Structural requirements for oxidation of low-density lipoprotein by thiols

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Abstract Oxidation of low-density lipoprotein (LDL) by macrophages, endothelial cells and smooth muscle cells, may be mediated by production of free thiols in the presence of transition metals. We examined the structural requirements, within a series of cysteinyl derivatives, for oxidation of thiols and of LDL in Hams F10 medium. The primary mechanism by which such thiols mediate oxidation of LDL is largely independent of superoxide production, but strongly correlated with the susceptibility of each thiol to iron-catalysed auto-oxidation. These effects are compared and contrasted with thiol-dependent oxidation of LDL by stimulated human monocytes and macrophages.

Key words: Atherosclerosis; Oxidized LDL; Macrophage; Smooth muscle cell; Thiol; Superoxide

1. Introduction

A primary event in the formation of atherosclerotic lesions is the accumulation of lipid-laden foam cells within the arterial wall [1]. Foam cells originate from monocyte-derived macrophages, which take up modified low-density lipoprotein (LDL) via scavenger receptor(s) and deposit cholesteryl esters within their cytoplasm [2,3]. Uptake of native LDL does not form foam cells, because accumulation of cholesteryl esters is prevented by down-regulation of the native LDL receptor [2,3]. Oxidized LDL is recognized by the macrophage scavenger receptor; evidence strongly suggests that oxidized LDL contributes to foam cell formation and atherogenesis in vivo [4–6].

Oxidation of LDL can be mediated in vitro, by all of the cell types associated with atherosclerotic lesions, including endothelial cells [7], smooth muscle cells [8], lymphocytes [9], platelets [10] and monocyte/macrophages [11,12]. The mechanism(s) by which oxidative modification of LDL occurs is not clear; most studies [6–10,12], but not all [11,13], have been performed in the presence of trace amounts of transition metals, which can propagate the peroxidation of lipids within the LDL particle [14]. A number of oxidizing species have been proposed, including lipoxygenases [11,15], nitric oxide [16] and/or superoxide [7,8]. However, although lipoxygenase-derived peroxides exert pro-oxidant effects on copper-oxidation of LDL [17], neither 5- or 15-lipoxygenase appear essential for macrophage-mediated oxidation of LDL [18,19]. Nitric oxide appears to protect against the oxidative modification of LDL by macrophages [20], while peroxynitrite, which can be formed in the reaction between macrophage-derived nitric oxide and superoxide [21], modifies LDL so that it can be recognized by the macrophage scavenger receptor [22]. Production of superoxide by human monocytes [23], regulated by the activity of protein kinase C [24], is reported to be required for lipid oxidation of LDL; in contrast, however, both human [25] and mouse [26] macrophage-mediated oxidation of LDL appears to be independent of superoxide production. Heinecke et al. [27] demonstrated that oxidation of LDL by monkey arterial smooth muscle cells required the production of superoxide by an L-cystine dependent process; more recently, oxidation of LDL by rabbit endothelial cells, and mouse peritoneal macrophages, were shown to be mediated by macrophage-dependent thiol production [28].

We have recently demonstrated that oxidation of LDL by human (THP-1) macrophages, in the presence of transition metals, was dependent upon a threshold level of extracellular thiol production [29], but independent of intracellular glutathione content [29,30]. The possible mechanism(s) by which thiols, interacting with transition metals, result in oxidation of LDL, include generation of thiyl radicals, production of superoxide and reduction of transition metal ions. Differing thiols are reported to oxidize LDL by both superoxide-dependent and -independent mechanisms [31], while conjugated diene formation can be increased or decreased by the presence of thiols [32]; these authors concluded that both thiol chain length and charge can influence iron-dependent oxidation of LDL in phosphate buffer [32].

This study examines the structural requirements for oxidation of thiols, and of LDL, in Hams F10 medium; in particular, the oxidation of LDL by cysteine and cysteinyl derivatives is compared with the half-life of each thiol. The role of superoxide in thiol-dependent oxidation of LDL was compared for both exogenously and endogenously (macrophage-derived) supplied thiols.

2. Experimental

2.1. Materials

Tissue culture medium and reagents, including Hams F10 formulated without iron sulphate and/or cystine, were purchased from Gibco BRL (Paisley, Scotland). Tissue culture plastics were purchased from Falcon (Becton Dickinson, Oxford, UK). All other chemicals, including Cu/Zn superoxide dismutase, were supplied by the Sigma Chemical Co. (Dorset, UK). Agarose electrophoresis gels were supplied by Beckman (High Wycombe, UK).

