

The action of defined oxygen-centred free radicals on human low-density lipoprotein

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The effects of defined oxygen-centred free radicals on human low-density lipoprotein (LDL) structure and receptor affinity are discussed in relation to the mechanisms of cell-mediated oxidative modification of LDL. Both hydroxyl (OH^\cdot) and hydroperoxyl (HO_2^\cdot) radicals caused depletion of endogenous α -tocopherol and formation of hydroperoxides. Superoxide ($\text{O}_2^{\cdot-}$) radicals produced only very limited oxidation, but could potentiate oxidation stimulated by the addition of Cu^{2+} . All these radicals enhanced the net negative charge of intact LDL and induced fragmentation of apolipoprotein B-100 (apo B). OH^\cdot also caused cross-linking of apo B. Radical attack decreased the affinity of LDL for the fibroblast apo B/E receptor, but did not enhance its endocytosis by mouse macrophages.

INTRODUCTION

There has recently been much interest in oxidative modifications to human low-density lipoprotein (LDL) which lead to its relatively uncontrolled uptake by macrophages, and which might contribute to the formation of foam cells in the developing atherosclerotic lesion *in vivo*. Many studies have shown that cell-mediated alterations, in which LDL is incubated with endothelial cells, smooth muscle cells or mononuclear phagocytes from a number of species, lead to the generation of modified form(s) of LDL which are more rapidly endocytosed by macrophages [1–4]. This is associated with changes in the LDL particle such as the formation of lipid peroxides, fragmentation of apolipoprotein B-100 (apo B), an increased net negative surface charge and the development of chemotactic and cytotoxic properties as well as enhanced endocytosis by macrophages [1, 5–7].

The molecular mechanisms underlying these alterations remain to be determined. In particular, the exact contribution of cells to the modification process has not been established. Some reports implicate cell-derived superoxide radicals ($\text{O}_2^{\cdot-}$) [8], while others do not [3].

To study the mechanism of LDL oxidative modification, we have examined the effects of several oxygen-centred free radicals on LDL structure and metabolism, using steady-state radiolysis to produce selectively $\text{O}_2^{\cdot-}$, hydroperoxyl (HO_2^\cdot) or hydroxyl (OH^\cdot) radicals in aqueous solutions of human LDL. We report here the effects of these radicals on the process of LDL modification, and their likely relevance *in vivo*.

EXPERIMENTAL

Materials

All chemicals were purchased from either BDH (Poole, Dorset, U.K.) or Sigma (Poole, Dorset, U.K.), and were of analytical grade where available. Tissue culture media

and sera were from Flow Laboratories (Rickmansworth, Herts, U.K.) and tissue culture plastics from Cel-Cult (Sterilin, Middx., U.K.) or Costar (Northumbria Biologicals, Cramlington, Northumbria, U.K.). Precast agarose gels were from Corning (Pao Alto, CA, U.S.A.). Carrier-free ^{125}I and $[1\text{-}^{14}\text{C}]$ oleate were from Amersham International (Aylesbury, Bucks., U.K.).

LDL preparation

Blood from normal volunteers was collected after overnight fasting by venipuncture into 50 ml centrifuge tubes containing EDTA and aprotinin (final concentrations of 4 mM and 90 kallikrein inhibitory units/ml respectively), and the plasma obtained by centrifugation. LDL (density 1.019–1.05 g/ml) was isolated by differential density ultracentrifugation [9] using KBr solutions for density adjustments. The LDL was washed by two further centrifugation steps at a density of 1.055 g/ml and dialysed for 24 h against at least five changes of 50–100 vol. of phosphate-buffered saline (PBS; Dulbecco A) containing EDTA (1 mg/ml) and chloramphenicol (0.1 mg/ml) [PBS/E/C]. All dialysis buffers and solutions for density adjustments were deoxygenated immediately before use by bubbling with N_2 and the dialyses were performed under essentially anaerobic conditions in stoppered bottles at 4 °C.

Iodination of LDL was performed by the iodine monochloride method [10]. The iodinated LDL was separated from unreacted iodide on a column of Sephadex G-25M (PD-10, Pharmacia) equilibrated with PBS containing EDTA (1 mg/ml) and dialysed for 48 h against deoxygenated PBS/E/C. Acetylation of LDL was as described [11].

