Changes of Compressibility of Low Density Lipoproteins Following Copper Mediated Oxidation

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Abstract. The methods of measuring the ultrasound velocity and density were used for study the adiabatic compressibility of low density lipoproteins (LDL) during their oxidation. We showed, that copper-mediated oxidation of LDL resulted in a decrease of apparent specific compressibility, φ_k/β_0, of lipoproteins. The changes of ultrasound velocity and φ_k/β_0 value started much earlier than the beginning of propagation phase corresponding to the fast increase in concentration of conjugated dienes, measured by absorption at 230 nm. It was assumed that the changes of compressibility could be in particularly due to increase in ordering of the phospholipids during reductive activation of Cu^{2+}.

Key words: Low density lipoproteins — Lipid oxidation — Adiabatic compressibility — Conjugated dienes

Introduction

Lipoproteins (LP) are supramolecular lipid-protein complexes that are responsible for lipid transport in blood plasma. Impaired lipoprotein metabolism in plasma and in cells, which depends on biophysical and biochemical properties of the particles, is associated with an increased risk of atherosclerosis. Among wide variety of lipoproteins a low density lipoproteins (LDL) are of special interest. The LDL are the major sterol transporters in the circulation between blood plasma and the cell membranes. They are composed of an apolar core of cholesteryl esters and triglycerides, surrounded by a monolayer of phospholipids, unesterified cholesterol and one protein component, apolipoprotein B-100 (apoB) (see Kostner and Laggner 1989).

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LDL shows a reversible thermotropic structural transition around 30 ℃, which is due to a cooperative order-disorder transformation of cholesteryl esters in the apolar core. This particle is supposed to be atherogenic (Kostner and Laggner 1989). The function of LP can be, however, disturbed by various factors. Among others, an oxidative stress is considered as one of the possible reasons for enhancement of atherosclerosis (Steinberg et al. 1989). So far the effort has focused mostly on finding the effective methods of detection of degree of *in vitro* LDL oxidation. Among the known methods the measurement of diene production by absorption at 230 nm (Puhl et al. 1994) and by fluorescence decay of 1,6-diphenyl-1,3,5-hexatriene (DPH)-modified phospholipids incorporated into the lipoproteins (Hofer et al. 1995) have been shown as most sensitive. LDL oxidation has been usually mediated by Cu^{2+} or by 2,2′-azobis (2-amidinopropane) hydrochloride (AAPH) as prooxidants. Less attention has been paid to study of physical properties of LDL during oxidation. At the same time the studies on biomembranes showed a considerable effect of oxidation on membrane ordering. Upon peroxidation, membrane proteins might be cross linked, and their rotational and lateral mobility is decreased (Richter 1987). It is assumed that the structural state of LP is directly related to the oxidative susceptibility of LDL. The focus in recent studies has been therefore made to study how the degree of human LDL oxidation depends on their thermodynamic properties (Ramos et al. 1995; Schuster et al. 1995). The results obtained by differential scanning calorimetry and absorption spectroscopy proved the existence of correlation between the phase state of LDL and degree of copper-mediated oxidation. However, it is not known yet, how the oxidation processes influence the physical properties of lipoproteins and what is the relation between the degree of oxidation, biochemical composition, structural state and physical properties of LDL. So far, the information about structural and physical properties of LP has been obtained mainly using different spectroscopic techniques (Kostner and Laggner 1989; Georges et al. 1995), gradient ultracentrifugation (Chapman et al. 1988), differential scanning calorimetry (DSC), density, X-ray diffraction methods (Kostner and Laggner 1989; Laggner 1995; Ramos et al. 1995). The interaction of LP with lipid bilayers has been studied by electrostriction method (Hianik and Passechnik 1995).

In our recent paper (Hianik et al. 1997) we have shown a high sensitivity of the method of measuring the velocity and absorption of ultrasound to study the phase behavior of LDL and high density lipoproteins (HDL). We have shown that the temperature phase transitions of LDL resulted in considerable changes of the adiabatic compressibility of lipoproteins. We can therefore expect that the method of molecular acoustics will be very informative also for the study the influence of oxidation processes on physical properties of LDL. In this paper we therefore studied the relation between changes of adiabatic compressibility of LDL determined by densitometry and velocity of ultrasound and the production of conjugated dienes measured by absorption spectroscopy at wavelength of 230 nm.

