FEBS Letters 433 (1998) 196–200 FEBS 20670

Enzymatic repair of oxidative damage to human apolipoprotein A-I

Alexander B. Sigalov[a,*], Lawrence J. Stern[b]

[a]*Biomedical Department, AMW Co. Ltd., 22-1-11 Tarusskaya Street, Moscow 117588, Russia*
[b]*Department of Chemistry, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Cambridge, MA 02139, USA*

Received 22 June 1998

Abstract Oxidative damage to apolipoprotein A-I that occurs in vivo commonly involves methionine oxidation, and is accompanied by alterations in structure, lipid association, and cholesterol efflux function. We have used the enzyme peptide methionine sulfoxide reductase (PMSR) to reverse this damage, and shown by a variety of criteria that enzyme treatment restores the primary, secondary, and tertiary structure and lipid association characteristic of the native unoxidized protein. Lipid-associated as well as lipid-free apolipoprotein A-I reacts with PMSR, suggesting that enzymatic reduction of oxidized apolipoprotein A-I in high density lipoproteins can result in restoration of biological activity and the ability to promote cholesterol efflux from cells.

Key words: Apolipoprotein A-I; Methionine oxidation; Enzymatic reduction; Peptide methionine sulfoxide reductase

1. Introduction

Serum levels of high density lipoproteins (HDL) inversely correlate with the risk of coronary heart disease [1], and the protective effect of HDL against atherosclerosis is thought to be due to its ability to mediate cholesterol efflux from cells in the process of reverse cholesterol transport [2]. Apolipoprotein (apo) A-I is the major protein constituent of HDL, comprising about 70% of the total HDL protein. Both lipid-bound and lipid-free forms of apo A-I play important roles in this process [3]. Two of the three methionines (Met-112 and Met-148) in apo A-I can readily be oxidized to methionine sulfoxides by chemical oxidizing agents in vitro [4,5]. Also, there is evidence for direct reduction of lipoprotein lipid hydroperoxides by apo A-I, with conversion of Met-112 and Met-148 to methionine sulfoxides [6,7]. Both the oxidized and unoxidized forms of apo A-I occur in vivo, and in fresh plasma samples the ratio oxidized/unoxidized apo A-I exhibits considerable interindividual variability [4]. Methionine oxidation can alter the ability of the apolipoprotein to associate with lipid [4,5], and oxidized apolipoprotein exhibits structural alterations as detected by circular dichroism spectroscopy and proteolytic susceptibility [5,8]. Recently, it has been shown that cellular cholesterol efflux mediated by HDL depends upon the ratio of unoxidized/oxidized apo A-I, and that the difference in the extent of efflux can reach 30% [9]. In addition, Met-112 and Met-148 of apo A-I are located within the central region of the molecule which is involved in the activation of the cholesterol esterifying enzyme lecithin:cholesterol acyltransferase [10].

Considering the deleterious effects of apo A-I oxidation and also that the oxidized form of apo A-I is contained in advanced human atherosclerotic plaques [7], the search for compounds which are able selectively to reduce methionine sulfoxides and to restore the structure and biological properties of both lipid-free and lipid-bound apo A-I will not only contribute to our understanding of the mechanisms of lipid metabolism, but could also lead to the development of novel approaches to the prevention and treatment of atherosclerosis and related cardiovascular diseases. However, previous attempts selectively to reduce methionine sulfoxides in oxidized apo A-I by chemical compounds, such as mercaptoethanol or dimethyl sulfide, were unsuccessful or incompletely characterized [4]. On the other hand, it is well known that methionine sulfoxide residues in proteins can be readily reduced by peptide methionine sulfoxide reductase (PMSR), an enzyme present in microorganisms and plants [11] and practically all mammalian tissues [12]. PMSR has been shown to reactivate the oxidized inactive derivatives of α1-protease inhibitor [13], ribosomal protein L12 [11] and Met-enkephalin [11].