Free radical generation

Radical species were generated in solution by irradiation of LDL using a 2000 Ci ^{60}Co source at a dose rate of 30–50 Gy/min at room temperature [12,13]. Prior to irradiation, the LDL was adjusted to a

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concentration of 1 mg of protein/ml (approx. $2.0 \mu\text{M}$ -apo B) and dialysed for 2 h (with two changes of 50–100 vol.) against Chelex-treated 10 mM-potassium phosphate buffer (pH 7.4). Aliquots (1.0 ml) of the LDL solution were top-gassed with the appropriate (water-saturated) gas for 15 min before irradiation and continuously during it. For generation of predominantly OH^\cdot radical, the gas was N_2O or $\text{N}_2\text{O}/\text{O}_2$ (5:1); gassing with air and the addition of 10 mM-formate to the solution permitted selective generation of either predominantly $\text{O}_2^{\cdot-}$ radical (at pH 7.4), or its conjugate acid, HO_2^\cdot radical (at pH 4.0).

Measurement of peroxidation

Immediately following irradiation, aliquots of LDL were incubated with catalase ($2.5 \mu\text{g}/\text{ml}$) for 30 min at

room temperature and then assayed on the same day for hydroperoxides, using a modification of the tri-iodide assay [14] adapted for an automated system [15].

Measurement of α -tocopherol

Samples of LDL (0.2–1.0 mg of protein/ml) were extracted into heptane [16] and analysed by h.p.l.c. on a Merck Lichrocart CN column (250 mm \times 4 mm) with hexane/propan-2-ol (99:1, v/v) as the mobile phase and a fluorescence detector (ex. 295 nm; em. 325 nm).

LDL electrophoresis

Agarose electrophoresis of LDL was performed in precast 1% agarose gels in barbitone buffer at pH 8.6. The dried gels were stained for protein using Coomassie

