Interaction of Sodium Dodecyl Sulfate with Pig Serum Low-Density Lipoprotein

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(Received December 20, 1988)

Synopsis. The interaction of sodium dodecyl sulfate (SDS) with the pig serum low-density lipoprotein (LDL) showed a subtle dependence on the SDS concentration. At the SDS concentrations below 0.01%, the binding isotherm conformed to the Langmuir's type, from which the maximum number of the binding site and the intrinsic binding constant were respectively estimated to be 1600 mol mol^{-1} and 6.5×10^3 (mol dm^{-3})$^{-1}$. At the SDS concentrations at or above 0.03%, the LDL structure irreversibly dissociated.

Sodium dodecyl sulfate (SDS), a well-known protein denaturant, has been widely used for various analytical purposes in protein chemistry. The protein denaturation by SDS is believed to involve the hydrophobic binding of SDS and consequent structural unfolding of protein molecules.[1,2] Interaction of SDS with lipoproteins, the micellar complexes of lipid and protein, is expected to be more complex, as both stabilizing and destabilizing effect can be resulted depending on the SDS concentration. The serum lipoproteins in general are quite unstable, readily undergoing irreversible aggregation, particularly after separation, and that often hampers the routine biochemical analyses of the lipoproteins. In this context, elucidation of the SDS-lipoprotein interaction is not only of interest but also profitable in view of possible stabilizing effect of SDS. This communication describes the effects of SDS on the structure of the pig serum low density lipoprotein (LDL) which is known to be closely similar to the human serum LDL in the physicochemical properties.[3]

The pig serum LDL was prepared by ultracentrifugal flotation and, where necessary, separated into two fractions (LDL_1 and LDL_2) by differential flotation.[3] The LDL fractions were exhaustively dialyzed against 0.1 mol dm^{-3} NaCl containing 0.02% EDTA in which most of the measurements were conducted. (For analysis of lithium dodecyl sulfate (LDS)-LDL interaction, 0.1 mol dm^{-3} LiCl was used as the background solution.) The binding data were obtained by the frontal gel chromatography[4] as well as by the equilibrium dialysis at an LDL concentration of 0.1% with the SDS concentration varying from 0.001 to 0.033%. The velocity sedimentation was carried out in a Hitachi model SCP 70H ultracentrifuge equipped with a UV scanner, and gel filtration was performed on a Superose 6 column (1×30 cm, Pharmacia) using an FPLC system (Pharmacia). The SDS-LDL mixtures for the sedimentation and gel filtration experiments were prepared by dializing an appropriate amount of LDL solution (0.1%) against a large volume of SDS solution for 48 hours at 20 °C. Therefore, the SDS concentrations in the mixtures, denoted as [s] in the results, refer to the equilibrium concentrations of free SDS. The distribution curve of the sedimentation coefficient, $g(s)$, was produced from the differential concentration diagram (dc/dx vs. x) according to the equation,[5]

$$g(s)=\frac{1}{c_0}\frac{dc}{dx}\left(\frac{x}{x_m}\right)^2(x\omega^2t),$$

where c_0 is the initial concentration, x the distance from the axis of rotation, x_m the distance to the meniscus, and t and ω the effective centrifugation time and angular velocity, respectively.

Figure 1 shows the binding curve for SDS-LDL in 0.1 mol dm^{-3} NaCl in which the data obtained at two temperatures, 5 ° and 20 °C, were plotted according to the Langmuir's adsorption isotherm in the form,[6]

$$\frac{1}{\theta}=\frac{1}{nk}\cdot\frac{1}{[s]}+\frac{1}{n},$$

where θ is the number of moles of bound SDS per mole of LDL, n the number of the binding site on LDL molecule, K the association constant and [s] the equilibrium concentration of free SDS, respectively. Two prominent features are revealed in Fig. 1: first, at the low concentrations of SDS ([s]<ca. 0.01%) the SDS-LDL binding conforms to the Langmuir's adsorption isotherm giving a linear plot, but it breaks at around [s]=0.01% above which the plot extrapolates to $n=\infty$ and second, the SDS-LDL binding is little affected by temperature. The binding parameters of SDS-LDL at the low SDS concentrations were:

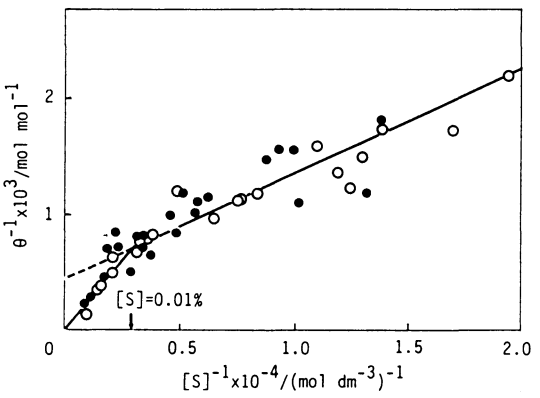

Fig. 1. Binding isotherms for SDS-LDL in 0.1 mol dm^{-3} NaCl at 20 °C (●) and 5 °C (○). θ is the number of moles of bound SDS per mole of LDL and [s] is the equilibrium concentration of free SDS. Concentration of LDL was fixed at 0.1%.

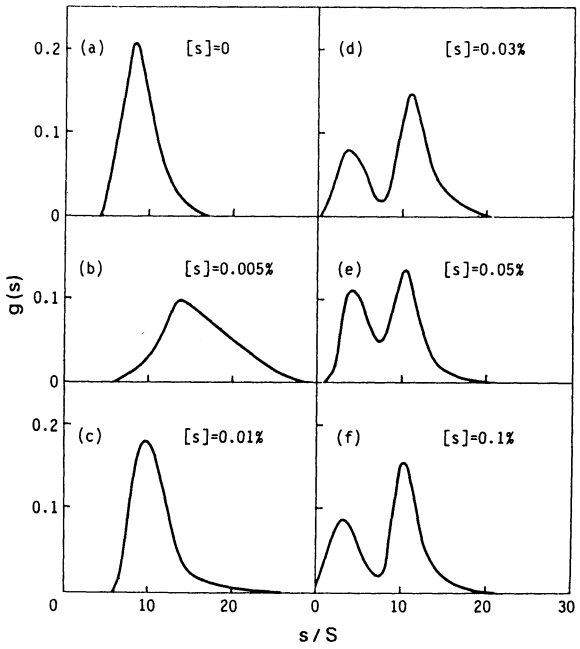

Fig. 2. Effect of SDS concentration on the distribution curve of sedimentation coefficient, $g(s)$, of LDL. [s] refers to the equilibrium concentration of free SDS in the LDL solutions. Concentration of LDL was 0.1%.

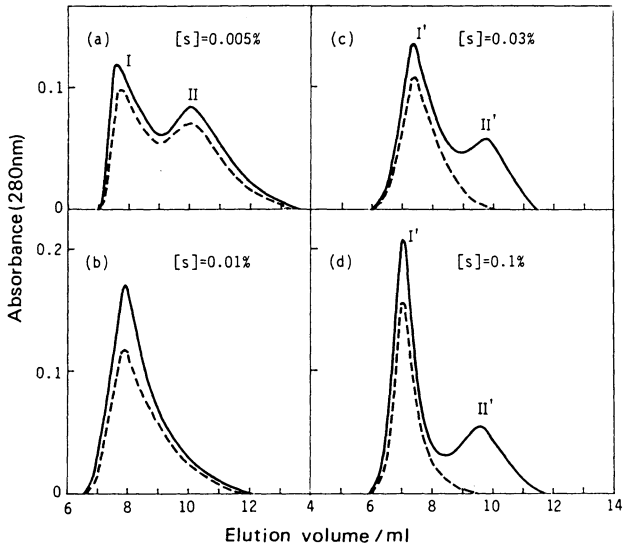

Fig. 3. Effect of SDS concentration on the gel filtration profile of LDL. [s] refers to the equilibrium concentration of free SDS. Gel filtration was performed on a column of Superose 6 (1×30 cm) preequilibrated with the eluent (0.1 mol dm^{-3} NaCl) containing SDS to the concentrations indicated by [s]. The loading concentration of LDL was 0.1%. ——, Absorbance at 280 nm; ----, Lowry's protein.