In the present report we describe, for the first time, the ability of a mammalian PMSR overexpressed in *Escherichia coli*, as well as of an enzyme contained in a crude spinach extract, to reduce methionine sulfoxides in the oxidized apo A-I and in this way to restore the structure and function of this apolipoprotein.

2. Materials and methods

2.1. Enzymes

A homogeneous preparation of bovine PMSR, EC 1.8.4.6, overexpressed in *E. coli* was a gift from Prof. Nathan Brot from the Hospital for Special Surgery affiliated with the New York Hospital and the Cornell University Medical College, New York, NY, USA, and provided as a solution in 25 mM Tris-HCl, pH 7.4 with a protein concentration of 0.37 mM. Chymotrypsin (TLCK treated, EC 3.4.21.1, 60.6 U/mg) was purchased from Fluka (Buchs, Switzerland). Cell-free spinach S-30 extract was prepared as described by Brot et al. [11] and filtered (0.22 μm filter).

2.2. Apolipoproteins

Apo A-I was isolated and purified as previously described [14] by delipidation and subsequent gel filtration and ion-exchange chromatography of HDL obtained from pooled human serum by differential ultracentrifugation. unoxidized and oxidized apo A-I forms were isolated from the initial apo A-I preparation by preparative HPLC using a BioCAD/SPRINT System from PerSeptive Biosystems (Cambridge,

*Corresponding author. Fax: (7) (095) 423-6868.
E-mail: alexsig@cityline.ru

Abbreviations: HDL, high density lipoproteins; apo A-I, apolipoprotein A-I; PMSR, peptide methionine sulfoxide reductase; DTT, dithiothreitol; DHPC, 1,2-diheptanoyl-*sn*-glycero-3-phosphocholine; CMC, critical micellar concentration; apo $A\text{-}I_{unox}$, unoxidized apo A-I contained in initial serum apo A-I; apo $A\text{-}I_{ox}$, oxidized apo A-I contained in serum apo A-I or obtained from unoxidized apo A-I using hydrogen peroxide; apo $A\text{-}I_{red}$, reduced apo A-I obtained by reduction of oxidized apo A-I using PMSR; DHPC, 1,2-diheptanoyl-*sn*-glycero-3-phosphocholine; POPC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine

PII: S0014-5793(98)00908-9

MA, USA), a Vydac C-18 column (22 mm×250 mm) and a two-solvent system: A, trifluoroacetic acid/water (1:1000, v/v); B, trifluoroacetic acid/acetonitrile/water (1:900:100, v/v). The column was heated to 50°C in a water bath and proteins were eluted at a flow rate of 15 ml/min with 28–49%, 49–53% and 53–73% gradient steps of solvent B over 12, 9 and 12 min, respectively. Then the content of solvent B was increased to 100% over 3 min, and finally decreased to 28% over 2 min. Peaks were identified by SDS-PAGE (12.5% acrylamide) using the standard Laemmli system [15] and by analytical HPLC (see below). Fractions containing apo A-I were frozen by liquid nitrogen and lyophilized. To verify that the isolation procedure did not contribute to the observed variation in the proportion of oxidized apo A-I, two samples were isolated from the same batch of pooled serum and found to have the same fractional oxidation (6% and 7%). Also, repetition of the delipidation and chromatography steps on a sample of purified apo A-I did not result in a change in the fractional oxidation. Naturally occurring apo A-I_{ox}, and apo A-I_{ox} obtained from unoxidized protein by oxidation with hydrogen peroxide, have the same primary structure and contain two methionine sulfoxide groups at Met-112 and Met-148 [4], so in the text we use the term 'oxidized' to designate both these species, while the terms 'unoxidized' and 'reduced' are used for naturally occurring apo A-I_{unox}, and for apo A-I obtained by reduction of the oxidized protein using PMSR, respectively.