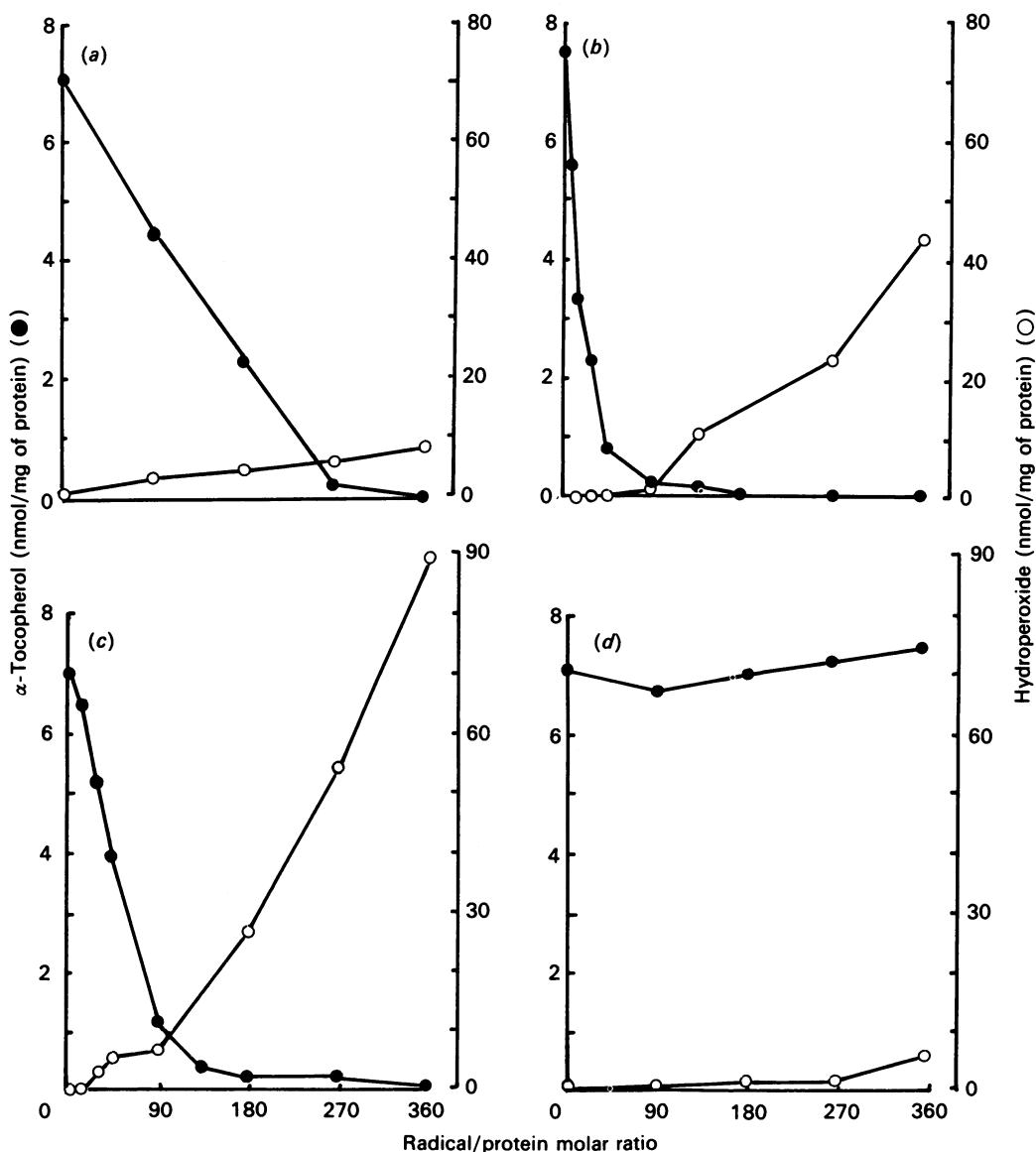


Fig. 1. Stimulation of LDL peroxidation by oxygen radical exposure

LDL solutions (1.0 mg/ml) were exposed to the indicated doses of radicals, then samples taken for extraction and assay of α -tocopherol or incubation with catalase ($2.5 \mu\text{g}/\text{ml}$) and measurement of hydroperoxide content, as described in the Experimental section. (a) $\text{O}_2^{\cdot-}$, (b) HO_2^\cdot , (c) $\text{OH}^\cdot/\text{O}_2$, (d) OH^\cdot . ○, Hydroperoxide; ●, α -tocopherol. Data are means of duplicate determinations that differed by no more than 5% within the experiment and are representative of three separate experiments.

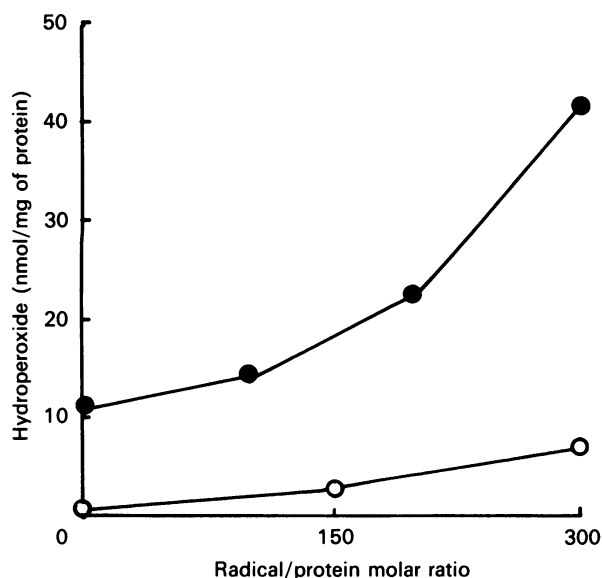


Fig. 2. Effect of superoxide radical exposure on the sensitivity of LDL to copper-catalysed peroxidation

LDL solutions (1.0 mg/ml) were exposed to the indicated doses of $O_2^{\cdot-}$, treated with catalase (2.5 $\mu\text{g}/\text{ml}$), then incubated without further addition (○), or with 100 μM - CuSO_4 (●) for 2 h at 37 °C. EDTA (2 mM) was added to all solutions, and the hydroperoxide content assayed immediately. Data are means of duplicate determinations that differed by no more than 9%.