Materials and Methods

Lipoprotein isolation

Low density lipoprotein was isolated from a single plasma sample from fasting normolipemic volunteers as described earlier (Steyrer and Kostner 1990). Immediately after blood drawing and centrifugation, the plasma was stabilised with EDTA and sodium azide (1 mg/ml) and subjected to density gradient ultracentrifugation in an SW-40 rotor (Beckmann) for 24 h at 40,000 rpm. LDL was harvested from a fraction corresponding to densities 1.025–1.055 g/ml of the density gradient and recentrifugated under identical conditions. All buffers and solutions used for lipoprotein preparation contained EDTA and sodium azide (1 mg/ml) and were deoxygenated in vacuo after saturation with nitrogen. All purification steps were performed at 4 ℃ and preparations were used within 1 week. The purity of the LDL fraction was assayed by double-decker rocket immunoelectrophoresis and sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis as described (Laurell 1966; Gaubatz et al. 1983; Armstrong et al. 1985). Protein was measured according to Lowry et al. (1951) in the presence of 0.5 % (w/w) sodium dodecyl sulphate. The protein concentration in suspension of LDL used for ultrasonic and density measurements was 1.2 mg/ml. Buffers were prepared using double distilled water. The LDL solution was dialysed over night against 10 mmol/l phosphate buffer (pH 7.4). Low salt concentrations are important for ultrasonic velocimetry, because velocity of ultrasound is sensitive to the concentration of salts (Sarvazyan 1991).

Oxidation measurements

The *in vitro* oxidation of LDL was determined by spectrophotometrical detection of conjugated dienes at 230 nm as described in the paper by Armstrong et al. (1985). This method has been shown to be effective for determination of the degree of oxidation of lipids and lipoproteins. The measurements were performed using a Specord 40 differential spectrometer (Germany). The sample temperature in the cuvettes was maintained by an external thermostatic bath at 37 ℃. Measuring cuvette was filled with LDL suspension (50 μg protein/ml), while the reference cuvette was filled by 10 mmol/l phosphate buffer (pH 7.4). The oxidation of lipoproteins was initiated by addition of $CuSO_4$ into both the lipoprotein suspension and the reference buffer at final concentration of 10 μmol/l.

Sound velocity and density measurements

The measurement of ultrasound velocity, u, allows us to evaluate the elastic properties of aqueous media, such as liposome or lipoprotein suspensions. This evaluation is based on a simple relationship:

$$\beta = 1/(\rho u^2) \tag{1}$$

where β is coefficient of adiabatic compressibility and ρ is the density. In the study of mechanical properties of solutions, measuring a relative change in a physical

characteristics *per* unit of solute concentration rather than its absolute value is often more important, precise and easier (Sarvazyan 1991). Such a relative concentration increments of compressibility, density and sound velocity can be defined as: $[\beta] \equiv (\beta - \beta_0)/\beta_0 C$; $[\rho] \equiv (\rho - \rho_0)/\rho_0 C$; and $[u] \equiv (u - u_0)/u_0 C$, where C is the molar concentration of the solute and values related to the solvent are denoted by a subscript 0. The relationship between these relative increments at low solute concentration can be obtained by differentiating the equation (1):

$$[\beta] = -2[u] - [\rho] \tag{2}$$

If taking into account the definition of the values of apparent molar volume $\Phi_\mathrm{v} \equiv (V - V_0)/CV = M/\rho_0 - [\rho]$ and the apparent molar compressibility $\Phi_\mathrm{k} \equiv (\beta V - \beta_0 V_0)/CV$, where M is the molar mass of the solute, then from above definitions and equation (2), the following relationship can be obtained:

$$\Phi_\mathrm{k}/\beta_0 = -2(u - u_0)/u_0 C - M/\rho_0 + 2\Phi_\mathrm{v} \tag{3}$$

and for specific values:

$$\varphi_\mathrm{k}/\beta_0 = -2[u] - 1/\rho_0 + 2\varphi_\mathrm{v} \tag{4}$$

where $\varphi_\mathrm{k}/\beta_0$, φ_v are the specific apparent compressibility and specific volume of solution respectively. The value of $[u]$ can be determined by ultrasonic velocity measurements and the value of φ_v (Kratky et al. 1973)

$$\varphi_\mathrm{v} = [1 - (\rho - \rho_0)/c]/\rho_0 \tag{5}$$

by means of density measurements (c is concentration in g/l).