2.3. Protein analytical techniques

Protein concentrations were measured using the bicinchoninic acid assay (Pierce, Rockford, IL, USA) with BSA as a standard. Analytical HPLC was performed using a Waters Automated Gradient Controller, a Waters 745B Data Processor and a Thermo Separation Products Spectra 100 UV-visible detector, coupled to a Vydac C-18 column (4.6 mm×250 mm) heated to 50°C and eluted with the same two-solvent system at a flow rate of 1.2 ml/min and 28–64% gradient of B over 33 min. Then the content of B was increased to 100% over 2 min, and finally decreased to 28% over 2 min. The HPLC column eluates were monitored by absorbance at 214 nm. Non-denaturing PAGE was carried out as for SDS-PAGE except SDS was omitted from all steps. Mass spectra were measured using a Voyager Elite STR mass spectrometer from PerSeptive Biosystems (Cambridge, MA, USA).

2.4. Chemical oxidation of apo A-I

To prepare apo A-I_{ox} containing sulfoxides at methionines 112 and 148, the unoxidized protein (approx. 25 mg) was dissolved in 1 ml of 3 M guanidine-HCl, pH 7.4, and then hydrogen peroxide was added to a final concentration of 300 mM. The mixture was incubated at room temperature for 15 min, and an oxidized protein was purified using preparative HPLC. As has been shown by von Eckardstein et al. [4], two methionine residues 112 and 148 (but not 86) in apo A-I are oxidized in parallel in a result of this procedure.

2.5. Enzymatic reduction of apo A-I methionine sulfoxide residues

Reduction of apo A-I_{ox} (30–90 μg) was carried out at 37°C in 33 mM Tris-HCl, 13 mM magnesium chloride, 13 mM dithiothreitol, pH 7.5, containing 4–12 M PMSR in a total volume of 30–90 μl. When using a cell-free spinach S-30 extract as the source of the enzyme, 2–6 μl of the extract was added instead of PMSR. Typically, the reaction mixtures were incubated for 60 or 120 min (overnight in the experiments with the spinach extract) and then analyzed by HPLC as described above or frozen using liquid nitrogen and stored at −70°C. When analyzing by HPLC, peak areas were quantitated with a Waters 745B Data Processor and the data were used to calculate the extent of reduction of apo A-I_{ox}. The peak containing apo A-I_{red} was collected into Eppendorf tubes (1.5 ml), frozen by liquid nitrogen and lyophilized for further analysis. In addition, the retention time of the chemically oxidized apo A-I was compared with a naturally occurring oxidized protein.

2.6. Cyanogen bromide (CNBr) treatment and proteolytic digestion with chymotrypsin

Twenty-five microgram each of unoxidized, oxidized and reduced proteins were dissolved in 88% formic acid (25 μl) and treated with 0.2 mg of CNBr at room temperature for 24 h. Then, the reaction mixtures were diluted with 250 μl of double distilled water and lyophilized for further analysis using SDS-PAGE (15% acrylamide).

Samples of unoxidized, oxidized and reduced apo A-I (10 μg) in 15 μl of PBS (0.02 M phosphate, 0.15 M NaCl, pH 7.2) were also treated with chymotrypsin at 37°C for 75 min using a protein:protease ratio of 1500:1 (w/w) and then analyzed by SDS-PAGE (15% acrylamide).

2.7. Circular dichroism

CD spectra were collected on solutions of 1.8–7.2 μM (0.05–0.2 mg/ml) apo A-I in 10 mM ammonium bicarbonate, 0.005% sodium azide, pH 7.8 with a 1 mm path-length cell using an AVIV 62DS spectropolarimeter. In some experiments DHPC (with CMC of 1.4 mM) or urea was added to the samples to a final concentration of 2.3 mM or 4 M, respectively. Data were collected at 25°C every nanometer from 195 to 260 nm with 1.0 s averaging per point and a 1 nm bandwidth. Spectra of at least five scans were signal averaged and baseline corrected by subtracting an averaged buffer spectrum. The spectra were normalized to molar residue ellipticity using a mean residue weight of 115.2 Da for human apo A-I. For comparison with previous results we calculated an apparent fractional percent α-helix content from the molar ellipticities at 222 nm by the method of Chen et al. ($[\phi]_{222}=-30\,300\,f_H-2340$, where f_H is the fraction of α-helical structure) [16]. Apo A-I spectra were concentration-independent in the concentration range from 1.8 to 7.2 μM indicating the lack of significant protein aggregation at these concentrations. In experiments on temperature-induced unfolding the data were collected on solutions of 7.2 μM (0.2 mg/ml) apo A-I in 10 mM ammonium bicarbonate, 0.005% sodium azide, pH 7.8 with a 1 mm path-length cell at 222 nm every 1°C from 25°C to 95°C with 5.0 s averaging per point and a 1 nm bandwidth.