Brilliant Blue R-250 in water/methanol/acetic acid (5:5:2, by vol.).

SDS/polyacrylamide gel electrophoresis was performed in 3–20% discontinuous gradient slab gels [17]. LDL samples (100 μg of protein) were lyophilized and delipidated with chloroform/methanol (2:1, v/v) [18]. The residue was heated at 95–100 °C for 2 min with sample buffer containing 10% mercaptoethanol, and aliquots containing 5–20 μg of protein were loaded on to the gels. Staining was performed using Coomassie Brilliant Blue R-250 in water/methanol/acetic acid (5:5:1, by vol.).

Binding assays

Binding of irradiated LDL to the fibroblast apo B/E receptor was determined by its ability to compete with ^{125}I -labelled native LDL for specific, high-affinity binding to surface receptors of Chinese hamster ovary (CHO-K1) fibroblasts [19]. Uptake of LDL by macrophages was measured by stimulation of [^3H]oleate incorporation into cholesteryl [^3H]oleate by Swiss TO mouse resident peritoneal macrophages [20].

Where indicated, data were subjected to a one-way analysis of variance (Student's *t*-test).

RESULTS

Lipid peroxidation and α -tocopherol depletion in radical treated LDLs

LDL solutions were exposed to radicals at room temperature, and peroxidation was determined in aqueous samples after catalase incubation to remove hydrogen peroxide generated during irradiation.

The effects of the free radicals on LDL peroxidation

are shown in Fig. 1. $O_2^{\cdot-}$ radicals produced very low levels of hydroperoxides, even at the highest dose supplied. In contrast, HO_2^{\cdot} radicals were much more reactive and caused significant peroxidation. OH^{\cdot} radicals were still more efficient at generating LDL hydroperoxides and this was entirely dependent on the presence of oxygen. For both OH^{\cdot} - and HO_2^{\cdot} -mediated oxidation, there was a radical dose below which little or no hydroperoxide formation was detectable. In other oxidative systems, such early resistance of LDL to oxidation is largely attributed to the preferential consumption of endogenous antioxidants such as α -tocopherol and β -carotene [21]. There was a similar relationship between α -tocopherol depletion and peroxidation (Fig. 1) when LDL was exposed to OH^{\cdot} or HO_2^{\cdot} radicals. Tocopherol was oxidized at a detectable but much lower rate by $O_2^{\cdot-}$ radicals. As previously reported for α -tocopherol in liposomes [22], the oxidizing abilities of the three radicals tested followed the sequence $\text{HO}_2^{\cdot} > \text{OH}^{\cdot} > \text{O}_2^{\cdot-}$. The extent of peroxidation produced in LDL, even by these relatively high doses of reactive oxygen species, was low in comparison with that obtained during the cell-mediated modification systems, as judged by relative amounts of thiobarbituric acid-reactive materials generated in the two systems (results not shown), and by direct measurements of hydroperoxide formation. Thus, for example, during macrophage-mediated modification of LDL, hydroperoxide levels of 600–800 nmol/mg of LDL protein were measured in samples incubated with cells for 6 h (W. Jessup, S. Rankin, C. V. de Whalley & D. S. Leake, unpublished work). At this time, detectable formation of high-uptake LDL has occurred [23].

Samples of LDL were also exposed to $O_2^{\cdot-}$ radicals, treated with catalase and incubated for a further 2 h with 100 μM - CuSO_4 . Fig. 2 shows that superoxide treatment increased the susceptibility of LDL to copper-catalysed oxidation in a dose-dependent manner.

Effect of radical attack on apo B integrity

Samples of radical-exposed LDLs were run on 3–20% acrylamide gels under reducing conditions. The 514 kDa band of intact apo B gradually diminished with increasing radical dose (Fig. 3), with the concomitant appearance of lower molecular mass fragments. In all the OH^{\cdot} -treated samples, insoluble Coomassie-staining material was present in the sample wells, which we presume arises from intermolecular cross-linking between apo B molecules. The occurrence of cross-linking in the absence of oxygen, where there is no peroxidation, indicates that a component of this process probably comprises radical–radical interactions. There was specificity in the cleavage by all radicals, with fragments of molecular weights 447 kDa, 190 kDa and 168 kDa produced by all systems. However, several other specific fragments were produced by $O_2^{\cdot-}$ and HO_2^{\cdot} (115, 97 and 66 kDa). All radicals in the presence of oxygen also produced a background smear of fragments; this was most extensive with OH^{\cdot} .