2.2. Cell culture

Human THP-1 macrophages were maintained exactly as described [30]. For experiments, cells were seeded at 1×10^6/well into 12-well plates, containing RPMI 1640 medium supplemented with glutamine (4 mM), penicillin and streptomycin (each 20 IU/ml), bovine fetal calf serum (10%, v/v) and phorbol 12-myristate 13-acetate (PMA) (100 ng/ml). Cells were maintained for seven days; media containing PMA were replaced at three day intervals. Rat A10 aortic smooth muscle cells

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Abbreviations: Low-density lipoprotein (LDL), ethylene diamine tetraacetic acid (EDTA), butylated hydroxytoluene (BHT), relative electrophoretic mobility (REM), superoxide dismutase (SOD), heat-inactivated superoxide dismutase (HI-SOD).

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were also cultured in RPMI medium containing foetal bovine serum, as above except that PMA was omitted. For experiments, smooth muscle cells were seeded at 1×10^5 cells/well in 12-well plates and grown until confluency was achieved. Human monocytes were prepared from freshly donated peripheral blood, collected in EDTA (0.27%); monocytes were isolated by centrifugation over Ficoll-Paque (Pharmacia) [11], and subsequent adherence to 12-well plates. Monocytes were seeded at 5×10^6 cells/well and stimulated by addition of PMA (100 ng/ml) in RPMI medium, as described above, for at least 4 h before oxidation of LDL and production of thiols were assessed.

2.3. Preparation of lipoproteins

Human LDL was prepared by ultracentrifugation in a discontinuous density gradient [33] and desalted using a PD10 prepacked column (Pharmacia). Isolated LDL was stored at 4°C and used within 7 days; under these conditions no significant changes in oxidizability of the LDL were noted [30]. The LDL was sterilized by passage through a 0.22 μm filter and the concentration expressed per mg of protein using bovine serum albumin as standard.

2.4. Measurement of LDL oxidation by thiols

All experiments were performed in Hams F10 modification medium (as defined below), formulated with or without 206 μM L-cysteine, which auto-oxidizes during storage to L-cystine; this medium is therefore referred to as Hams F10 with or without cystine. Each thiol was prepared immediately before use and added at a final concentration of 500 μM. After incubation for 8 h at 37°C, butylated hydroxytoluene (BHT) was added to the medium (final concentration 50 μM) to prevent further oxidation, and the electrophoretic mobility of each sample determined.

2.5. Measurement of LDL oxidation by cells

Oxidation of LDL, by human monocytes, THP-1 macrophages or rat A10 smooth muscle cells, was performed in Hams F10 medium (formulated with or without cystine) containing glutamine and penicillin/streptomycin, supplemented with 3 μM $FeSO_4$, 10 μM EDTA and 100 μg/ml LDL (final volume 1 ml), for 18 h at 37°C exactly as previously described [30]. These conditions have previously been demonstrated to be optimal for LDL oxidation by mouse peritoneal macrophages [34]; the addition of EDTA should also inhibit any LDL oxidation mediated by traces of copper (90 nM) in Hams F10 medium [34]. In most cases, parallel cell-free control incubations were also carried out. The electrophoretic mobility of samples of LDL (10 μl) were determined on agarose gels using the lipoprotein electrophoresis system supplied by the Beckman company.

2.6. Measurement of thiol concentration

The half-life of each thiol was determined in Hams F10 medium, formulated without cystine and $FeSO_4$. In experiments requiring the presence of transition metals, this medium was supplemented with $FeSO_4$ (6 μM) and EDTA (10 μM). Each thiol was prepared immediately before use, and added to the medium at a final concentration of 100 μM (final vol. 1 ml); experiments were performed in 12-well plates incubated at 37°C. Thiol concentrations were determined at hourly intervals during incubations of 6–8 h. The measurement of extracellular thiols, produced by macrophages or smooth muscle cells, was determined after incubation of Hams F10 modification medium (formulated with or without cystine) for 6 h at 37°C. In order to determine the half-life of macrophage-produced thiol, cells were incubated with Hams F10, formulated with cystine but without $FeSO_4$, for 24 h. The medium was then removed from the cells, replaced in cell-free 12-well plates, and the half-life determined in the presence or absence of iron and EDTA, as described above. Measurements of thiol concentration were determined using the thiol-specific reagent 5,5′-dithio-bis(2-nitrobenzoic acid) (DTNB) exactly as described [30].

2.7. Heat inactivation of superoxide dismutase

Copper/zinc superoxide dismutase (SOD) was heat inactivated by heating to 100°C for 60 min, a process previously shown to remove >95% of SOD activity [26]; additions of both active and heat-inactivated SOD were made to a final concentration of 50 μg/ml.