Ultrasonic velocity was measured using a differential fixed-path velocimeter consisting of two acoustic resonators as described elsewhere (Sarvazyan 1982; Hianik et al. 1997; Halstenberg et al. 1998).

For precise density measurements the vibrating tube principle was used to determine the apparent partial specific volumes, φ_v, (see equation (5)) of lipoproteins (Kratky et al. 1973), employing the densitometer system DMA 60 with DMA 602M (Anton Paar KG, Graz, Austria). In order to obtain a higher resolution, the measurements were performed with two sample cells DMA 602M. One contained the lipoprotein suspension and the other one – the reference buffer. The cells for both ultrasonic and density measurements were thermostated with a Lauda RK 8 CS ultrathermostat with accuracy of 0.02 ℃.

Both the measurements of sound velocity and density were started after addition of Cu^{2+} (final concentration 10 μmol/l) into the measuring and reference cells upon continuous stirring. The measurements were performed every 10 min.

Experimental errors

The accuracy of determination of the sound velocity increment, $[u]$, and the specific partial volume, φ_v, was better than 10^{-3} ml/g. The accuracy of the determination of the density was better than 10^{-3} g/ml. Each series of measurements was performed at least 3 times.

Results

In order to detect the oxidative susceptibility of LDL, we used standard procedure of measurement production of conjugated dienes (Puhl et al. 1994). The measurements of 230 nm absorption were started immediately after addition of prooxidant ($CuSO_4$). Absorption was measured at intervals of 1.5 min. The typical plot of absorbance as a function of time is presented on Fig. 1a. Three phases can be clearly seen on the figure: initial lag phase (A), propagation phase (B) and decomposition phase (C). At longer time, a second increase following phase C takes place.

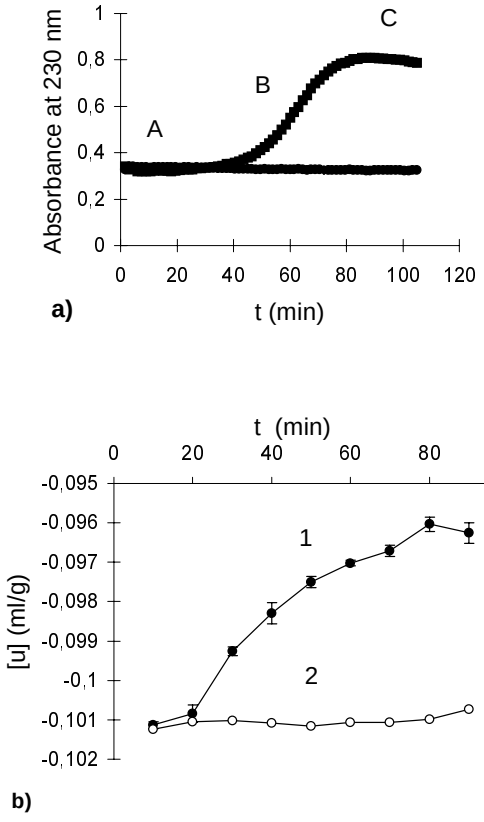

Figure 1. Time course of the changes in (a) absorbance measured at 230 nm and (b) concentration increment of ultrasound velocity $[u]$, for the suspension of LDL following their oxidation in the presence of 10 μmol/l Cu^{2+} (curve 1) and without Cu^{2+} (curve 2), $T = 37\,^{\circ}C$. A, lag phase; B, propagation phase; C, decomposition phase of oxidation process. The error bars in Fig. 1b correspond to S.D. calculated from 5 measurements of resonance frequency in identical samples. If not indicated the error bars did not exceed the size of symbols. Duration of each measurement cycle of the ultrasound velocity was not longer than 20 s. The S.D. for absorbance measurements did not exceed 5 %.