Alterations in electrophoretic mobility

Radical exposure of LDL increased its anodic mobility in a dose- and radical-dependent manner (Table 1). The increases in mobility followed the order $\text{OH}^{\cdot}/\text{O}_2^{\cdot-} > \text{OH}^{\cdot} > \text{HO}_2^{\cdot}$. This order does not correlate with the degree of lipid peroxidation induced, and therefore is unlikely to be due simply to alterations in surface charge

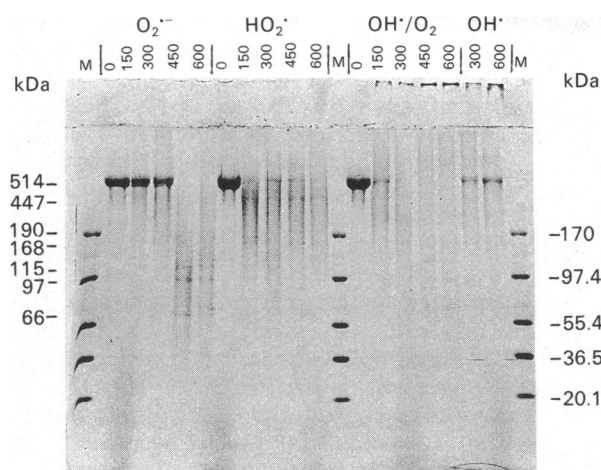


Fig. 3. Integrity of apo B following oxygen radical exposure

LDL solutions (1.0 mg/ml) were exposed to radicals as described in the Experimental section, and then 2 mM-EDTA was added. Post-irradiation samples (100 μ l) were lyophilized and delipidated [18], and heated in 200 μ l of sample buffer under reducing conditions. Samples (20 μ l; 10 μ g of LDL protein) were run on discontinuous 3–20% gradient SDS/polyacrylamide-gel electrophoresis. Standards were: α -macroglobulin (170 kDa), phosphorylase *b* (97.4 kDa), glutamate dehydrogenase (55.4 kDa), lactate dehydrogenase (36.5 kDa) and soybean trypsin inhibitor (20.1 kDa). Figures above the lanes are the calculated radical/protein molar ratios; M, molecular mass markers.

consequent on peroxidation of LDL lipids, or to the binding of reactive products of lipid peroxidation, such as malonaldehyde or 4-hydroxynonenal [24]. In contrast, the increase in mobility which is seen in LDL incubated with redox-active metals, or with modifying cell cultures, is blocked by inhibitors of peroxidation [3], which suggests that these processes are dependent on the binding of lipid peroxy and alkoxy radicals, or other more stable reactive products of peroxidation to apo B.

Affinity of radical-exposed LDLs for the apo B/E and scavenger receptors

We measured the affinity of radical-treated LDLs for

Table 1. Electrophoretic mobilities of free-radical-exposed low-density lipoprotein

The mobilities of the LDLs were measured immediately after irradiation in 1% agarose gels at pH 8.6 for 60 min. Data are expressed relative to the mobility of unirradiated control samples in each case, and are from a single representative experiment.

Radical species	Theoretical radical/protein molar ratio...	Relative electrophoretic mobility				
		0	150	300	450	600
$O_2^{\cdot-}$		1.00	1.04	1.04	1.04	1.12
HO_2^{\cdot}		1.00	1.10	1.24	1.38	1.38
OH^{\cdot}		1.00	1.13	1.48	1.57	1.57
OH^{\cdot}/O_2		1.00	1.20	1.60	1.70	1.90

Table 2. Competitive inhibition of native LDL binding to fibroblast apo B/E receptors by radical-exposed LDLs

^{125}I -labelled native LDL was incubated at 20 μ g of protein/ml at 4 $^{\circ}C$ for 2 h with confluent cultures of CHO-K1 fibroblasts, which had been upregulated by pre-incubation in lipoprotein-deficient serum for 48 h [19]. Unlabelled, irradiated (1000 Gy; radical/protein molar ratio 300) or control LDLs were added at 20 μ g of protein/ml, as indicated. Specific binding was measured as described in the Experimental section. In the absence of any competing lipoproteins, the measured binding (100%) was 46.9 ± 3.1 ng/mg of cell protein. Data are means \pm S.D. of triplicate determinations. * $P < 0.05$ compared with control.