K=5×10^3 (mol dm^{-3})$^{-1}$ and n=2×10^3 mol mol^{-1}. The binding isotherm for lithium dodecyl sulfate (LDS)-LDL interaction was much similar to that of SDS-LDL interaction yielding n=2×10^3 mol mol^{-1} and K=5×10^3 (mol dm^{-3})$^{-1}$. Figure 2 shows the distribution profiles of the sedimentation coefficient, $g(s)$, of SDS-LDL (0.1%) mixtures with various SDS concentrations. At [s]=0.005% (Fig. 2b), the $g(s)$ curve was notably broad as compared to that at [s]=0 (Fig. 2a), but by increasing [s] to 0.01% it nearly restored the original profile with somewhat increased sedimentation coefficient (Fig. 2c). It is important to note that the broadening and sharpening of the $g(s)$ curves observed at the two concentrations could readily be reversed each other by adjusting [s] by dialysis. Further increases of [s] to above 0.03% caused an irreversible structural dissociation leading to bimodal $g(s)$ curves (Figs. 2d—f).

Figure 3 shows the result of gel filtration obtained for SDS-LDL (0.1%) mixtures with [s] varying from 0.005% to 0.1%. It is to be noted that, due to an LDL-gel interaction, the elution of LDL required the presence of at least 0.005% SDS in the eluent, and hence that the apparent elution volume cannot be directly related to the molecular size. The SDS effect manifested in Figs. 3a and 3b at the low concentrations ([s]<0.01%) was completely reversible, but that observed at the higher concentrations (Figs. 3c and 3d) could not be reversed. The two fractions of Fig. 3a (I and II) both contained protein (the Lowry's protein) and all the lipid components of LDL, i.e., phospholipids, neutral lipids, cholesterol and its ester, and sphingolipids. (The lipid components were identified by thin-layer chromatography after extraction in a chloroform(2)-methanol(1) mixture.) In contrast, as shown in Figs. 3c and 3d, protein was found only in fraction I′, although both fractions (I′ and II′) contained the lipid components.

The present study shows that the mode of the interaction between LDL and SDS depends on the concentration of the latter. At the low SDS concentrations ([s]=0.005—0.01%), the binding isotherm conformed to the Langmuir's type, and the relevant thermodynamic parameters were estimated to be: ΔF°=−5.0 kcal mol^{-1}, ΔH°=0, ΔS°=17 e.u., respectively. The entropic nature of the interaction suggests involvement of the hydrophobic effect. However, even within the narrow range of [s](0.005—0.01%), the state of LDL molecule varies with the SDS concentration: as evident from Fig. 2 (b and c) and Fig. 3 (a and b), at [s]=0.005% LDL reversibly associates to form their oligomers which, upon increasing [s] to 0.01%, dissociates into the monomeric units possessing somewhat greater sedimentation coefficients than the intact LDL. This increment in the sedimentation coefficient may be attributed to the SDS binding. The nature of the reversible association-dissociation of LDL is not clear, but it may be worth to note that a similar behavior was observed for the mixtures of the human serum LDL and dextran sulfate (DS), i.e., aggregation of LDL-DS complexes at low DS/LDL ratios and disaggregation at high DS/LDL ratios.[7,8] The former was attributed to the ionic interactions between LDL-SDS complexes and LDL molecules, and the latter to disruption of the ionic interactions consequent upon an increased binding of DS leading to an increased negative charge density on the LDL molecules. It may tentatively be assumed that a sim-

ilar mechanism operates in the present system.

Upon further increases in [s] to above 0.01%, e.g. 0.03%, the LDL structure appears to irreversibly disintegrate. Breake of the linearity of the binding isotherm at around 0.01% of [s], leading to $n=\infty$ (Fig. 1), may be indicative of a structural dissociation of LDL molecule with subsequent micellar complex formation between SDS and the dissociated LDL fragments. Indeed, this postulate is strongly supported by the sedimentation and gel filtration experiments which revealed that at high SDS concentrations ([s]$\geq$0.03%) LDL irreversibly dissociated into two fragments, an apoprotein containing fraction and a nonprotein fraction (Figs. 2d—2f, 3c, and 3d).

Since there is no additional peak detectable other than the main peak in both sedimentation and gel filtration experiments at [s]=0.01% (Figs. 2c and 2b), one may reasonably conclude that LDL molecules retain their original structures with their hydrophobic surfaces covered by the bound SDS. As expected, SDS of this concentration was found to protect LDL from the disruptive effect of NaSCN,[9] a highly chaotropic salt.

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