Effect of Guanidine Hydrochloride on the Conformation of Egg Yolk Low Density Lipoprotein

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The denaturation of egg yolk low-density lipoprotein (LDL) induced by guanidine hydrochloride (GdnHCl) has been investigated by using circular dichroism (CD) and fluorescence measurements. The transition curves were obtained from changes of the residue ellipticity at 222 nm and the fluorescence intensity of the aromatic residues, tyrosine and fluorescence. Different transition curves were obtained at the molarities of GdnHCl between 2 and 6 M, and between 1 and 4 M from the CD and the fluorescence measurements, respectively. The noncoincidence of the transition curves as well as the asymmetry of the transition curve obtained from the fluorescence measurements suggests that the denaturation process of LDL consists of several transition states. A very low apparent free energy of denaturation ($\Delta G_{app}^{H_2O} = 3.0$ kcal mol$^{-1}$) obtained by CD data suggests that LDL has an unstable folded structure in its native state.

Keywords egg yolk low-density; lipoprotein; denaturation; guanidine hydrochloride; circular dichroism; fluorescence

Plasma low-density lipoprotein (LDL, $d = 1.02 - 1.06$ g/ml) contains an apolipoprotein (apo B, molecular weight: ca. 550 kDa) whose state is important in the secretion, intravascular processing and catabolism of LDL$^{1-3}$. The application of LDL as a site specific drug carrier in the drug delivery system has been studied,$^3$ and it is important to clarify the structural characteristics of LDL to understand its function in vivo and to make use of it as a drug carrier. Optical studies employing the circular dichroism (CD) method$^4-10$ have revealed the features of the secondary structure of human LDL in its natural lipid environment or its delipidated state, although the thermostability of LDL is not yet understood because of its heat coagulation.$^9$ In these CD and fluorescence studies we sought to evaluate the structural stability of LDL against a denaturant which is powerful to most proteins, guanidine hydrochloride (GdnHCl).

MATERIALS AND METHODS

Egg yolk LDL (QPFL-10, Lot No. YK 2001) was purchased from Funakoshi Co. Ltd., Tokyo, and analytical grade GdnHCl and other reagents were obtained from Wako Pure Chemical Industries, Ltd., Osaka. The stock solution of LDL (in 2.7% NaCl) was diluted one hundred times with 0.1 M sodium phosphate buffer, pH 7.5 before use. Concentration of protein was determined by the Lowry method$^{11}$ using bovine serum albumin as a standard according to Chen and Kane.$^9,10$ CD measurements in the spectral region between 250–210 nm were carried out at 20°C in a Jasco J-720 spectropolarimeter using a quartz cell with 1 mm path length and a protein concentration of 0.112 mg·ml$^{-1}$. The data were expressed in terms of mean residue ellipticity [$\theta$] in units of deg·cm$^2$·dmol$^{-1}$ using a mean residue weight (MRW) of 112 according to the relation,$^9$

$$[\theta] = \frac{\theta^c \times MRW}{10cL}$$

where $\theta^c$ is the observed ellipticity, $L$ is the cell path length in cm and $c$ is protein concentration in g·ml$^{-1}$. The percentage of helical structure of LDL was approximated from the treatment of Greenfield and Fasman$^{12}$ or a similar modified procedure.$^{13}$

Fluorescence measurements were performed with a Hitachi 850 spectrofluorometer. The fluorescence excitation wavelength was 280 nm. The temperature for CD and fluorescence measurements was controlled within 0.1°C by the use of a hollow cell holder through which water from a constant temperature bath was circulated, and this was measured directly using a Takara thermistor D641.

Free energy of denaturation was calculated assuming a two state mechanism; the equilibrium constant, $K$, was estimated from both the change in ellipticity at 222 nm and the change in the fluorescence intensity at the maximum fluorescence according to the relationship,$^{14}$

$$K = \exp(-\Delta G_{app}^{RT}) = \left[\frac{[\theta]}{[\theta]^N}\right] \left[\frac{[\theta]}{[\theta]^D}\right]$$

where $[\theta]^N$ and $[\theta]^D$ are the residue ellipticity and fluorescence intensity at each GdnHCl concentration, respectively. $[\theta]^N$, $[\theta]^D$ are the ellipticity and fluorescence intensity at the native and the denatured state, respectively, corresponding to the values at low and high GdnHCl concentrations. According to Green and Pace$^{14}$ and Tanford,$^{15}$ the apparent free energy of denaturation, $\Delta G_{app}$, varies linearly with GdnHCl concentration and is given by the following equation,

$$\Delta G_{app} = \Delta G_{app}^{H_2O} - m[GdnHCl]$$

where $\Delta G_{app}^{H_2O}$ is the apparent free energy in the absence of denaturant obtained from the direct linear extrapolation of $\Delta G_{app}$ to [GdnHCl] = 0, and $m$ is the gradient which is the function of the difference in the number of denaturant molecules bound to the denatured and native states of the molecule. A least-squares analysis was used to fit data.