Radical species	Binding of native LDL (% of uninhibited control binding)
None (control)	53.0 ± 5.7
$O_2^{\cdot-}$	56.4 ± 6.4
HO_2^{\cdot}	63.6 ± 11.9
OH^{\cdot}	$72.4 \pm 5.9^*$
OH^{\cdot}/O_2	$67.0 \pm 2.5^*$

the apo B/E receptor by comparing their ability to compete with ^{125}I -labelled native LDL for binding to fibroblast surface receptors at 4 $^{\circ}C$ (Table 2). LDL exposed to 300 mol of radicals/mol of protein gave a significant decrease in affinity for the LDL receptor only in the OH^{\cdot} -treated samples, despite the fact that degradation of the apo B was detectable in all of the conditions (Fig. 3).

The rate of uptake of radical-treated LDLs by mouse resident peritoneal macrophages was also determined. These cells endocytose acetylated LDL more rapidly than native LDL (Table 3), and much of cell-modified LDL is taken up by the same ('scavenger') receptor [1]. But none of the radicals tested was able to produce a species of LDL which was endocytosed more rapidly than native LDL.

Table 3. Stimulation of cholesterol esterification in macrophages by altered LDL

Mouse resident peritoneal macrophages were incubated with [^{14}C]oleyl albumin in the presence of 25 μ g of protein/ml of the indicated LDL species for 24 h at 37 $^{\circ}C$, and [^{14}C]cholesteryl oleate formation was measured as described in the Experimental section. Data are the means \pm S.D. of triplicate determinations. All radical species were supplied at a radical/protein molar ratio of 300. * $P < 0.05$ compared with control.

LDL pretreatment	[^{14}C]Oleate esterification (nmol/mg of cell protein)
None (control)	0.210 ± 0.026
$O_2^{\cdot-}$	0.287 ± 0.091
HO_2^{\cdot}	0.190 ± 0.051
OH^{\cdot}	0.185 ± 0.034
OH^{\cdot}/O_2	0.213 ± 0.087
Acetylation	$20.92 \pm 5.556^*$

DISCUSSION

All of the oxygen-centred radicals examined in this study were capable of producing some of the changes in LDL structure associated with the cell-mediated modification process, although none could completely reproduce it. This is not unexpected, since redox-active metals, which are an essential feature of the cell modification system [2], were not added in most of these studies *in vitro*. By using this system, it was possible to study the individual reactivities of $O_2^{\cdot-}$, HO_2^{\cdot} and OH^{\cdot} towards LDL. All of these radicals may be produced, directly or indirectly, by modifying cell cultures.

$O_2^{\cdot-}$ induced little peroxidation in LDL, which is consistent with the low reactivity of this species towards unsaturated lipids [25]. Like those authors, we found the protonated form of $O_2^{\cdot-}$ (HO_2^{\cdot}) was much more active in inducing hydroperoxide formation. HO_2^{\cdot} may initiate peroxidation by abstracting hydrogen from LDL lipids, but it has also been suggested that it may act by decomposing traces of lipid peroxides (see [26]). These species are almost certainly present in low amounts in freshly isolated LDL, and their existence is probably required to prime cell-free Cu^{2+} -mediated oxidation of LDL.