3. Results and discussion

The initial apo A-I preparation isolated from pooled human serum and used in this study was a mixture of both apo A-I_{ox} (~20%) and apo A-I_{unox} (~80%) protein species, as shown by analytical reversed-phase HPLC (Fig. 1A). This protein mixture migrated as a single band on SDS-PAGE (data not shown) and its chromatographic profile and retention times of apo A-I_{ox} and apo A-I_{unox} were consistent with previously reported data [4,5]. The percentage of apo A-I_{ox} in eight other apo A-I preparations obtained from pooled human sera (about 200 individual specimens in each pool) using the same isolation technology had varied from 3% to 25%, providing further indirect evidence of considerable interindividual variability of the ratio oxidized/unoxidized apo A-I [4]. The two components (apo A-I_{ox} and apo A-I_{unox}) in the initial serum apo A-I preparation were separated by preparative HPLC. The isolated apo A-I_{unox} exhibited a single peak by analytical HPLC (Fig. 1B) and a single band on SDS-PAGE (Fig. 2A, lane 1). Treatment of apo A-I_{unox} with hydrogen peroxide resulted in the formation of apo A-I_{ox} which exhibited the same retention time as the apo A-I_{ox} component of the initial preparation (Fig. 1C). The electrophoretic pattern of apo A-I_{ox} on SDS-PAGE (Fig. 2A, lane 2) did not differ from that for the unoxidized form (Fig. 2A, lane 1) while on non-denaturing PAGE the oxidized form exhibited a band with about 1.8 times higher electrophoretic mobility in comparison with a relevant band of apo A-I_{unox} (data not shown).

Incubation of apo A-I_{ox} with purified PMSR in the presence of DTT resulted in the appearance of a new peak on analytical HPLC chromatogram (apo A-I_{red}) with the same retention time as for apo A-I_{unox} (Fig. 1D). Similar results were obtained when a spinach S-30 cell-free extract was added as the source of the enzyme (data not shown) With the purified PMSR enzyme preparation, the reaction yielded 35–40% apo A-I_{red} (as a percentage of a total apo A-I amount) after 5 min incubation. Longer incubation time (up to 2.5 h), increased apo A-I concentration (12.5–125 μM), or increased