RESULTS AND DISCUSSION

Figure 1 shows the CD spectra of LDL at various
GdnHCl concentrations in the wavelength region of 210—240 nm. The native LDL has a characteristic negative CD band near 220 nm, and the negative trough was raised with increasing GdnHCl concentration, reflecting a decrease of the ordered structure. The residue ellipticity near 220 nm remarkably resembled human LDL, and, therefore, the estimated helical contents of 22—35% were also similar to the values for human LDL of 24—33% in natural lipid environment.\textsuperscript{4,7,9} In human LDL, the ellipticity near 220 nm decreased about 20% in the delipidated LDL (apo B).\textsuperscript{6,7} indicating the partial degradation of the secondary structure of LDL by delipidation and therefore a stabilizing role of lipids in the conformation of LDL protein. Figure 2 shows the changes in the molar ellipticity of LDL at 222 nm with GdnHCl concentration at 20°C. A large conformational transition occurs at the molarities of GdnHCl between 2 and 6 M. Figure 3 shows the change of the fluorescence of the aromatic residues, tyrosine and tryptophan, of LDL induced by GdnHCl. The tentative transition curve obtained from fluorescence measurements is shown in Fig. 4. The overall transition occurred at the molarities of GdnHCl between 1 and 4 M. The transition curve is, however, asymmetrical and seems to consist of several stages. This complexity of the transition curve obtained from the fluorescence measurements may be due to the sensitive fluorescence changes which probably reflect the small local structural changes including the disruption of the interaction between apo B and lipids. Aromatic residues, tyrosine and tryptophan, may be exposed to the solvent at an early stage of the denaturation process, and unfolding of the helical regions of the polypeptide chain may occur at a late stage. Noncoincidence of the transition curves obtained from CD and fluorescence measurements indicates the existence of several stages of the structural transition in the process of denaturation. Such noncoincidence of the transition curves has been observed for many proteins which have intermediate states in the denaturation process.\textsuperscript{16—19}

To estimate the apparent free energy of denaturation in H\textsubscript{2}O, $\Delta G_{\text{app}}^{\text{H}_{2}O}$, at 20°C in the absence of denaturant, the apparent free energy change of denaturation, $\Delta G_{\text{app}}$, was obtained from Eq. 1 and plotted against GdnHCl according to Eq. 2, as shown in Fig. 5. The values of $\Delta G_{\text{app}}^{\text{H}_{2}O}$ were estimated by linear extrapolation to zero GdnHCl concentration in the figure. The value of $\Delta G_{\text{app}}^{\text{H}_{2}O}$ was $3.0 \pm 0.1$ kcal mol\textsuperscript{-1}, which may correspond to the stabilization energy of the entire molecule. On the other hand, for the transition studied by fluorescence, the plot of $\Delta G_{\text{app}}$ against
GdnHCl did not give a simple linear line as expected from the complexity of the transition curve, and $\Delta G_{\text{app}}^{H_2O}$ could not be correctly estimated. The value of $\Delta G_{\text{app}}^{H_2O}$ of 3 kcal·mol$^{-1}$ is considerably lower than the reported values of $\Delta G_{\text{app}}^{H_2O}$ for most globular proteins, which range from 9 to 25 kcal·mol$^{-1}$. It is noted, however, that low stabilizing free energy similar to our data has been observed in some apolipoproteins. Mantulin et al. reported the values of $\Delta G_{\text{app}}^{H_2O}$ as 2.8 kcal·mol$^{-1}$ obtained from GdnHCl denaturation followed by CD study of apolipoprotein C-II (apo C-II) of human very low density lipoprotein (VLDL). Edelstein and Scanu reported that $\Delta G_{\text{app}}^{H_2O}$ of 3.7 kcal·mol$^{-1}$ could be obtained from GdnHCl denaturation following CD study of apolipoprotein A-I (apo A-I) of human high density lipoprotein (HDL). Each of these low values of stabilization free energy suggests that all the apolipoproteins have an unstable folded structure in solution. Mantulin et al. stated that the low $\Delta G_{\text{app}}^{H_2O}$ of apo C-II is compatible with the view that there are limited regions of hydrophobic interactions stabilizing tertiary structure, but the precise reason for the low free energy is still unclear. However, the noticeable similarity of the apparent low free energy change for both the intact LDL and the delipidated LDL may lead to the conclusion that the lipids do not have much intrinsical contribution to stabilize the apolipoprotein secondary structure itself. The effect of the lipids on the process of denaturation of LDL ought to be investigated in more detail in further study.

References