This effect is due to decomposition products that have absorption at 230 nm range (see Puhl et al. 1994 for more detail). We did not observe significant changes in absorbance of LDL suspension without Cu^{2+} (Fig. 1a, curve 2).

The length of lag phase is related to oxidative susceptibility of lipoproteins. LDL contain antioxidant (vitamin E), that preserve their oxidation. The time corresponding to lag phase can be easily determined at the intercept of the tangent with the time axis (Puhl et al. 1994). For our sample of LDL the value of lag phase was 48 min (±5% S.D.). Another important characteristic, that can be derived from Fig. 1a is the maximum rate of oxidation, which is determined by propagation phase. This value can be derived from the slope of the tangent ($\Delta A/\mathrm{min}$) of the curve (Fig. 1a) and knowing the molar absorbance $\varepsilon_{230\ \mathrm{nm}}$ for conjugated dienes of 29.000 $\mathrm{l{\cdot}mol^{-1}{\cdot}cm^{-1}}$. The rate in μmol/l of conjugated dienes formed *per* liter and minute is given by $(\Delta A/\mathrm{min})\ (\varepsilon_{230\ \mathrm{nm}})^{-1} = 0.524\ \mu\mathrm{mol/l{\cdot}min}$, which is in a good agreement with that reported by Puhl et al. (1994). The maximum amount of dienes can be calculated using the maximum increase in absorbance:

$$A(\varepsilon_{230\ \mathrm{nm}})^{-1} = 16.6\ \mu\mathrm{mol/l}$$

The dependence, similar to that presented on Fig. 1a has been published in a number of papers. The question arises, however, what changes of physical properties of the LDL take place during the oxidation process. Fig. 1b, curve 1, represents the changes of $[u]$ value following copper mediated oxidation of LDL as a function of time. The experiment was performed in similar conditions to that of absorption measurements. From the Fig. 1b, it can be seen that for the dependence of $[u]$ *vs.* time there are also three regions with the changes characteristic of value $[u]$: lag phase, propagation phase and saturation with subsequent decrease. However, in contrast to absorption curve, the changes of $[u]$ started already at the time, when no substantial changes of absorption occurred. For comparison, on Fig. 1b, curve 2, there is also the dependence of $[u]$ plotted *vs.* time for LDL without Cu^{2+}. We can see, that $[u]$ value practically does not changes and only slight increase is observed at longer times, which might be connected with oxidation of LDL by oxygen. The changes of $[u]$ without prooxidant are, however, considerably smaller than that observed at the presence of Cu^{2+}.

As can be seen from eq. (1), the velocity of ultrasound together with the density of solution are related to the coefficient of adiabatic compressibility of solution. In order to characterize the volume compressibility of LDL, the convenient parameter is apparent specific compressibility, $\varphi_\mathrm{k}/\beta_0$, (see eq. (4)). Therefore, also the density, ρ, of LDL following oxidation was measured and the specific volume, φ_v, of LDL particles was determined. In contrast to resonatory cells for measurement of sound velocity, the cells for density measurements does not allow stirring the LDL suspension during measurements. Due to the sedimentation of LDL, the long term measurements of its density could leads to errors. We therefore incubated LDL suspension with $CuSO_4$ and took the small volume of the LDL at certain interval of time (approx. 5 min) prior to the measurements. This procedure, however, does

