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Interaction of low-density lipoproteins of serum with hemin*†

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SUMMARY

The interaction of low-density lipoproteins of human serum with hemin and the nature of the oxidative denaturation of low-density lipoproteins of serum catalyzed by hemin was studied by means of spectrophotometric and ultracentrifugal analyses and by manometric methods. The results indicated that a complex was formed *in vitro* between hemin and isolated serum low-density lipoproteins. The peroxidation of the low-density lipoproteins was enhanced in the presence of low concentrations of hemin, approximately 1 to 10 mg of hemin per gram of lipoproteins. At higher concentrations, the catalytic effect of the hemin was depressed. Progressive increases in the amount of hemin associated with the lipoproteins and progressive decreases in the flotation rate of the lipoproteins were noted as more hemin was added. In unfractionated serum, a preferential association of hemin with the albumin fraction and with high-density lipoproteins minimized the association of hemin with low-density lipoproteins. Although the mode of association between isolated low-density lipoproteins and hemin was not clarified, most of the hemin molecules appeared to associate loosely with the protein moieties of low-density lipoproteins.

The lability of low-density lipoproteins when isolated from serum has been widely recognized (1). Upon storage of low-density lipoproteins for a prolonged period, a series of oxidative changes has been observed (2, 3). Furthermore, the presence of cupric ion greatly accelerated these changes as verified by ultracentrifugal analysis (4, 5). The lipoproteins could be stabilized by addition of antioxidants or copper-complexing agents. We have previously shown that the hydroperoxide of methyl linoleate markedly increased the lability of low-density lipoproteins of serum (6). Initial or primary association of the hydroperoxide of methyl linoleate with low-density lipoproteins was noted prior to their denaturation. These results, therefore, indicated that the formation, and subsequent degradation or polymerization, of hydroperoxides of unsaturated fatty acids of their lipid moieties were responsible for the oxidative denaturation of the low-density lipoproteins of serum.

Hemin and various hemoproteins are known to catalyze peroxidation of lipids *in vitro* (7–9). Since various hemoproteins are widely distributed in biological systems, under certain physiological disturbances, hemoproteins or the oxidized form of their prosthetic group, ferriprotoporphyrin, may become available to catalyze oxidative denaturation of the low-density lipoproteins of serum. In the present studies, the interaction of low-density lipoproteins with hemin was determined by spectrophotometric and ultracentrifugal analyses, and the catalytic effect of hemin on the oxidative denaturation of the lipoproteins was followed by spectrophotometric and manometric methods.

METHODS

Pooled samples of human serum obtained from normal male volunteers were subjected to an initial centrifugation at 79,420 × *g* for 24 hr, and the top fraction, composed of chylomicrons and lipoprotein molecules of the S_f 20–400 class, was removed (10). The solution density of the sera was then adjusted to 1.063 with saline solution, and the low-density lipoprotein fraction was isolated and analyzed according to the method described by De Lalla and Gofman (11).

To study the association of lipoproteins with hemin and its progressive changes under an atmosphere of

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oxygen, hemin[1] was recrystallized twice (12) and dissolved in 0.1 N sodium hydroxide and immediately diluted to a sodium hydroxide concentration of 0.01 N. Exactly 90 μl of 4×10^{-3} M hemin was placed in Warburg flasks, and 0.5 ml of buffered low-density human lipoprotein solution (pH 7.4, 14.5 mg/ml in saline solution of density 1.063) and 0.36 ml of 0.1 M potassium phosphate (pH 7.4) were added. The solution was adjusted to a density of 1.063 and a final volume of 3.6 ml by addition of saline solution.

For the spectrophotometric determinations, 1 volume of the above solution was diluted with 4 volumes of 0.15 M saline solution containing 0.01 M potassium phosphate buffer (pH 7.4). The progressive changes in the absorption spectrum of the hemin-lipoprotein complex were followed at 20° under an atmosphere of oxygen for a period of 48 hr. For determination of the absorption spectrum in the ultraviolet range, 0.17 ml of the hemin-lipoprotein mixture was diluted to 5 ml with 0.15 M saline solution containing 0.01 M potassium phosphate buffer (pH 7.4). The hemin-lipoprotein mixture was extracted with absolute alcohol and diluted to 5 ml, the proteins and inorganic salts were removed by filtration, and the ultraviolet absorption spectrum of the filtrate was determined.