2.8. Statistics

All statistical tests were performed using the Student's *t*-test (as indicated in the legends to Figures and Tables); a statistical difference was reported when $P < 0.05$. All measurements were made in triplicate within each experiment; numbers of independent experiments are indicated in the legends to Figures and Tables.

3. Results

3.1. The role of thiols in oxidation of LDL by differing cell types

We examined the importance of thiol production, by rat A10 smooth muscle cells, human (THP-1) macrophages and human monocytes, on LDL oxidation in Hams F10 medium (final concentration 6 μM $FeSO_4$, 10 μM EDTA). As described previously [29,30], the L-cystine-dependent production of thiols by THP-1 macrophages was associated with increased LDL oxidation (Table 1). In marked contrast, rat A10 smooth muscle cells did not exhibit cystine-dependent thiol production; these cells oxidized LDL at a low, but significant, rate. Human monocytes, stimulated with PMA (100 ng/ml) produced thiols and oxidized LDL in a thiol-dependent manner; however, both THP-1 macrophages and human monocytes also exhibited cystine-independent oxidation, suggesting more than one oxidative mechanism operates in these cell types.

3.2. Oxidation of thiols in Hams F10 medium

We established the half-life of a range of thiols – L- and D-cysteine, D,L-homocysteine, cysteinyl-glycine, threonine-valine-cysteinyl-glycine, *N*-acetyl-L-cysteine, mercaptoethylamine mercaptoethanol and dithiothreitol – in Hams F10 medium (formulated without cystine), supplemented with or without $FeSO_4$ (6 μM) and EDTA (10 μM). These additions will rapidly oxidize in air to Fe^{3+}-EDTA [35], which is probably the primary species interacting with thiol auto-oxidation (and LDL oxida-

Table 1
Thiol-dependent oxidation of LDL by differing cell types

Cell type	Thiol (μM)		LDL oxidation (R.E.M.)	
	−Cystine	+Cystine	−Cystine	+Cystine
THP-1 macrophages	0.53 ± 0.07 (3)	10.23 ± 0.63 (3)[a]	0.55 ± 0.11 (10)	1.58 ± 0.11 (10)[a]
Human monocytes (PMA stimulated)	0.89 ± 0.38 (3)	23.92 ± 0.83 (3)[a]	0.63 ± 0.10 (3)	1.63 ± 0.12 (3)[a]
Rat A10 smooth muscle cells	0.52 ± 0.10 (4)	0.47 ± 0.06 (4)	n.d.	0.18 ± 0.05 (3)

Oxidation of LDL (100 μg/ml) was performed in Hams F10 medium, formulated with or without cystine, supplemented with $FeSO_4$ (6 μM) and EDTA (10 μM), and measured by increases in electophoretic mobility over 18 h, as described in section 2; cell-free controls were subtracted to give specific cellular oxidation. Measurements of extracellular thiol concentrations were made after 6 h, as described in section 2; cell-free controls were subtracted from measurements made in the presence of cells. Values are means ± S.E.M. for the number of experiments shown in parentheses.
[a] Values significantly different from incubations in the absence of cystine; all incubations performed in the presence of cells resulted in LDL which was oxidized significantly ($P < 0.05$) more than the cell-free controls.

