycaprolactone (PCL) solutions containing the H_2S donor *N*-(benzoylthio)benzamide (NSHD1)[42] to create microfibers which release H_2S when immersed in an aqueous solution containing 1 mM cysteine. The authors employed both energy dispersive X-ray (EDX) and Fourier transform infrared spectrometry (FTIR) to confirm the presence of the H_2S donor in the fibrous matrix. The H_2S release from the fibres was reported to occur over extended timescales, although the overall amount was far less than observed from the NSHD1 donor alone. Furthermore, the microfibers were shown to significantly decrease ROS production in H_2O_2 treated cells and to promote the expression of wound healing related genes collagen types I and III.

In an extension to this study, the same group employed pH responsive nanofibers[43] using the H_2S donor JK1[44] (Table 1). This approach benefits from being independent from the intracellular concentration of thiol. In this study, the obtained PCL-JK1 hybrid nanofibers showed pH regulated H_2S release with lower pH leading to greater and faster release of H_2S.

Conclusions and Future Outlook

H_2S is a critically important molecule in mammalian physiology, and its full catalogue of biological functions is constantly expanding. Given the breadth and depth of these functions, there is a critical need to develop new donors wherein delivery of the molecule can be spatiotemporally controlled. To this end, the development of macromolecular approaches for the controlled and triggered delivery of H_2S may offer new opportunities for employing this potent yet promiscuous signalling molecule in particular applications. To date, donors with various macromolecular structures have been developed, such as conjugates, micelles, hydrogels and nanofibers. Macromolecular donors offer numerous potential benefits over small molecule donors, such as improved solubility, reduced cytotoxicity, and the potential to readily tune the pharmacokinetics and hence locus of action. As such there is a strong motivation for developing further H_2S donating macromolecules as potential therapeutics. Moreover, the demonstration that appropriately designed macromolecular H_2S donors can be incorporated into lipid nanoassemblies offers a further avenue for tailoring the release properties and preparing pharmaceutically relevant H_2S releasing formulations.[33]

Whilst investigations into the effects of H_2S-donating compounds on physiological systems continue to multiply, research into the delivery of hydrogen sulfide using a macromolecular approach is only in its infancy as a field. As a result, the focus and nature of such studies remains broad. For example, some research groups have focused on evaluating the differences between small molecule donors vs. conjugated forms whilst others have concentrated on comparing different polymer types and structural forms of the macromolecular donors. Some have focused on comparing release rates while others have investigated cellular toxicity. Undoubtedly, as the field expands it is likely that research will move towards studying the materials in a biological setting to better understand their applicability as therapeutic agents.

Of course, in addition to materials that release H_2S there is also scope to investigate materials that can potentially sequester H_2S, and thereby mimic the effect of down-regulating H_2S producing enzymes. Indeed, there is already some investigation underway in this area.[45]

H_2S is, together with nitric oxide and carbon monoxide, one of the three so-called gasotransmitters. As such, these molecules provide important precedents as to how the field might evolve in coming years. Given that appreciation of the role of H_2S in mammalian physiology has expanded in much the same way as it did for nitric oxide, so too we might anticipate the investigation of H_2S-releasing materials to follow a similar trajectory to that of NO-releasing materials.[46,47] Should that be the case, then the future of research into macromolecular H_2S donors looks very bright indeed.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

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