To determine the association of hemin with specific classes of serum lipoproteins, 0.5 ml of 1.2×10^{-3} M hemin was added to each of four samples of serum (5 ml) and the densities of the solutions were adjusted to 1.006, 1.063, 1.107, and 1.220, respectively, by the addition of sodium bromide and 1.1% sodium chloride. The final volume of all solutions was 8 ml. The samples were then subjected to preparative ultracentrifugation under nitrogen gas at 79,420 $\times$ g for 24 hr, and the lipoproteins that floated at each of the four solvent densities were quantitatively separated. No attempt was made to separate the individual classes of lipoproteins from the same sample of serum since prolonged centrifugation of serum in the presence of hemin caused oxidative denaturation of lipoproteins. The hemin associated with the lipoproteins separated at each of the four solution densities was calculated from the absorption spectrum of the lipoproteins by using as a blank lipoprotein solutions similarly separated from the same amount of serum without the addition of hemin. The amount of hemin associated with each of the lipoprotein classes $D^{1.006}$, $D^{1.063}_{1.006}$, $D^{1.107}_{1.063}$, and $D^{1.220}_{1.107}$ (13) was calculated by subtracting the value for the lipoproteins separated at each solution density from the value obtained for the lipoproteins separated at the succeeding solution density. The bottom layer, which contained proteins separated at a solution density of 1.220, was used to determine the amount of hemin sedimented with serum proteins.

The mixture of serum and hemin was also subjected to continuous-flow paper electrophoresis by using a Spinco Model CP cell and a Spinco Model CPD Constat power supply (14). Veronal buffer, pH 8.6, 0.02 ionic strength, was permitted to flow down a paper curtain, and the sample was applied at a feeding rate of 0.225 ml/hr. A current of 50 m.a. was used. The fractions that dripped off the curtain were collected during a 6-hr period in 32 test tubes placed along the bottom of the curtain. The amount of hemin present in these tubes was determined spectrophotometrically; the paper curtain was stained with bromophenol blue to locate the position of fractionated proteins.

To determine the effect of the concentration of hemin on the rate of peroxidation, 0.5 ml of low-density lipoproteins (15 mg/ml) was placed in Warburg flasks, and amounts of 1.2×10^{-3} M hemin varying from 0.0 to 0.5 ml were placed in the side arm. The total volume of the reaction system in the Warburg flask was adjusted to 2.4 ml in 0.1 M potassium phosphate buffer, pH 7.4. After temperature equilibration, the contents were mixed and oxygen uptake was measured manometrically at 20° for a period of 24 hr.

For ultracentrifugal separation of the hemin–low-density lipoprotein complex, 0.5 ml of low-density lipoproteins of serum (15 mg/ml) was added to 0.0 to 0.5 ml of 1.2×10^{-3} M hemin solution, and the total volume was brought up to 8 ml with saline solution. The solution density was 1.063, and the final concentration of added potassium phosphate buffer was 0.1 M. The samples were centrifuged for 24 hr at 79,420 $\times$ g, a 1-ml portion of the low-density lipoprotein fraction was separated from each sample, and the amount of hemin associated in the lipoprotein fractions was determined spectrophotometrically with a blank solution containing the same amount of lipoproteins.

To determine the flotation rate of the hemin–lipoprotein complex, a freshly prepared hemin–lipoprotein mixture was used instead of the hemin–lipoprotein complex separated by the above preparative ultracentrifugation, since oxidative denaturation of the complex might have proceeded during the centrifugation. One-half milliliter of the low-density lipoproteins of serum was added to 0.0 to 0.5 ml of 1.2×10^{-3} M hemin solution and diluted to 2.5 ml with saline solution. The density of the solution and the final concentration of the

[1] Crystalline hemin was obtained from California Corporation for Biochemical Research, Los Angeles, California. Hemin solution or hemin molecule in text refers to ferriprotoporphyrin IX in which the associated anion is probably a hydroxyl group under present experimental conditions. Freshly prepared hemin solution was used throughout the studies.