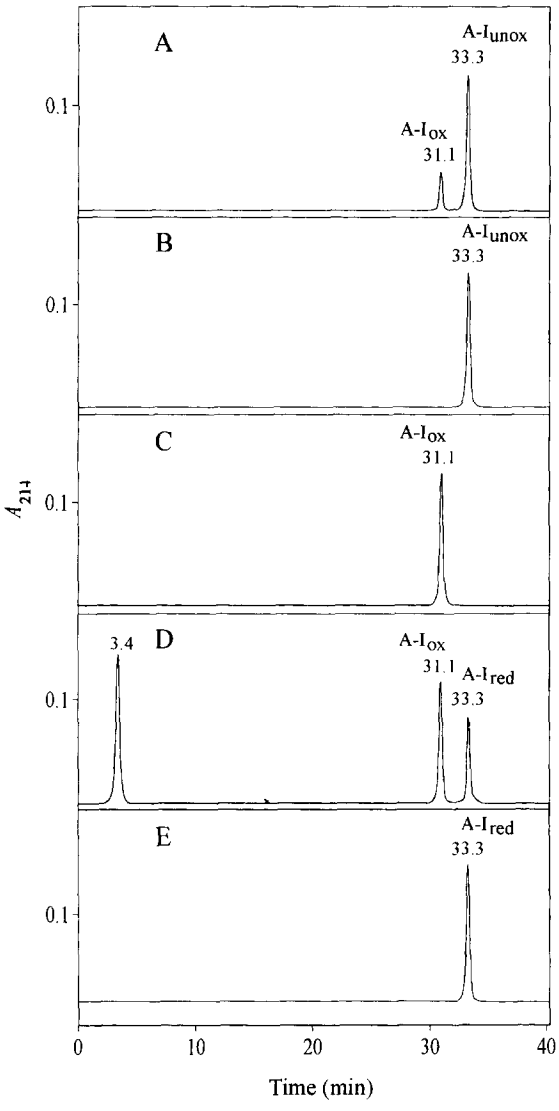

Fig. 1. Analytical reversed-phase HPLC profiles of initial apo A-I (A), apo A-I_{unox} isolated from initial apo A-I by preparative HPCL (B), apo A-I_{ox} obtained by treatment of apo A-I_{unox} with hydrogen peroxide for 15 min and subsequent isolation by preparative HPLC (C), apo A-I_{ox} after incubation with PMSR for 60 min (D) and apo A-I_{red} isolated by HPLC (E). Retention times (in minutes) are shown above each peak.

DTT concentration (13–26 mM) did not significantly increase the final yield of reduced protein. When using the spinach extract, the reaction had a linear relationship between activity and amount of extract but maximum yield of reduced protein also did not exceed 35–40%. The reasons for the lack of a more complete conversion of apo A-I_{ox} to apo A-I_{red} by PMSR are not clear. Apo A-I_{red} protein formed as a result of enzymatic reduction was isolated as a homogeneous preparation (Fig. 1E), having the same electrophoretic mobility as apo A-I_{unox} on SDS-PAGE (Fig. 2A, lane 3) and on non-denaturing PAGE (data not shown). This material was used in further studies. Reoxidation of the isolated apo A-I_{red} using hydrogen peroxide resulted in the formation of oxidized protein form with a chromatographic pattern similar that for apo A-I_{ox} (data not shown).

Matrix-assisted laser desorption mass spectrometry revealed that apo A-I_{ox} was 32 mass units greater than the initial apo A-I_{unox} and apo A-I_{red}. Therefore, under the conditions used in this study, both of the labile methionine residues Met-112 and Met-148 are oxidized by H_2O_2, consistent with previously reported data [4], and both are reduced by PMSR. CNBr cleaves proteins by conversion of methionine to homoserine lactone with concomitant peptide bond cleavage, but methionine sulfoxides are resistant to reaction. We used CNBR to probe for the presence of methionine and methionine sulfoxide residues in apo A-I preparations. The results of CNBr digestion of unoxidized, oxidized and reduced apo A-I are shown in Fig. 2B (lanes 1, 2 and 3, respectively). Unoxidized and reduced apo A-I had the same electrophoretic pattern and were almost completely by CNBr while apo A-I_{ox} was much more resistant as previously reported by Anantharamaiah et al. [5]. These results indicate that unoxidized and reduced apo