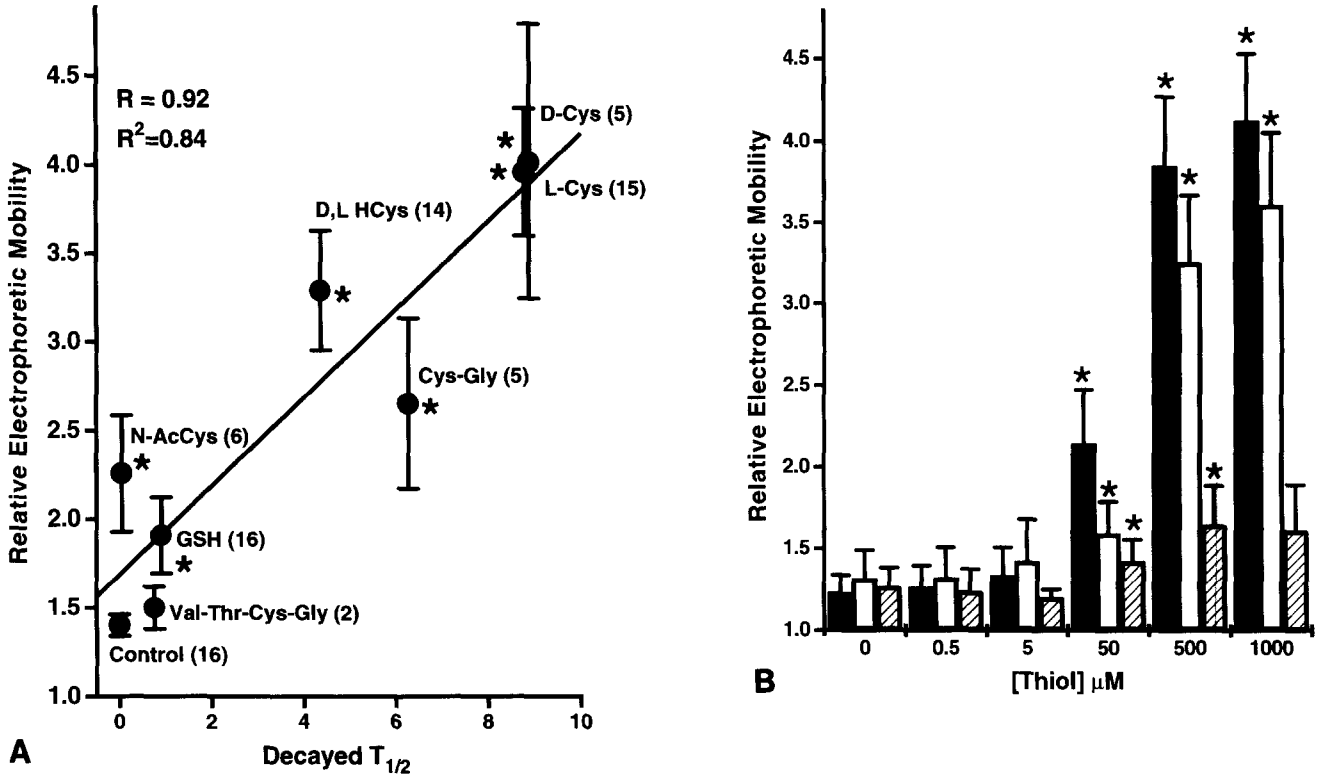

Fig. 1. (A) Relationship between thiol half-life and LDL oxidation. Oxidation of LDL (100 μg/ml), measured by increases in electrophoretic mobility, correlates with the number of decayed thiol half-lives during an 8 h incubation at 37°C. *Indicates values significantly ($P < 0.05$) from the control incubation, expressed as means ± S.E.M. (or ranges for two experiments) for the numbers of independent experiments indicated in parentheses. (B) Effect of thiol concentration of LDL oxidation. Oxidation of LDL, assessed as above, was measured following incubation for 8 h at 37°C, with the indicated concentrations of cysteine (■), homocysteine (□) or glutathione (▨) in Hams F10 medium supplemented with iron/ EDTA. Values are means ± S.E.M. for three independent experiments. *Indicates values significantly different ($P < 0.05$) from the control incubation.

tion) in this system. Cysteine and cysteine-containing peptides demonstrated increased stability (evidenced by increased half-life) as the length of the peptide chain increased (Table 2). Acetylation of cysteine increased its half-life substantially, while increasing the length of the sulphydryl side chain, as in homocysteine, decreased the susceptibility of this thiol to iron-catalyzed oxidation by around 2-fold. Removal of the γ-glutamyl residue from glutathione, giving cysteinyl-glycine, increased susceptibility to oxidation; the addition of two hydrophobic amino acids to cysteinyl-glycine substantially decreased the oxidation of this thiol. Replacement of the hydroxyl group of mercaptoethanol with an amine group, in mercaptoethylamine, markedly decreased the half-life of this thiol, especially in the presence of iron. The thiol(s) produced by THP-1 macrophages was also sensitive to iron-catalysed oxidation, and gave results which were virtually identical to exogenously added cysteine.

3.3. Oxidation of LDL by thiols in Hams F10 medium

The thiol-dependent oxidation of LDL in Hams F10 medium, supplemented with $FeSO_4$ (6 μM) and EDTA (10 μM), was measured, after incubation for 8 h at 37°C, by increases in electrophoretic mobility. The presence of mercaptoethanol, mercaptoethylamine or dithiothreitol did not significantly increase the oxidative modification of LDL, compared with the control incubation (REM values of 1.14 ± 0.10, 1.11 ± 0.08, 1.59 ± 0.28, respectively; means ± ranges, $n = 2$); the evident oxidation of these thiols in Hams F10 medium (Table 2) obviously did not translate into pro-oxidant effects on the modification of apoprotein B in LDL. In contrast, cysteine, modified forms of cysteine and cysteine-containing peptides, oxidized LDL in a manner which directly correlated ($R = 0.92$, $R^2 = 0.84$) with the number of thiol half-lives decayed during the 8h incubation (Fig. 1A). The susceptibility of each thiol to iron-catalysed oxidation (Table 2) also correlated positively with the degree of LDL oxidation ($R = 0.80$, $R^2 = 0.65$).