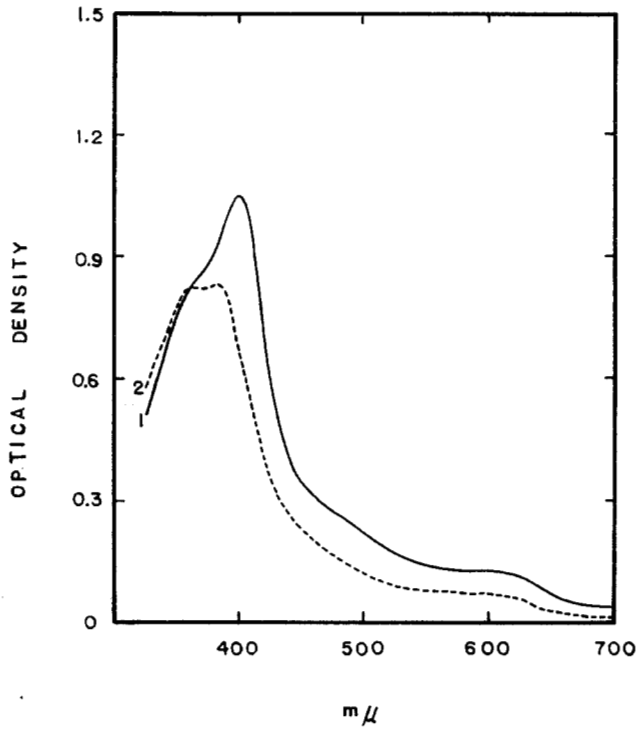

FIG. 1. Absorption spectra of hemin and hemin–lipoprotein complex. Curve 1 is the adsorption spectrum of the hemin–lipoprotein mixture determined with a blank consisting of a lipoprotein solution of the same concentration in a buffered saline solution; curve 2 is the absorption spectrum of hemin alone determined with a blank consisting of the buffered saline solution, which was equivalent to the use of the lipoprotein solution blank.

added phosphate buffer were the same as for preparative ultracentrifugations. The solutions were subjected to ultracentrifugal analysis in a Spinco Model E ultracentrifuge at a speed of 52,640 rpm with an acceleration time of 5 min 20 sec. The bar angle was 45°.

In order to observe whether the lipid moieties of the low-density lipoproteins of serum contributed to the formation of a hemin–lipoprotein complex, 0.5 ml of the low-density lipoproteins (15 mg/ml) was extracted with 25 volumes of chloroform–methanol 2:1 (v/v). The precipitated proteins were removed by filtration, and the solvent evaporated under vacuum. The lipid fraction thus obtained was emulsified with the aid of 1 mg of Tween-60 and added to 0.5 ml of 1.2×10^{-3} M hemin solution; the total volume was diluted to 8 ml with saline solution. The density of the solution was 1.063, and the final concentration of added potassium phosphate buffer was 0.1 M. The solution was then subjected to preparative ultracentrifugation as described previously.

RESULTS

The addition of low-density lipoproteins of serum to a solution of hemin resulted in the formation of a complex. The existence of a complex was indicated by the replacement of the broad absorption maximum of hemin at 380 mμ by a narrow band of absorption with a maximum at around 400 mμ together with an intensification of the absorption (Fig. 1). The lipoprotein solution blank showed negligible absorption at the wavelengths used. Further proof for complex formation was shown by ultracentrifugal separation of the complex from unassociated hemin. At a solution density of 1.063 and at 79,420 × g, the complex floated at the top of the tube while the hemin sedimented to the bottom.

The intensity of the absorption maximum at 400 mμ of the original hemin–lipoprotein complex (Fig. 2, Curve 1) stayed constant for the first 24 hr of incubation at 20° under an atmosphere of oxygen. In the next 6 hr, the absorption maximum rapidly decreased due to the accumulation of lipohydroperoxide in the hemin–lipoprotein complex (Curve 2). Although the intensity of absorption at 400 mμ gradually increased