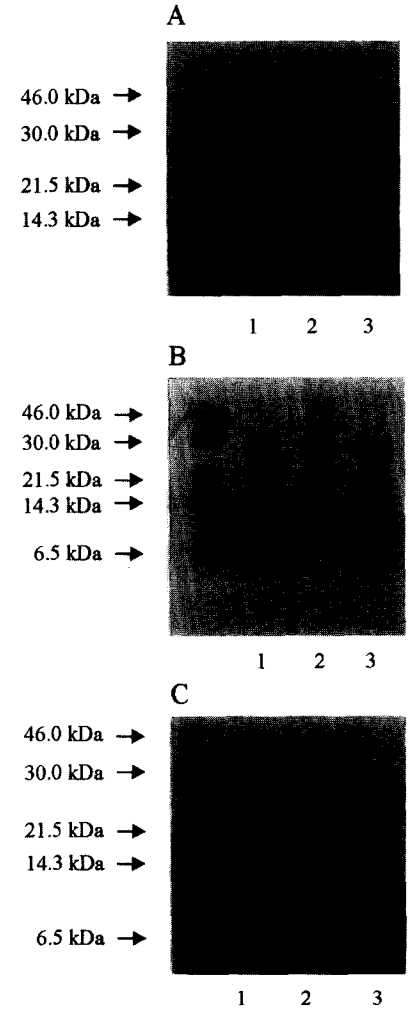

Fig. 2. SDS-PAGE analysis of unoxidized, oxidized and reduced apo A-I and digestion with chymotrypsin and CNBr. Undigested apo A-I (A) or apo A-I digested with CNBr (B) or chymotrypsin (C) were analyzed by 12.5% (A) or by 15% (B and C) acrylamide SDS-PAGE and detected by staining with Coomassie blue R-250. Lane 1, apo A-I_{unox}; lane 2, apo A-I_{ox}; lane 3, apo A-I_{red}. The positions and molecular masses of protein standards are indicated on the left of the gels.

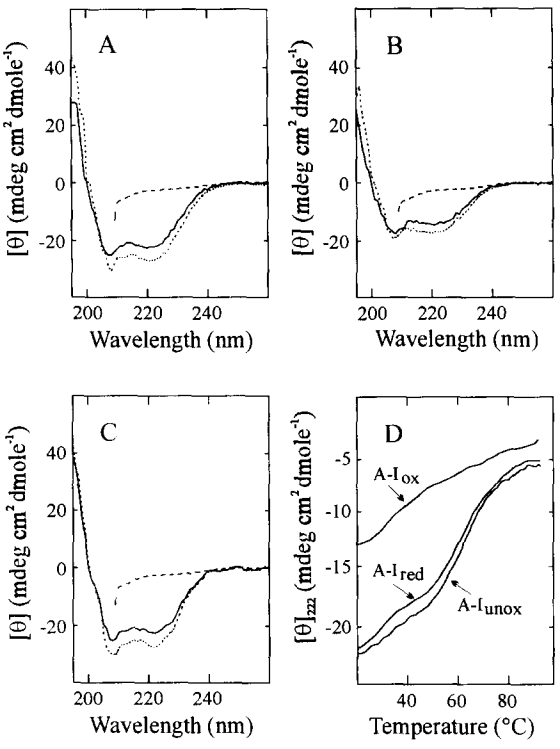

Fig. 3. Far-UV circular dichroism spectra and temperature-induced unfolding of unoxidized, oxidized, and reduced apo A-I. Circular dichroic spectra were measured for unoxidized (A), oxidized (B) and reduced (C) apo A-I (7.2 μM) with (dotted line) and without (solid line) 2.3 mM DHPC or in the presence of 4 M urea (dashed line) in 10 mM ammonium bicarbonate, 0.005% sodium azide, pH 7.8; in a 1 mm path-length cell at 25°C, with 1 nm bandwidth and 1.0 s averaging per point. Temperature-induced unfolding data (D) were collected in a temperature range of 25–95°C at 222 nm on a solution of 7.2 μM (0.05–0.2 mg/ml) apo A-I in 10 mM ammonium bicarbonate, 0.005% sodium azide, pH 7.8; in a 1 mm path-length cell; with 1 nm bandwidth, 1°C temperature increment, and 5.0 s averaging per point.

A-I contain the reduced methionine residues in contrast with apo A-I_{ox}. This together with the mass spectrometric and HPLC data confirm that the reduction of apo A-I_{ox} by PMSR results in the restoration of the primary apo A-I structure damaged by the oxidative modification induced by hydrogen peroxide.