However, thiols may exert both pro- and anti-oxidant effects in the presence of iron, depending upon the concentration of thiol employed [36]. We therefore examined the effect of a range of concentrations of glutathione, homocysteine and cysteine upon LDL oxidation (Fig. 1B). Low concentrations of thiol did not exert pro-oxidant effects on LDL oxidation in this system; oxidation was significantly ($P < 0.05$) enhanced at 50 μM thiol. We also did not observe inhibition of LDL oxidation at higher concentrations of cysteine, homocysteine and glutathione, in contrast to Heinecke et al. [31]. It is noteworthy that the the efficacy of each thiol in mediating LDL oxidation was maintained i.e. cysteine > homocysteine >> glutathione. This supports our argument that the half-life of auto-oxidation of each thiol, which will obviously remain constant, is a determining factor in thiol-mediated LDL oxidation.

Finally, we compared the effect of Hams F10 supplemented with iron (final concentration 6 μM), but without the addition of EDTA, with the thiol-mediated oxidation of LDL described

above. In the absence of EDTA, addition of cysteine, homocysteine or glutathione (500 μM final concentration) gave R.E.M. values of 4.03 ± 0.14, 3.11 ± 0.57 and 1.38 ± 0.08 (mean ± S.E.M., $n = 5$). Clearly the presence of EDTA in this system exerts only minor influences on thiol-mediated oxidation of LDL, and does not alter the ability of each thiol to mediate oxidation of LDL.

3.4. The effect of superoxide dismutase on oxidation of LDL by thiols

The presence of superoxide dismutase (SOD) partially inhibited the L-cystine-dependent oxidation of LDL by THP-1 macrophages; heat inactivation of SOD abrogated this effect, as expected (Table 3). Superoxide production by macrophages could be due to both extracellular thiol oxidation and to activation of NADPH oxidase [28,40]. However, the presence of diphenylene iodonium (30 μM), which inhibits NADPH oxidase, did not significantly inhibit LDL oxidation by THP-1 macrophages, compared with the control incubation (4.03 ± 0.09 versus 4.47 ± 0.16, respectively; means ± S.E.M., $n = 3$). In the absence of L-cystine, SOD had no effect on macrophage oxidation; however, heat-inactivated SOD (HI-SOD) was markedly pro-oxidant under these conditions, probably due to the release of copper from the active site of the enzyme [26]. The addition of SOD inhibited cysteine-induced oxidation of LDL by around 30% and D,L-homocysteine-mediated oxidation by around 25%, while glutathione-mediated oxidation of LDL was unaffected by the addition of SOD. Superoxide dismutase substantially increased the rate of oxidation of cysteine, giving half-life values of 0.64h ± 0.02 and 1.06h ± 0.03 in the presence or absence of iron/EDTA respectively (means ± ranges, $n = 2$). This may be due to dismutation of superoxide to hydrogen peroxide, which can then increase formation of radicals from cysteine [37]. In the two experiments performed in the presence of cysteine and HI-SOD, HI-SOD was about half as effective as SOD; in the light of the argument discussed above, however, it is difficult to ascribe this effect to a loss of superoxide production.

4. Discussion

The production of free thiol by monocytes and macrophages seems to be one mechanism by which these cells can propagate the oxidation of LDL. This study demonstrates that the half-life of thiol oxidation can be directly correlated with the degree of LDL oxidation in Hams F10 medium. The juxtaposition of a free amino group with the sulphydryl side chain appears to increase the oxidation rate of each thiol – increasing the distance between these two groups, or acetylation of the amino group decreases the rate of thiol oxidation substantially. However, as results with mercaptoethanol and mercaptoethylamine demonstrate, susceptibility to iron-catalysed auto-oxidation was not sufficient to ensure thiol-mediated oxidation of LDL. The presence of a free carboxyl group, in cysteine and cysteinyl derivatives, appears essential for thiol-mediated oxidation of LDL; in the presence of a free carboxyl group, LDL oxidation then appeared to be influenced by the oxidizability of each thiol. Oxidation of LDL did not appear to be related to the lipophilicity of each thiol – mercaptoethanol and dithiothreitol did not significantly increase LDL oxidation and the addition of hydrophobic amino acids to cysteinyl-glycine (Thr-Val-Cys-Gly) reduced LDL oxidation. Stimulated human monocytes have been shown to produce extracellular cysteine [38,39]; the strong similarity between the half-lives of macrophage-derived thiol and cysteine allowed us to confirm that this was the major thiol species responsible for macrophage-mediated LDL oxidation.