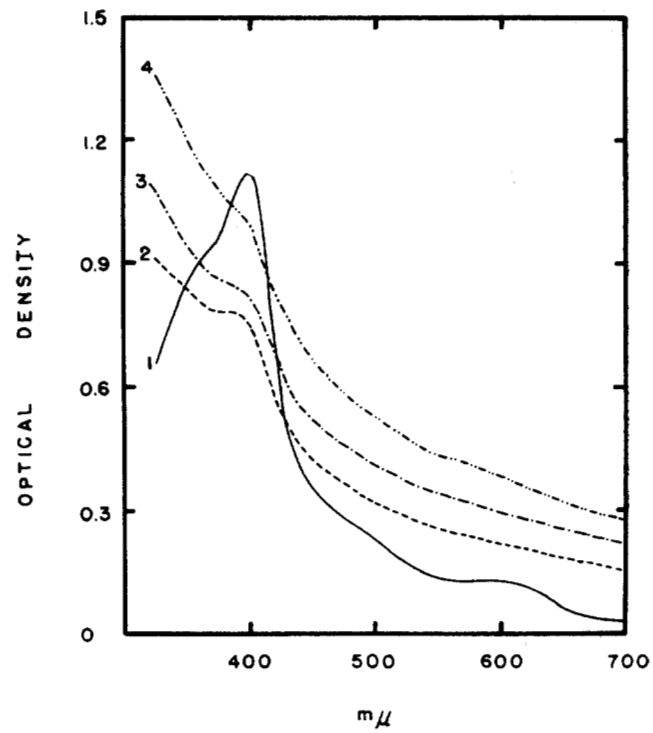

FIG. 2. Changes in the absorption spectrum of a hemin–lipoprotein mixture. Curve 1 is the original absorption spectrum of the hemin–lipoprotein mixture; curves 2, 3, and 4 are the absorption spectra of the mixture at 30, 36, and 48 hr after incubation. The spectrophotometric determinations were made with a blank consisting of an equivalent concentration of buffer and saline solution.

during further incubation (Curves 3 and 4), this phenomenon seemed to be accompanied by an increase in absorption in the ultraviolet range and by the development of visible turbidity apparently due to oxidative denaturation of the hemin–lipoprotein complex and its subsequent aggregation. Determination of the ultraviolet absorption spectrum of a hemin–lipoprotein mixture indicated a considerable increase in absorption at around 233 mμ and at 265–285 mμ after 48 hr incubation (Fig. 3). Furthermore, the spectrum of the alcohol extract of the lipoproteins (Fig. 4) indicated that the increase in absorption appeared to be caused by the formation of conjugated dienoic hydroperoxide and by the formation of carbonyl compounds in the lipid moieties of the lipoproteins (15). The conjugated dienoic acid was presumably formed by peroxidation of linoleic acid, the predominant unsaturated fatty acid of low-density lipoproteins (39% by gas-liquid chromatographic analysis) (16).

When hemin was added into serum, approximately 86% of the hemin was sedimented together with serum proteins, and 14% of the hemin was bound to serum lipoproteins that were separated by ultracentrifugal

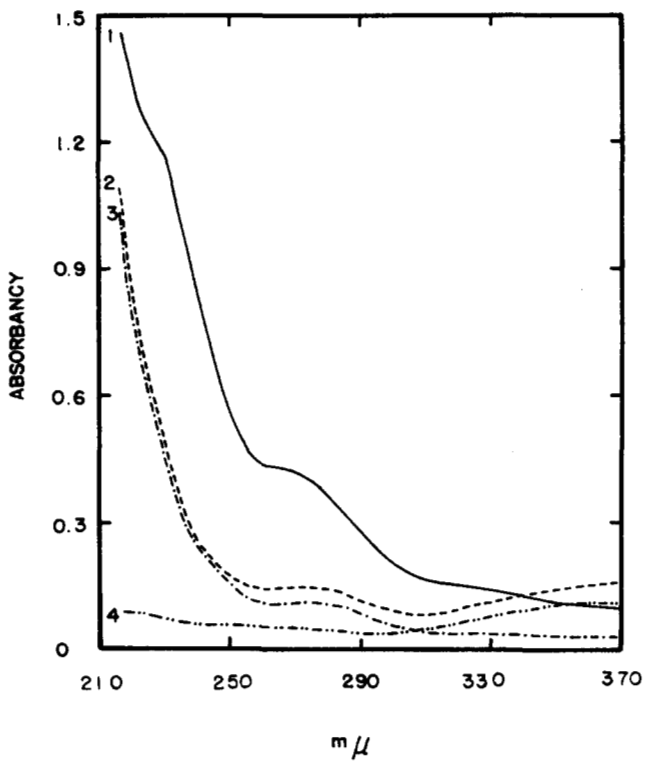

FIG. 3. Ultraviolet absorption spectra of the hemin–lipoprotein mixture. Curve 1 is the absorption spectrum of the hemin–lipoprotein mixture 48 hr after incubation; curve 2 is the spectrum of the mixture before incubation; curves 3 and 4 are the spectra of lipoprotein and hemin, respectively, at the same concentration in a buffered saline solution.