To examine whether the reduction of apo A-I_{ox} using PMSR restores protein secondary structural features, CD spectra were measured for unoxidized, oxidized and reduced apo A-I (Fig. 3). The CD spectra of all three proteins were characterized by double minima at ~208 nm and ~222 nm and a maximum about 195 nm, features that are typical of proteins with a high helical content. The spectrum of apo A-I_{ox} (Fig. 3B) was considerably less intense than that of the unoxidized form (Fig. 3A), indicating a reduction of apo A-I helical content upon oxidation, consistent with previous reports (Table 1). In contrast, in the presence of 4 M urea the denatured proteins exhibited similar CD spectra indicative of a lack of any defined secondary structure. Reduction of the methionine sulfoxide residues in apo AI_{ox} with PMSR completely restored the CD spectrum (Fig. 3C) and calculated fractional helicity values (Table 1) characteristic of the unoxidized protein.

To examine whether the reduction of apo A-I_{ox} by PMSR also restores protein tertiary structural features, the temperature-induced unfolding of unoxidized, oxidized and reduced apo A-I was observed by CD spectroscopy (Fig. 3D). Apo A-I_{unox} was characterized by a weakly cooperative unfolding with midpoint temperature 65 ± 2°C indicative of a globular, folded structure [17], while apo A-I_{ox} did not exhibit any cooperative unfolding transition, suggestive of a largely unfolded structure. Reduction of A-I_{ox} with PMSR completely restored the characteristic thermal denaturation of the native unoxidized protein (Fig. 3E).

We carried out also a comparative study of the protease susceptibility of unoxidized and oxidized apo A-I using chymotrypsin. Oxidation of the two methionine residues of apo A-I_{ox} results in a dramatic increase in the protease susceptibility (Fig. 2C, lane 2) in comparison with the unoxidized form (Fig. 2C, lane 1). The oxidized protein is rapidly and completely digested to small fragments, consistent with the largely unfolded structure suggested by the thermal denaturation data. A similar increase of apo A-I protease susceptibility upon oxidation of the methionine residues 112 and 148 to sulfoxides has been recently reported [8]. Again, the behavior of the native unoxidized protein was restored upon reduction of the methionine sulfoxide with PMSR, and apo A-I_{red} is essentially completely resistant to chymotrypsin (Fig. 2C, lane 3). This together with the CD results confirm that the reduction of A-I_{ox} by PMSR results in restoration of the secondary and tertiary apo A-I structures damaged by the oxidative modification induced by hydrogen peroxide.

Apo A-I associates with lipids to form HDL particles. A conformational change in apo A-I has been correlated with lipid association [18]. To examine this conformational change and its dependence on the apo A-I oxidation state, we used the short-chain lipid DHPC as a convenient model lipid to study lipid-bound apo A-I structural features. CD spectra of apo A-I_{unox} taken in the presence of 2.3 mM DHPC indicate a substantial increase in helical content relative to the lipid-free form (Fig. 3A, Table 1), consistent with previous studies using another lipid system [5,17]. The very high helical content of lipid-associated apo A-I calculated by the method of Chen et al. [16] is supported by the recently determined crystal structure of a large fragment of apo A-I_{unox} (residues 44–243) believed to be in the lipid-associated state, which is essentially completely helical [19]. In contrast to previous study using a different lipid [5], we observed a small but significant increase in the helical content of apo A-I_{ox} upon lipid association (Fig.

Table 1
Apparent fractional helical content of unoxidized, oxidized and reduced apo A-I, in the absence or presence of lipids

Protein	This study		Previous studies			
	Buffer alone	+DHPC	Buffer alone	+POPC	+DMPC	Reference
Apo A-I_{unox}	66	81	68	82	–	[17]
Apo A-I_{ox}	37	48	30	–	29	[5]
Apo A-I_{red}	68	82	–	–	–	–

3B, Table 1). After reduction of apo A-I_{ox} with PMSR, the CD spectra (Fig. 3C) and calculated helical content (Table 1) of apo A-I_{red} in the presence of DHPC were indistinguishable from those of apo A-I_{unox}, indicating that methionine sulfoxide reduction restores the native lipid-dependent conformational change. The addition of DHPC (up to final concentration of 2.3 mM) to the reaction mixture containing apo A-I_{ox} and PMSR did not affect the reaction rate of the yield of the apo A-I_{red} (data not shown). This fact strongly suggests that apo A-I_{ox} in HDL can be also reduced by PMSR and by this way restored its ability to promote cholesterol efflux from cells, a crucial step in the process of reverse cholesterol transport.