Formation of thiyl radicals can initiate lipid peroxidation [40] by abstraction of hydrogen from unsaturated fatty acids or by the formation of sulphonyl radicals which can form adducts with double bonds [41]. However, the reactivity of thiyl radicals towards polyunsaturated fatty acids is increased by (i) an increased distance between the reactive $S^{\cdot}$-centre and the ionic groups in the attacking molecule, (ii) a decreased number of ionic functions and (iii) increased lipophilicity of the attacking thiyl radical [41]. These results contrast directly with the oxidation of LDL mediated by thiols in Hams F10 medium, suggesting to us that initiation of lipid peroxidation by thiyl radicals may not play a major role in this process.

The direct correlation between susceptibility of cysteinyl-thiols to iron-catalysed oxidation and extent of LDL oxidation, suggests that LDL oxidation may be enhanced either by thiol-mediated reduction of transition metals or by the increased production of oxidizing species generated during thiol oxidation. Auto-oxidation of thiols can generate superoxide [42], which can promote oxidation of LDL in the presence of transi-

Table 2
Thiol auto-oxidation in Hams F10 medium

Thiol	Half-life (h)		Iron-catalysis of thiol oxidation (%)
	F10 (without cystine or iron)	F10 (without cystine) + iron/EDTA	
Mercaptoethylamine	0.95 ± 0.00	0.24 ± 0.00	395.8
L-Cysteine	2.79 ± 0.01	0.91 ± 0.01	306.6
D-Cysteine	2.79 ± 0.01	0.90 ± 0.01	310.0
Cysteinyl-glycine	2.75 ± 0.04	1.24 ± 0.00	221.8
Dithiothreitol	0.67 ± 0.00	1.53 ± 0.00	43.8
D,L-Homocysteine	2.43 ± 0.43	1.97 ± 0.44	123.3
Mercaptoethanol	3.37 ± 0.21	3.11 ± 0.28	108.3
Glutathione	8.97 ± 0.39	8.85 ± 0.76	101.3
Valine-threonine-cysteinyl-glycine	11.33 ± 0.23	10.55 ± 0.09	107.4
N-Acetyl-L-cysteine	>20	>20	n.d.
Macrophage-derived free thiol	2.89 ± 0.08	0.94 ± 0.08	308.5

Thiols (100 μM) were added to Hams F10 medium, formulated without cystine, supplemented with or without $FeSO_4$ (6 μM) and EDTA (10 μM) at 37°C for 6–8 h. The free thiol concentration was measured at intervals of one hour using DTNB (0.1 mM, final concentration) as described in section 2; values are means ± ranges of two independent experiments.

Table 3
The role of superoxide in thiol-dependent LDL oxidation

	LDL Oxidation (R.E.M.)		
Condition:	Control	SOD	HI-SOD
Cell experiments			
Hams F10 (−cystine)	1.51 ± 0.08 (3)	1.59 ± 0.20 (3)	3.25 ± 0.39 (3)[b]
Hams F10 (+cystine)	4.47 ± 0.64 (3)[a]	2.40 ± 0.55 (3)[b]	4.30 ± 0.35 (3)
Cell-free experiments			
No addition	1.35 ± 0.07 (5)	1.21 ± 0.08 (5)	1.18 ± 0.05 (2)
L-Cysteine	4.54 ± 0.15 (6)[a]	3.52 ± 0.27 (5)[b]	3.33 ± 0.11 (2)
D,L-Homocysteine	3.94 ± 0.12 (3)[a]	3.20 ± 0.10 (3)[b]	n.d.
Glutathione	1.87 ± 0.08 (4)[a]	1.92 ± 0.20 (4)	n.d.

The addition of superoxide dismutase (SOD) (50 μg/ml), or heat-inactivated superoxide dismutase (HI-SOD) on oxidation of LDL (100 μg/ml) by thiols (500 μM) or by THP-1 macrophages (in the presence or absence of L-cystine) was assessed in Hams F10 medium supplemented with $FeSO_4$ (6 μM) and EDTA (10 μM). Incubation of LDL with thiols (8 h) or THP-1 macrophages (17 h) resulted in oxidation of LDL measured by increases in electrophoretic mobility. Values are means ± S.E.M., or means ± ranges, for the numbers of experiments shown in parentheses.
[a] Indicates values significantly different from the control incubation where no additions were made.
[b] Indicates values significantly different from each respective control.