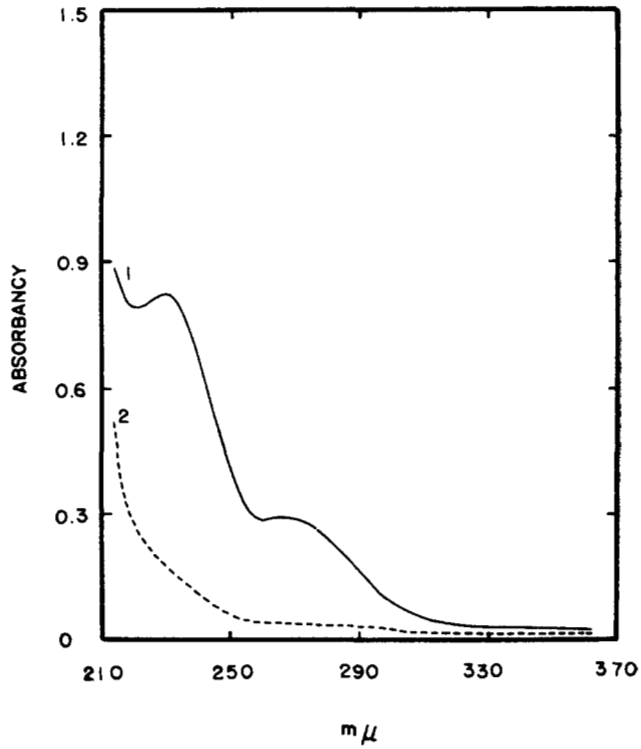

FIG. 4. Ultraviolet absorption spectra of alcohol extracts of hemin–lipoprotein mixtures. Curve 1 is the ultraviolet absorption spectrum of the alcohol extract of the hemin–liproprotein mixture 48 hr after incubation; curve 2 is the absorption spectrum of an alcohol extract of the mixture before incubation.

methods (Table 1). The high-density lipoproteins of an approximate hydrated density of 1.145 were preferentially bound with hemin molecules, and only 1% of the hemin was bound to the low-density lipoproteins of S_f 0–20. When hemin was added into a low-density lipoprotein solution isolated from the serum, however, approximately 92% of the added hemin was bound to the lipoproteins. Furthermore, when a serum–hemin mixture was subjected to a paper-strip electrophoresis (6) (Veronal buffer, pH 8.6, 0.075 ionic strength, and a current of 5 m.a.), most of the hemin migrated as a brown zone together with the serum albumin fraction, and no hemin was retained at the zone of application. In the absence of serum, free hemin did not migrate and thus the entire hemin was retained at the original zone of application. Therefore, it was suspected that most of the hemin that sedimented with serum proteins at a solution density of 1.220 (Table 1) might have been bound with serum proteins, especially with the serum albumin fraction.

A continuous-flow paper electrophoresis of a serum–hemin mixture indicated that the serum albumin fraction was associated with almost 70% of the added hemin molecules (Fig. 5). We have noted that, in the absence

TABLE 1. ASSOCIATION OF HEMIN WITH LIPOPROTEINS OF SERUM

Serum Constituents	Density Range	Optical Density at 400 mμ*	Approx. Percentage of Associated Hemin†
Low-density lipoproteins, S_f 20 and higher	$D^{1.006}$	0	0
Low-density lipoproteins, S_f 0–20	$D_{1.006}^{1.063}$	0.13	1
High-density lipoproteins, 1.075	$D_{1.063}^{1.107}$	0.02	...
High-density lipoproteins, 1.145	$D_{1.107}^{1.220}$	1.10	13
Ultracentrifugal residue	$D^{1.220}$‡	1.52	86

* For the lipoproteins, 1 ml of the top fraction obtained by centrifugation was diluted to 5 ml and used for measurement of optical density. For ultracentrifugal residue, 5 ml of the bottom fraction was diluted to 25 ml and used for measurement.

† For the calculation of approximate percentage of associated hemin, the optical density at 400 mμ was assumed to be proportional to the amount of hemin in each fraction. For ultracentrifugal residue, the optical density 1.52 was multiplied by 5 to correct for the dilution factor.

‡ The protein fraction, which was sedimented at a solution density of 1.220, was designated as ultracentrifugal residue (1).

of serum, free hemin always moved faster than serum albumin in a continuous-flow paper electrophoresis with Veronal buffer at an ionic strength from 0.02 to 0.75. On the other hand, in paper-strip electrophoresis under the same conditions, free hemin did not move from the point of application. It would appear that free hemin, possibly in the form of micelle, adsorbs firmly onto the filter paper and that the voltage that can be applied to a Spinco Model R Series D paper-strip electrophoresis cell may not be sufficiently high to overcome the adsorption. In the experiments in which serum was separated by continuous-flow paper electrophoresis, the concentration of hemin was four times higher than the concentration used in the ultracentrifugal fractionation. This increased level was used to compensate for the dilution of the sample by the buffer solution permitted to flow down the paper curtain during the continuous electrophoresis. The fourfold increase in the level of added hemin might have decreased the percentage of hemin associated with the serum albumin fraction while increasing the percentage of hemin associated with serum lipoproteins. It is interesting to note that the low-density lipoproteins of serum were less stable than high-density lipoproteins or serum proteins. Therefore, the preferential association of hemin with serum proteins, especially the albumin fraction, or with high-density lipoproteins may protect low-density lipoproteins against oxidative denaturation catalyzed by hemin.