The relationships between the depletion of α -tocopherol and the rate of peroxidation of LDL indicate that the type of radical to which LDL is exposed may influence its susceptibility to oxidation. Thus, OH^{\cdot} was able to induce some peroxide formation even when substantial amounts of tocopherol were still present, whereas HO_2^{\cdot} appeared to react rather more selectively with tocopherol at low doses. Though charged, even $O_2^{\cdot-}$ was able to oxidize tocopherol in LDL, at a rate approx. 10-fold greater than that which could be accounted for by the (1%) protonated radicals present at this pH. Cell-derived superoxide may therefore have a direct role in promotion of LDL oxidation, both by depleting endogenous antioxidants and by inducing the formation of low amounts of peroxides. The increase in the pro-oxidant activity of Cu^{2+} towards LDL which was induced by $O_2^{\cdot-}$ pretreatment may result from both of these effects. In areas of locally low pH, such as the endosome [27] and at the surfaces of membranes [28], such as the plasma membrane, the conversion of $O_2^{\cdot-}$ to HO_2^{\cdot} by equilibrium could lead to much more extensive peroxidation.

Oxygen-centred radicals can fragment proteins in solution [29] and lipid environments [30,31]. Loss of intact apo B was seen after oxidation of LDL *in vitro* [32,33], during cell-modulated modification [6] and in this study. All the radicals produced some specific fragmentation. In the presence of oxygen, all radicals also produced a background smear of fragments. The extent to which this occurred was related to the amount of peroxidation induced by the radicals, which is consistent with the view that hydroperoxides can promote protein cleavage [30,31]. We also found evidence of OH^{\cdot} -mediated cross-linking in the absence of oxygen, as has been noted for other soluble and membrane proteins [13,29,31].

The alterations to apo B which occur during oxidative modification of LDL probably account for the changes which occur in the affinities of the particle for the LDL (apo B/E) receptor and macrophage receptors. The critical events are not understood, but are thought likely

to be the result of conformational changes in critical binding domains, or in the derivatization of essential amino acid residues by products of lipid peroxidation [35,36]. The affinity of radical-treated LDLs for the apo B/E receptor was little altered, even though substantial loss of intact apo B was detected in most treatments. Either the receptor-binding domain was largely unaffected by the conditions used, or any cleavages which did occur did not produce gross conformational changes in the binding region.

Free radical attack was also insufficient to generate a species of LDL which is rapidly endocytosed by macrophages. This is despite the fact that those cell systems which can develop modified LDL with a high affinity for macrophage receptors, such as the scavenger receptor, produce much smaller quantities of $O_2^{\cdot-}$ than were used in this study. For example, arterial smooth muscle cells produce only approx. 10% of the average dose we supplied during productive modification of LDL [37]. The cell-mediated system seems to rely on a quite extensive degree of redox-metal-catalysed peroxidation, leading to greater oxidative decomposition of LDL lipids than we inflicted in our system. The inability of OH^{\cdot} alone to produce a modified LDL suggests that the requirement for iron or copper by the cell modification system is for catalytic decomposition of peroxides, rather than the conversion of $O_2^{\cdot-}$ to more reactive species such as OH^{\cdot} .

REFERENCES

- Henricksen, T., Mahoney, E. M. & Steinberg, D. (1981) *Proc. Natl. Acad. Sci. U.S.A.* **78**, 6499–6503
- Heinecke, J. W., Rosen, H. & Chait, A. (1984) *J. Clin. Invest.* **74**, 1890–1894
- Hinsbergh, V. W. M., Scheffer, M., Havekes, L. & Kempen, H. J. M. (1986) *Biochim. Biophys. Acta* **878**, 49–64
- Parathasarathy, S., Printz, D. J., Boyd, D., Joy, L. & Steinberg, D. (1986) *Arteriosclerosis* **6**, 505–510
- Morel, D. W., DiCorleto, P. E. & Chisholm, G. M. (1984) *Arteriosclerosis* **4**, 357–364
- Steinbrecher, U. P., Parathasarathy, S., Leake, D. S., Witzum, J. L. & Steinberg, D. (1984) *Proc. Natl. Acad. Sci. U.S.A.* **81**, 3883–3887
- Quinn, M. T., Parathasarathy, S. & Steinberg, D. (1985) *Proc. Natl. Acad. Sci. U.S.A.* **82**, 5949–5953
- Steinbrecher, U. P. (1988) *Biochim. Biophys. Acta* **959**, 20–30
- Havel, R. J., Eder, H. H. & Bragdon, J. H. (1955) *J. Clin. Invest.* **34**, 1345–1353
- Bilheimer, D. W. S., Eisenberg, S. & Levy, R. I. (1972) *Biochim. Biophys. Acta* **60**, 212–221
- Basu, S. K., Anderson, R. G. W., Goldstein, J. L. & Brown, M. S. (1976) *Proc. Natl. Acad. Sci. U.S.A.* **73**, 3178–3182
- Willson, R. L. (1978) in *Biochemical Mechanisms of Liver Injury* (Slater, T. F., ed.), pp. 123–224, Academic Press, New York
- Dean, R. T., Roberts, C. R. & Jessup, W. (1985) in *Intracellular Protein Catabolism* (Khairallah, E. A., Bond, J. S. & Bird, J. W. C., eds.), pp. 341–350, A. R. Liss, New York
- Hicks, M. & Gebicki, J. M. (1978) *Anal. Biochem.* **99**, 249–253
- Thomas, S. M., Jessup, W., Gebicki, J. M. & Dean, R. T. (1988) *Anal. Biochem.* **176**, 353–359
- Burton, G. W., Webb, A. & Ingold, K. U. (1985) *Lipids* **20**, 29–39

17. Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685
18. Weisgraber, K. H., Innerarity, T. L. & Mahley, R. W. (1978) *J. Biol. Chem.* **253**, 9053–9062
19. Kreiger, M., Brown, M. S. & Goldstein, J. L. (1981) *J. Mol. Biol.* **150**, 167–184
20. Brown, M. S., Goldstein, J. L., Kreiger, M., Ho, Y. K. & Anderson, R. G. W. (1979) *J. Cell Biol.* **82**, 597–613
21. Esterbauer, H., Jurgens, G., Quehenberger, O. & Koller, E. (1987) *J. Lipid Res.* **28**, 495–509
22. Fukuzawa, K. & Gebicki, J. M. (1983) *Arch. Biochem. Biophys.* **226**, 242–251
23. Rankin, S. M. & Leake, D. S. (1988) *Agents Actions Suppl.* **26**, 233–239
24. Jurgens, G., Hoff, H., Chisholm, G. M. & Esterbauer, H. (1987) *Chem. Phys. Lipids* **45**, 315–336
25. Gebicki, J. M. & Bielski, B. J. H. (1981) *J. Am. Chem. Soc.* **103**, 7020–7022
26. Halliwell, B. & Gutteridge, J. M. C. (1985) *Free Radicals in Biology and Medicine*, Oxford University Press, Oxford
27. Ohkuma, S. & Poole, B. (1978) *Proc. Natl. Acad. Sci. U.S.A.* **75**, 3327–3331
28. Davis, J. T. & Rideal, E. K. (1963) in *Interfacial Phenomena*, p. 95, Academic Press, New York
29. Wolff, S. P. & Dean, R. T. (1986) *Biochem. J.* **234**, 399–403
30. Hunt, J. V., Simpson, J. A. & Dean, R. T. (1988) *Biochem. J.* **250**, 87–93
31. Dean, R. T., Thomas, S. M., Vince, G. & Wolff, S. P. (1986) *Biomed. Biochim. Acta* **45**, 1563–1573
32. Schuh, J., Fairclough, G. F. & Haschmeyer, R. H. (1978) *Proc. Natl. Acad. Sci. U.S.A.* **75**, 3173–3177
33. Montgomery, R. R., Nathan, C. F. & Cohn, Z. A. (1986) *Proc. Natl. Acad. Sci. U.S.A.* **83**, 6631–6635
34. Dean, R. T., Thomas, S. M. & Garner, A. (1986) *Biochem. J.* **240**, 489–494
35. Parathasarathy, S., Fong, L. G., Otero, D. & Steinberg, D. (1987) *Proc. Natl. Acad. Sci. U.S.A.* **84**, 537–540
36. Steinbrecher, U. P. (1987) *J. Biol. Chem.* **262**, 3603–3608
37. Heinecke, J. W., Baker, L., Rosen, H. & Chait, A. (1986) *J. Clin. Invest.* **77**, 757–761

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