tion metals [43] or react with Fe^{3+} (or Fe^{3+}-EDTA) to generate Fe^{2+} (or Fe^{2+}-EDTA) [35,44]. Superoxide dismutase partially inhibited LDL oxidation mediated by macrophages, cysteine or homocysteine, but was ineffective against glutathione-mediated LDL oxidation. The relatively small reductions shown in the presence of thiols, however, suggests that superoxide does not play a major role in thiol-mediated LDL oxidation. The increased oxidation of cysteine in the presence of SOD, has previously been attributed to the production of hydrogen peroxide which can then enhance cysteine oxidation [37]. Production of hydrogen peroxide, in the presence of iron, could then generate hydroxyl radicals, via the Fenton reaction, which can increase the oxidation of LDL [43]. The greater efficacy of SOD in inhibiting cellular oxidation of LDL could be due to the removal of hydrogen peroxide by intracellular catalase, thereby inhibiting hydrogen peroxide mediated increases in thiol oxidation. However, the relevance of this oxidative pathway in the absence of extracellular SOD is doubtful, as the catalase inhibitor, aminotriazole, does not increase LDL oxidation in these macrophages (data not shown), and hydroxyl radical scavengers such as mannitol [8] do not decrease cellular oxidation of LDL. Defining even a partial role for superoxide in thiol-dependent oxidation of LDL would require the absence of such effects in the presence of heat-inactivated SOD; however, the prooxidant effects of HI-SOD make this impossible.

Superoxide-independent oxidation of LDL, exhibited by all of the thiols tested, suggested that the primary role of these agents in oxidizing LDL may be the reduction of transition metal ions, thereby facilitating the decomposition of seeding peroxides (LOOH) within the LDL particle (where LH represents an unsaturated fatty acid).

$$RS^- + Fe^{3+} \rightarrow RS^\bullet + Fe^{2+}$$
$$Fe^{2+} + LOOH \rightarrow Fe^{3+} + LO^\bullet + OH^-$$
$$LO^\bullet + LH \rightarrow L^\bullet + LOH$$
$$L^\bullet + O_2 \rightarrow LOO^\bullet$$

These peroxides may be formed during the preparation of LDL or, in the presence of cells, by the thiol-independent oxidation of LDL seen with human monocytes and THP-1 macrophages; however, the reactivity of cysteinyl radicals may also be sufficient to initiate lipid peroxidation [41]. The physiological relevance of transition metal reduction by macrophage-produced thiols within the arterial wall still needs to be demonstrated; significantly, however, Hirano et al. [45] have shown that homocysteine can oxidatively modify LDL in the presence of ferritin, possibly by the reductive release of Fe^{2+} from the protein [35]. The nature of the thiol-independent oxidation of LDL requires further investigation; Garner et al. [46] have reported that macrophages can mediate the reduction of transition metal ions in thiol-deficient medium. Macrophage-mediated oxidation of LDL could therefore involve two interacting processes – an initiating or seeding event followed by the thiol-dependent propagation of LDL oxidation.

References

[1] Gerrity, R.G. (1981) Am. J. Pathol. 103, 181–190.
[2] Brown, M.S. and Goldstein, J.L. (1983) Annu. Rev. Biochem. 52, 223–261.
[3] Goldstein, J.L., Ho, Y.K., Basu, S.K. and Brown, M.S. (1979) Proc. Natl. Acad. Sci. USA 76, 333–337.
[4] Steinbrecher, U.P., Zhang, H. and Lougheed, M. (1990) Free Rad. Biol. Med. 9, 155–168.
[5] Palinski, W., Rosenfeld, M.E., Yla-Herttuala, S., Gurtner, G.C., Socher, S.S., Butler, S.W., Parthasarathy, S., Carew, T.E., Steinberg, D. and Witztum, J.L. (1989) Proc. Natl. Acad. Sci. USA 86, 1372–1376.
[6] Yla-Herttuala, S., Palinski, W., Rosenfeld, M.E., Parthasarathy, S., Carew, T.E., Butler, S., Witztum, J.L. and Steinberg, D. (1989) J. Clin. Invest. 84, 1086–1095.
[7] Steinbrecher, U.P. (1988) Biochim. Biophys. Acta 959, 20–30.
[8] Heinecke, J.W., Baker, L., Rosen, H. and Chait, A. (1986) J. Clin. Invest. 77, 757–761.
[9] Lamb, D.J., Wilkins, G.M. and Leake, D.S. (1992) Atherosclerosis 92, 187–192.
[10] Aviram, M. (1987) Clin. Biochem. 29, 91–95.
[11] McNally, A.K., Chisolm, G.M., Morel, D.W. and Cathcart, M.K. (1990) J. Immunol. 145, 254–259.
[12] Jessup, W., Rankin, S.M., De Whalley, C.V., Hoult, J.R.S., Scott, J. and Leake, D.S. (1990) Biochem. J. 265, 399–405.
[13] Navab, M., Hama, S.Y., Van-Lenten, B.J., Drinkwater, D.C., Laks, H. and Fogelman, A.M. (1993) J. Clin. Invest. 91, 1225–1230.
[14] Thomas, C.E. and Jackson, R.L. (1991) J. Pharmacol. Exp. Ther. 256, 1182–1188.
[15] Parthasarathy, S., Wieland, E. and Steinberg, D. (1989) Proc. Natl. Acad. Sci. USA 86, 1046–1050.
[16] Chang, C.J., Woo, P., Honda, H.M., Ignarro, L.J., Young, L., Berliner, J.A. and Derner, L.L. (1994) Arterioscler. Thromb. 14, 1808–1814.
[17] O'Leary, V.J., Darley-Usmar, V.M., Russell, L.J. and Stone, D. (1992) Biochem. J. 282, 631–634.