It must be noted that peroxidation of the low-density lipoproteins of serum is not necessarily proportional to the concentration of hemin added into the reaction system. The oxygen uptake of the low-density lipoproteins measured in Warburg flasks at 20° increased progressively with an increase in the amount of added hemin (Fig. 6). Oxygen uptake reached a maximum by the addition of 0.1 ml of 1.2×10^{-3} M hemin, or approximately 10 mg of hemin per gram of the low-density lipoproteins. Further increases in the concentration of hemin caused a decrease in oxygen uptake.

We have noted that an increase in the amount of hemin added into the isolated low-density lipoproteins linearly increased the amount of hemin associated with the lipoproteins, which were then re-isolated by ultracentrifugal methods. Approximately 92% of hemin was found to be associated with 0.5 ml of low-density lipoproteins (15 mg/ml) when the quantity of 1.2×10^{-3} M hemin solution was varied from 0.1 to 0.5 ml. Furthermore, the increase in the amount of hemin progressively decreased the flotation rate of the lipoproteins (Fig. 7). Since, at a solution density of 1.063, the hemin–lipoprotein complex floated to the top of the tube while free hemin was sedimented, the progressive decrease in the flotation rate was apparently caused by the increased association of hemin molecules with low-density lipoproteins. Over prolonged periods, oxidative degradation of the low-density lipoproteins catalyzed by hemin progressively removed the lipid, which

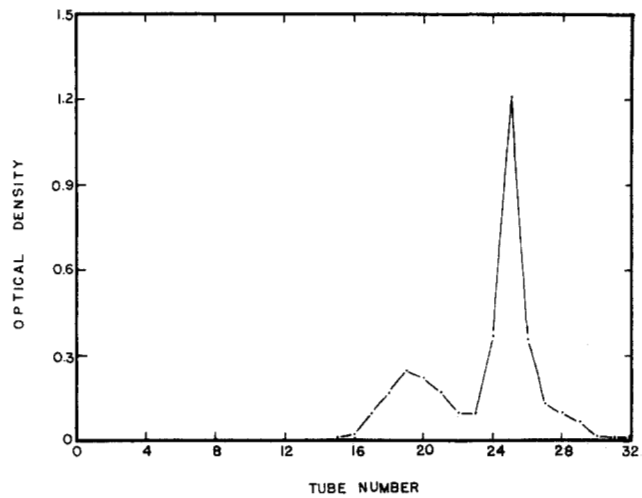

FIG. 5. Continuous-flow paper electrophoresis of serum-hemin mixture. The curve represents the optical density at 400 mμ of each fraction diluted to 10 ml. Tubes 10–13, 15–18, 19–22, and 23–27 contained γ-globulin, β-globulin, α_2 and α_1 globulins, and albumin fractions, respectively. The percentage of the total area under the peak between Tube 23–29 represents the percentage of added hemin associated with the serum albumin fraction. In the absence of serum, free hemin was recovered from tubes 31 and 32 under the same conditions.

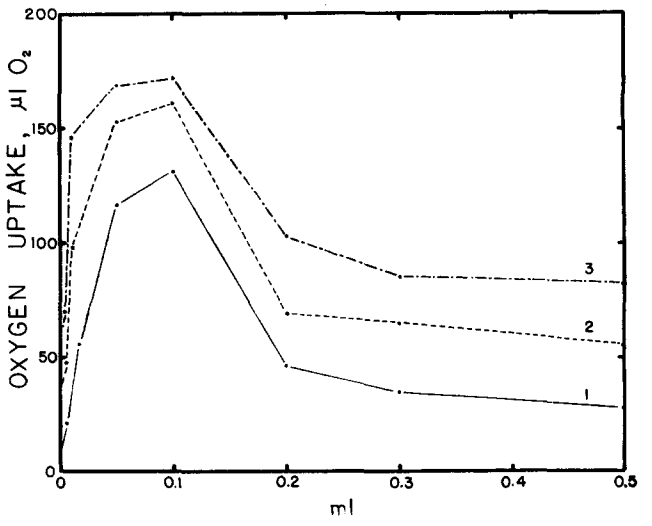

FIG. 6. Changes in oxygen uptake as a function of the concentration of hemin. Curves 1, 2, and 3 represent oxygen uptake by 7.5 mg low-density lipoproteins of serum in the presence of 0.0–0.5 ml of 1.2×10^{-3} M hemin solution at 6, 12, and 24 hr incubation, respectively.