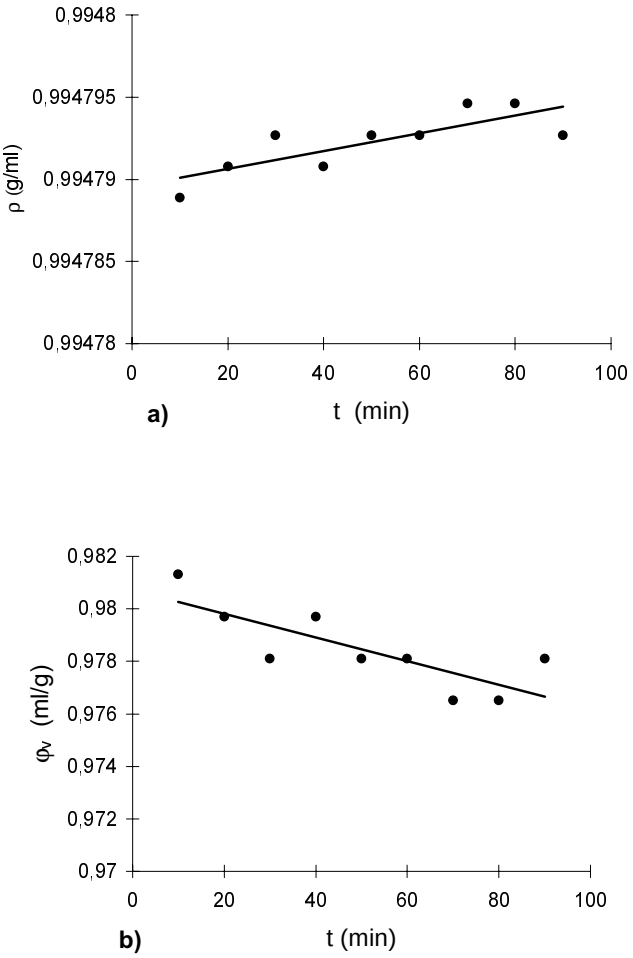

Figure 2. Time course of the density, ρ (a) and specific volume, φ_{v} (b) for the suspension of LDL following their oxidation in the presence of 10 μmol/l Cu^{2+}, $T = 37$℃.

not allow to obtain an accuracy of the density determination better than $\sim 10^{-3}$ g/ml. This is because of certain dispersion of experimental density points around average linear regression line (Fig. 2a). The same holds for calculated specific volume as a function of time (Fig. 2b). The general tendency of increase in density and decrease in specific volume with time (i.e. with increasing in degree of LDL oxidation) was, however, reproducibly observed in three independent experiments with LDL. Having both $[u]$ and φ_{v} and using eq. (4) it is easy to calculate the value of $\varphi_{\mathrm{k}}/\beta_0$. The value of $\varphi_{\mathrm{k}}/\beta_0$ for not oxidized LDL was 1.16 ± 0.06 ml/g. This is in good agreement with that published earlier for LDL isolated from normoleptic

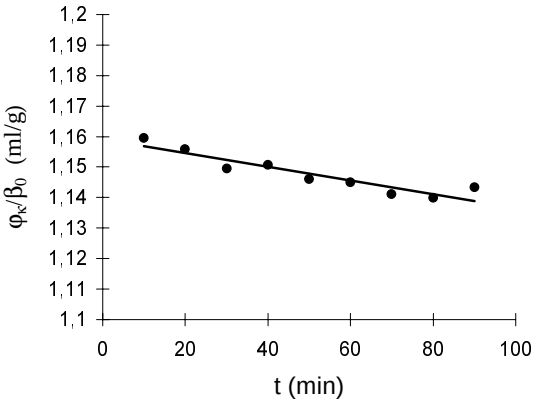

Figure 3. Time course of the specific apparent compressibility, φ_k/β_0, for the suspension of LDL following their oxidation in the presence of 10 μmol/l Cu^{2+}, T = 37 ℃.

donors and measured at the same temperature 37 ℃ (1.17 ± 0.09 ml/g) (see Hianik et al. 1997). The plot of φ_k/β_0 value *vs.* time is presented on Fig. 3. It is seen that with oxidation of LDL the apparent specific compressibility of LDL decreases. However, in contrast with kinetic measurement of concentration increment of sound velocity, $[u]$, (Fig. 1b, curve 1), the value of φ_k/β_0 did not reveal well expressed saturation at larger times. This is also due to the dispersion of specific volume, used for calculation of φ_k/β_0. Thus, during oxidation the LDL particles become more rigid.