Acknowledgements: We wish to thank Prof. Nathan Brot (Hospital for Special Surgery affiliated with the New York Hospital and the Cornell University Medical College, New York, NY) for sending a PMSR sample, Ms. Mia Frayzer for her excellent technical assistance, Dikran Aivazian for his help in obtaining the CD data, Aaron Sato for isoelectric focusing and Jennifer Ogrodnick for mass spectral analyses. The research described in this publication was made possible in part by Award No. RC1-196 of the U.S. Civilian Research and Development Foundation for the Independent States of the Former Soviet Union (CRDF).

References

[1] Gordon, D.J. and Rifkind, B.M. (1989) New Engl. J. Med. 321, 1311–1316.
[2] Schmitz, G. and Williamson, E. (1991) Curr. Opin. Lipidol. 2, 177–189.
[3] Barter, P.J. and Rye, K.-A. (1996) Curr. Opin. Lipidol. 7, 82–87.
[4] von Eckardstein, A., Walter, M., Holz, H., Benninghoven, A. and Assmann, G. (1991) J. Lipid Res. 32, 1465–1476.
[5] Anantharamaiah, G.M., Hughes, T.A., Iqbal, M., Gawish, A. and Neame, P.J. (1988) J. Lipid Res. 29, 309–318.
[6] Garner, B., Witting, P.K., Waldeck, A.R., Christison, J.K., Raftery, M. and Stocker, R. (1998) J. Biol. Chem. 273, 6080–6087.
[7] Garner, B., Waldeck, A.R., Witting, P.K., Rye, K.-A. and Stocker, R. (1998) J. Biol. Chem. 273, 6088–6095.
[8] Roberts, L.M., Ray, M.J., Shin, T.-W., Hayden, E., Reader, M.M. and Brouillette, C.G. (1997) Biochemistry 36, 7615–7624.
[9] Sigalov, A.B., Petrichenko, I.E. and Kolpakova, G.V. (1997) Eur. J. Clin. Chem. Clin. Biochem. 35, 395–396.
[10] Uboldi, P., Spoladore, M., Fantappiè, S., Marcovina, S. and Catapano, A.L. (1996) J. Lipid Res. 37, 2557–2568.
[11] Brot, N., Weissbach, L., Werth, J. and Weissbach, H. (1981) Biochemistry 78, 2155–2158.
[12] Moskovitz, J., Jenkins, N.A., Gilbert, D.J., Copeland, N.G., Jursky, F., Weissbach, L. and Brot, N. (1996) Proc. Natl. Acad. Sci. USA 93, 3205–3208.
[13] Abrams, W.R., Weinbaum, G., Weissbach, L., Weissbach, H. and Brot, N. (1981) Proc. Natl. Acad. Sci. USA 78, 7483–7486.
[14] Sigalov, A., Alexandrovich, O. and Strizevskaya, E. (1991) J. Chromatogr. 537, 464–468.
[15] Laemmli, U.K. (1970) Nature 277, 680–685.
[16] Chen, Y.H., Yang, J.T. and Martinez, H.M. (1972) Biochemistry 11, 4120–4131.
[17] Rogers, D.P., Brouillette, C.G., Engler, J.A., Tendian, S.W., Roberts, L.M., Mishra, V.K., Anantharamaiah, G.M., Lund-Katz, S., Philips, M.C. and Ray, M.J. (1997) Biochemistry 36, 288–300.
[18] Atkinson, D. and Small, D.M. (1986) Annu. Rev. Biophys. Biophys. Chem. 15, 403–456.
[19] Borhani, D.W., Rogers, D.P., Engler, J.A. and Brouillette, C.G. (1997) Proc. Natl. Acad. Sci. USA 94, 12291–12296.