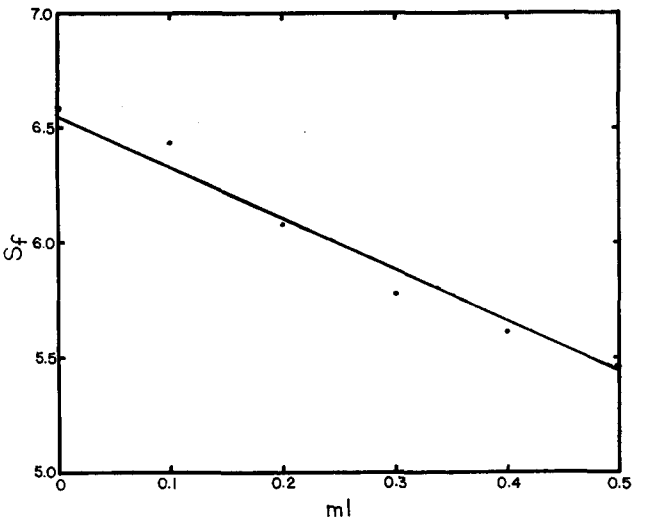

FIG. 7. Flotation rate of low-density liproproteins of serum in the presence of hemin. The curve represents progressive decreases in flotation rate of 0.5 ml lipoproteins added to 0.0–0.5 ml of 1.2×10^{-3} M hemin solution and total volume brought to 2.5 ml.

lowered the flotation rate of the native lipoproteins (16). In the present study, freshly mixed samples of lipoprotein and hemin were always used, and oxidative degradation was therefore not significant.

Most of the hemin molecules in the hemin–lipoprotein complex seemed to be loosely associated with low-density lipoproteins. Upon addition of 25 volumes of chloroform–methanol 2:1 (v/v) to the hemin–lipoprotein mixtures containing 0.1–0.5 ml of 1.2×10^{-3} M hemin solution and 0.5 ml of the low-density lipoproteins, approximately 80% of the hemin molecules together with the lipid moieties of the lipoproteins were extracted into the organic phase. The remaining hemin molecules were coprecipitated with the protein moieties of the lipoproteins. All of the precipitated hemin molecules, however, were not necessarily tightly bound with the protein moieties of the lipoproteins; some of them might have been simply occluded in, or absorbed on the surface of, the denatured proteins. When a solution of free hemin was added to 25 volumes of chloroform–methanol, the hemin remained completely soluble.

It is interesting to consider whether the lipid moieties of the low-density lipoproteins contributed to the association of hemin with the low-density lipoproteins. If any firm association exists between hemin and the lipid moieties of the lipoproteins, some hemin molecules should stay with the lipid in the chloroform layer formed upon addition of water into the methanol–chloroform extract of the low-density lipoprotein–hemin mixture. We found, however, that all the hemin present in the methanol–chloroform extract was transferred into the aqueous layer by the addition of one-fifth volume of water. This was also true for free hemin dissolved in methanol–chloroform. Therefore, no firm association appeared to exist between hemin and the lipid derived from the low-density lipoproteins. It must be emphasized that the failure to observe a transfer of the hemin into the chloroform layer does not exclude the possibility of a loose association between hemin and the lipid moiety of the intact lipoprotein molecule.

When the lipid emulsion prepared from the methanol–chloroform extract of low-density lipoproteins of serum was added to a solution of hemin, no complex was formed. The lipid emulsion, which was floated to the top of the tube by ultracentrifugation at $79{,}420 \times g$ at a solution density of 1.063, was not associated with hemin molecules as determined by spectrophotometric analysis. However, the association of hemin with the lipid moiety of intact lipoproteins and the association of hemin with the extracted, disoriented lipids in an aqueous environment may not necessarily represent similar situations. Although the present results suggested that the protein moiety of low-density lipoproteins of serum may primarily be involved in the association of hemin with the lipoproteins, the exact nature of the association between hemin and the intact lipoprotein molecule has not been clarified.