Discussion

The main and novel results presented in this paper demonstrate that changes in LDL compressibility started at very earlier stage of LDL oxidation, i.e. when the concentration of conjugated dienes did not show any substantial changes. We should note that similar effect has been observed recently by fluorescence spectroscopy method (Hermetter A. et al., 1996). Using specific fluorescence probes they observed faster oxidation of the hydrophobic core of LDL which did not contain vitamin E as compared to surface lipid oxidation. Two effects can contribute to the changes of the ultrasound velocity: changes in hydration and/or structural changes of LDL. It is assumed that Cu^{2+} ions bind to discrete sites on apoB and form apoB-Cu^{2+} complexes. Reductive activation of Cu^{2+} to Cu^{+} ($Cu^{2+} \rightarrow Cu^{+}$) mediated by LDL can lead to formation of complex which can initiate lipid peroxidation (Esterbauer and Jürgens 1993). Cu^{2+}-ions probably penetrate into the LDL phospholipid monolayer at the interface between apoB and phospholipids. During this penetration the ions should lose the hydrated shell. However, if the changes of hydration represent an exclusive factor influencing $[u]$, then we can expect decrease of $[u]$ and not increase this value. It is known, that hydrated shell around cations is well ordered and its coefficient of adiabatic compressibility is lower than that of bulk solution (Buckin 1988). Therefore, the lost of hydrated shell should result in

overall increase in adiabatic compressibility, which is not the case. We can therefore assume, that observed increase in $[u]$ value and decrease in apparent specific compressibility is due to changes in structural state of LDL.

It is clear, that due to presence of antioxidants, at the initial stage of oxidation, there is very limited numbers of lipid peroxides in LDL. However, the peroxidation might effect both the compressibility of apoB and the lipids. We can therefore assume, that decrease of adiabatic compressibility of lipoproteins might be due to formation of highly ordered clusters of phospholipids around apoB during reductive activation of Cu^{2+}. As soon as the peroxidation propagates, the changes in phospholipid ordering could spread over the entire phospholipid surface in LDL. Due to peroxidation, both restriction of mobility of protein and lipids could take place. Consistently, a decrease in mobility of proteins and an increase in ordering of membrane phospholipids has been shown in the past by various methods (Richter 1987; Tirosh et al. 1997).

Thus, our results show that copper-mediated oxidation of LDL is accompanied by a continuous decrease in volume compressibility of LDL. Presently we can not decide what processes in LDL have dominant contribution to this effect – the changes in compressibility of apoB due to binding of Cu^{2+} or lipid peroxidation, or both of these factors. High sensitivity of molecular acoustic methods for study protein-lipid interactions (Tata and Dunn 1992) and even compressibility of proteins (Sarvazyan and Kharakoz 1977) allowed us to show that during oxidation of a model protein – bovine serum albumin by AAPH the adiabatic compressibility of this protein decreased (unpublished results). Therefore we can also expect oxidation induced changes in compressibility of apoB. The changes in compressibility of apoB could be also due to the possible changes of protein conformation induced by binding of Cu^{2+} to the certain groups (e.g. —SH, —COOH, —OH, etc.) of the amino acid residues. However, further studies focusing on the determination of physical properties of apoB and amphiphilic compounds of LDL would be necessary for a deeper understanding of the observed phenomena.

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References

Armstrong V. W., Walli A. K., Seidel D. (1985): Isolation, characterization and uptake in human fibroblasts of an apo(a)-free lipoprotein obtained on reduction of lipoprotein(a). J. Lipid Res. **26**, 1314—1323

Buckin V. A. (1988): Hydratation of nucleic bases dilute aqueous solutions. Apparentmolar adiabatic and isothermal compressibilities, apparent molar volumes and their temperature slopes. Biophys. Chem. **29**, 283—292

Chapman M. J., Laplaud P. M., Luc G., Forgez P., Bruckert E., Goulinet S., Lagrange D. (1988): Further resolution of the low density lipoprotein spectrum in normal

human plasma: physicochemical characteristics of discrete subspecies separated by density gradient ultracentrifugation. J. Lipid Res. **29**, 442—458

Esterbauer H., Jürgens G. (1993): Mechanistic and genetic aspects of susceptibility of LDL to oxidation. Curr. Opin. Lipidology **4**, 114—124

Gaubatz J. W., Heideman C., Gotto A. M. Jr., Morrisett J. D., Dahlen G. H (1983): Human plasma lipoprotein(a). Structural properties. J. Biol. Chem. **258**, 4582—4589

Georges R., Hofer G., Sommer A., Stütz H., Grillhofer H., Kostner G. M., Paltauf F., Hermetter A. (1995): Transfer of phospholipase A-resistant pyrene-dialky-glycerophosphocholine to plasma lipoproteins: differences between Lp(a) and LDL. J. Lipid Res. **36**, 251—259