[18] Jessup, W., Darley-Usmar, V., O'Learr, V. and Bedwell, S. (1991) Biochem. J. 278, 163–169.
[19] Sparrow, C.P. and Olszewski, J. (1992) Proc. Natl. Acad. Sci. USA 89, 128–131.
[20] Jessup, W., Mohr, D., Gieseg, S.P., Dean, R.T. and Stocker, R. (1992) Biochim. Biophys. Acta 1180, 73–82.
[21] Ischiropoulos, H., Zhu, L. and Beckman, J.S. (1992) Arch. Biochem. Biophys. 298, 446–451.
[22] Graham, A., Hogg, N., Kalyanaraman, B., O'Leary, V., Darley-Usmar, V. and Moncada, S. (1993) FEBS Lett. 330, 181–185.
[23] Cathcart, M.K., McNally, A.K., Morel, D.W. and Chisolm, G.M. (1989) J. Immunol. 142, 1963–1969.
[24] Li., Q. and Cathcart, M.K. 91994) J. Biol. Chem. 269, 17508–17515.
[25] Garner, B., Dean, R.T. and Jessup, W. (1994) Biochem. J. 301, 421–428.
[26] Jessup, W., Simpson, J.A. and Dean, R.T. (1993) Atherosclerosis 99, 107–120.
[27] Heinecke, J.W., Rosen, H., Suzuki, L.A. and Chait, A. (1987) J. Biol. Chem 262, 10098–10103.
[28] Sparrow, C.P. and Olszewski, J. (1993) J. Lipid Res. 34, 1219–1228.
[29] Wood, J.L. and Graham, A. (1995) Biochem. Soc. Trans. 23, 242S.
[30] Graham, A., Wood, J.L., O'Leary, V.J. and Stone, D. (1994) Free Rad. Res. 21, 295–308.
[31] Heinecke, J.W., Kawamura, M., Suzuki, L. and Chait, A. (1993) J. Lipid Res. 34, 2051–2061.
[32] Lamb, D.J., Rees, E. and Leake, D.S. (1994) Atherosclerosis 109, 44.
[33] Chung, B.H., Wilkinson, T., Geer, J.C. and Segrest, J.P. (1980) J. Lipid Res. 21, 284–291.
[34] Lamb, D.J. and Leake, D.S. (1992) Atherosclerosis 94, 35–42.
[35] Halliwell, B. and Gutteridge, J.M.C. (1989) in: Free Radicals in Biology and Medicine, Oxford University Press.
[36] Rowley, D.A. and Halliwell, B. (1982) FEBS Lett. 138, 33–36.
[37] Saez, G., Thornalley, P.J., Hill, H.A.O., Hems, R. and Bannister, J.V. (1982) Biochim. Biophys. Acta 719, 24–31.
[38] Droge, W., Eck, H.-P., Gmunder, H. and Mihm, S. (1991) Am. J. Med. 91 (3C) 140S–144S.
[39] Gmunder, H., Eck, H.-P, Benninghoff, B., Roth, S. and Droge, W. (1990) Cell Immunol. 129, 32–46.
[40] Searle, A.J.F. and Willson, R.L. (1983) Biochem. J. 212, 549–554.
[41] Schoneich, C., Dillinger, U., von Bruchhausen, F. and Asmus, K-D. (1992) Arch. Biochem. Biophys. 292, 456–467.
[42] Misra, H.P. (1974) J. Biol. Chem. 249, 2151–2155.
[43] Bedwell, S., Dean, R.T. and Jessup, W. (1989) Biochem. J. 262, 707–712.
[44] Minotti, G. and Aust, S.D. (1992) Lipids 27, 219–226.
[45] Hirano, K., Ogihara, T., Miki, M., Yasuda, H., Tamai, H., Kawamura, N. and Mino, M. (1994) Free Rad. Res. 21, 267–277.
[46] Garner, B., Dean, R.T. and Jessup, W. (1994) Atherosclerosis 109, 49.