DISCUSSION

The catalytic effect of metallic ion, such as the cupric ion, on the oxidative denaturation of the low-density

lipoproteins of serum has been widely recognized (4, 5, 17). It is possible that a metallic ion, which is abundantly present in prosthetic groups of various hemoproteins, such as ferriprotoporphyrins, may be more important in the *in vivo* oxidative denaturation of low-density lipoproteins than trace amounts of cupric ion. The role of ferriprotoporphyrin in the *in vitro* oxidative denaturation of low-density lipoproteins of serum was shown in the present study.

The catalytic effect of hemin on oxidative denaturation of low-density lipoproteins of serum seemed dependent on the amount of hemin molecules associated with low-density lipoproteins. The molecular and surface properties of both low-density lipoproteins and hemin molecules seemed to be directly involved in this phenomenon.

Ferriprotoporphyrin is known to exist in solution as large micelles, within which there are both monomeric and dimeric units (18). The change in the absorption spectrum of hemin upon addition of low-density lipoproteins of serum may indicate that the state of dispersion of hemin in solution was drastically changed. It is possible that, at low concentrations of hemin, the hemin molecules may be associated with low-density lipoproteins in such a way as to prevent intermolecular interaction between hemin molecules, thus exerting its full catalytic activity. Such configuration may allow an efficient free radical chain reaction and may result in the co-oxidation of both hemin and of the unsaturated lipids in the low-density lipoproteins. On the other hand, at high concentrations of hemin, hemin molecules may be arranged as stable micelles on the surface of low-density lipoproteins or may be associated with low-density lipoproteins in such a way as to prevent the orientation of hemin molecules at the lipid-medium interface. A possible decrease in the solubility of oxygen in the medium and an inhibition of diffusibility of oxygen from medium to the unsaturated lipid moieties by hemin micelles may also partially contribute to the decreased oxygen uptake by the lipoproteins.

It has been shown that interaction of hemin with human serum albumin leads to the formation of methemalbumin, which is an abnormal component of blood plasma in certain diseases associated with excessive hemolysis (19). It is interesting to note, from the present study, that serum albumin and, to a lesser extent, high-density lipoproteins of approximate hydrated density of 1.145 combined predominantly with hemin in plasma, thus providing a means of removing free hematin compounds from the blood stream, and minimized the association of hemin with the low-density lipoproteins of serum.

One might expect the extent of oxidative denaturation of low-density lipoproteins catalyzed by hemin to be affected by dietary constituents, such as the degree of unsaturation in a dietary fat and the amount of natural antioxidants or synergists available in the diet. In fact, we have noted previously that a decrease in the content of unsaturated dietary fat effectively prevented the oxidative denaturation of low-density lipoproteins of serum (16). A dietary source of tocopherol only partially prevented the oxidative denaturation of low-density lipoproteins. The concentration of C_{18} dienoic and C_{20} tetraenoic acids in the low-density lipoproteins was found to be a major factor governing the rate and degree of lipohydroperoxide formation in the lipid moieties. The lag period before absorption of the hemin–lipoprotein complex decreased at 400 mμ, and the extent of the subsequent oxidative denaturation of the lipoproteins seemed to depend on the rate of lipohydroperoxide formation in the highly unsaturated fatty acids. Although the lability of low-density lipoproteins to oxidation was suspected to be due partially to free sulfhydryl groups in the protein moieties of the lipoproteins, neither sulfhydryl group nor cysteine was detected in the lipoproteins or in the hydrolysates of the protein moieties of the lipoproteins (20).

Since some hemoproteins exist in a lipid-rich media or are present as lipoproteins, various or multiple stabilizing systems may exist to prevent their peroxidative effect on lipids or on lipoproteins. These stabilizing systems may thus allow the hemoproteins to maintain their normal *in vivo* functions (i.e., oxygen transport by hemoglobins; electron transport by cytochromes; and normal enzymatic function by catalase, peroxidase, and other metalloporphyrin-containing enzymes). Under certain physiological disturbances, however, these hemoproteins or the oxidized form of their prosthetic group, ferriprotoporphyrin, may become available to initiate free radical oxidation of unsaturated lipid moieties of various lipoproteins resulting in denaturation or degradation. Release of hemoglobins and their heme group from erythrocytes, in hemorrhage or thrombosis, may lead to the above phenomena.

We have found that not only hemin but also various hemoproteins formed different types of complexes with lipoproteins, depending upon the conditions employed in the reaction systems. The details in these studies and their possible significance in biological systems will be presented in subsequent papers.

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