Halstenberg S., Heimburg T., Hianik T., Kaatze U., Krivanek R. (1998): Cholesterol induced variations in the volume and enthalpy fluctuations of lipid bilayers. Biophys. J. **75**, 264—271

Hianik T., Passechnik V. I. (1995): Bilayer Lipid Membranes. In: Structure and Mechanical Properties. Kluwer Academic Publishers, Dordrecht, Boston, London

Hianik T., Rybar P., Kostner G. M., Hermetter A. (1997): Molecular acoustic as a new tool for the study of biophysical properties of lipoproteins. Biophys. Chem. **67**, 221—228

Hofer G., Lichtenberg D., Hermetter A. (1995): A new fluorescence method for the continuous determination of surface lipid oxidation in lipoproteins and plasma. Free Radic. Res. **23**, 317—327

Hofer G., Lichtenberg D., Kostner G. M., Hermetter, A. (1996): Oxidation of fluorescent glycero- and sphingophospholipids in human plasma lipoproteins: alkenylacyl subclasses are preferred targets Clin. Biochem. **29**, 445—450.

Kostner G. M., Laggner P. (1989): Chemical and physical properties of lipoproteins. In: Human Plasma Lipoproteins (Eds. J. C. Fruchart and J. Shephert), pp. 23—54, Walter de Gruyer, Berlin, New York

Kratky O., Leopold H., Stabinger H. (1973): The determination of the partial specifi volume of proteins by the mechanical oscillator technique. Methods Enzymol. **27**, 98—110

Laggner P. (1995): X-ray and neutron small-angle scattering on plasma lipoproteins. In: Modern Aspects of Small-Angle Scattering (Ed. H. Brumberger), pp. 371—386, Kluwer Academic Publishers, Dordrecht, Boston, London

Laurell C. B. (1966): Quantitative estimation of proteins by electrophoresis in agarose gel containing antibodies. Anal. Biochem. **15**, 45—52

Lowry O. H., Rosebrough N., Farr A. L., Randall R. J. (1951): Protein measurement with the folin phenol reagent. J. Biol. Chem. **93**, 265—275

Puhl H., Waeg G., Esterbauer H. (1994): Methods to determine oxidation of low-density lipoproteins. Methods Enzymol. **233**, 425—441

Ramos P., Gieseg S. P., Schuster B., Esterbauer H. (1995): Effect of temperature and phase transition on oxidation resistance of low density lipoprotein. J. Lipid Res. **36**, 2113—2128

Richter C. (1987): Biophysical consequences of lipid peroxidation in membranes. Chem. Phys. Lipids **44**, 175—189

Sarvazyan A. P. (1982): Development of methods of precise ultrasonic measurements in small volumes of liquids. Ultrasonics **20**, 151—154

Sarvazyan A. P. (1991): Ultrasonic velocimetry of biological compounds. Annu. Rev. Biophys. Biophys. Chem. **20**, 321—342

Sarvazyan A. P., Kharakoz D. P. (1977): Acoustics study of conformational state of pro-

teins in water. In: Molecular and Cell Biophysics (Ed. G. M. Frank), pp. 93—106, Nauka, Moscow (in Russian)

Schuster B., Prassl R., Nigon F., Chapman M. J., Laggner P. (1995): Core lipid structure is a major determinant of the oxidative resistance of low density lipoprotein. Proc. Natl. Acad. Sci. U.S.A. **92**, 2509—2513

Steinberg D., Parthasarathy S., Carew T. E., Khoo J. C., Witztum J. L. (1989): Beyond cholesterol. Modifications of low-density lipoprotein that increase its atherogenicity. N. Engl. J. Med. **320**, 915—924

Steyrer E., Kostner G. M. (1990): The interaction of lipoprotein Lp(a) with the B/E-receptor. J. Lipid Res. **31**, 1247—1253

Tata D. B., Dunn F. (1992): Interaction of ultrasound and model membrane systems: Analyses and predictions. J. Phys. Chem. **96**, 3548—3555

Tirosh O., Kohen R., Katzhendler J., Alon A., Barenholz Y. (1997): Oxidative stress effect on the integrity of lipid bilayers is modulated by cholesterol level of bilayers. Chem. Phys. Lipids **87**, 17—22

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