Interaction of dextran sulfate with low-density lipoproteins of plasma

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SUMMARY The interaction between dextran sulfate and low-density lipoproteins of the S_f 0–10 class in phosphate buffer of pH 7.4 and ionic strength 0.1 was studied by means of analytical ultracentrifugation. The sedimentation pattern of the dextran sulfate–lipoprotein mixture at high dextran sulfate/lipoprotein weight ratios showed a boundary indicative of a soluble complex with a high sedimentation rate, and a free dextran sulfate boundary with a lower sedimentation rate. The amount of free dextran sulfate seemed to determine the distribution of various transition states of polymers, ranging from disintegrated units to large insoluble aggregates.

KEY WORDS dextran sulfate · low-density lipoproteins · complex · soluble · insoluble · human plasma · ultracentrifugal sedimentation · sulfated polysaccharides

THE BIOLOGICAL importance of sulfated polysaccharides in relation to fat transport has been widely recognized since the intravenous injection of heparin was found to induce production of an enzyme called "post-heparin lipoprotein lipase" and to result in changes in the lipoprotein spectrum of the serum. The interaction of sulfated polysaccharides with lipoproteins has been the subject of numerous investigations well reviewed by Cornwell and Kruger (1).

Both soluble and insoluble complexes are formed by the interaction of lipoproteins and polyanions. The formation of insoluble complexes has been extensively studied by many investigators. Precipitation methods for the isolation and purification of plasma lipoproteins (2–5) and turbidimetric procedures for their quantitative estimation (6–8) depend on the formation of such insoluble complexes in which the polyanions are sulfated polysaccharides.

The formation of soluble complexes has previously been studied by moving boundary electrophoresis, since the electrophoretic mobility of a soluble lipoprotein–sulfated polysaccharide complex is greater than that of free lipoproteins (2, 9, 10). By this method Bernfeld, Donahue, and Berkowitz (10) found that with β-lipoproteins two types of soluble complexes, reversible and irreversible, are formed with sulfated polysaccharides depending upon the nature of the latter. A similar study on the interaction of β-lipovitellin and heparin was reported by Sugano (11).

The present experiment was designed to study the ultracentrifugal behavior of the soluble complex formed between low molecular weight dextran sulfate and low-density lipoproteins of the S_f 0–10 class in the presence of excess dextran sulfate in phosphate buffer of pH 7.4 and ionic strength 0.1.

MATERIALS AND METHODS

Dextran sulfate was prepared from dextran with an average molecular weight of 180,000 according to the method described by Ricketts (12), and isolated as the sodium salt. This salt was free from nitrogen and was shown by ultracentrifugal analysis to consist of only one component. The sulfur content of this dextran sulfate sodium was measured colorimetrically (13) and found to be 17.1%, which corresponds to 1.93 sulfate groups per hexose unit.

To prepare low-density lipoproteins, pooled samples of human plasma separated from citrated whole human blood were subjected to centrifugation at 79,490 × g for 24 hr, and the top fraction, composed of chylomicrons and lipoprotein molecules of the S_f 20–400 class, was removed (14). The solution density of the plasma was adjusted to 1.063 with sodium chloride, and the low-density lipoprotein fraction was isolated and purified by preparative ultracentrifugation according to

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the method of Gofman et al. (15) in the presence of 0.02% EDTA. The lipoproteins thus obtained were shown to belong to the S_f 0–10 class.

To study interaction of dextran sulfate with low-density lipoproteins of the S_f 0–10 class in phosphate buffer, the lipoprotein fraction was first dialyzed against 1 liter of cooled distilled water at 5° for 3 days and then against 1 liter of cooled phosphate buffer of pH 7.4 and ionic strength 0.1, with daily change of the buffer, for 4 days. The external solutions used in the dialysis contained 0.02% EDTA and were saturated with nitrogen. Under these conditions, the lipoprotein solution did not develop turbidity. Ray, Davisson, and Crespi (16) previously observed by ultracentrifugal analysis that oxidative denaturation of low-density lipoproteins during prolonged dialysis was effectively prevented by adding EDTA to the external solution.

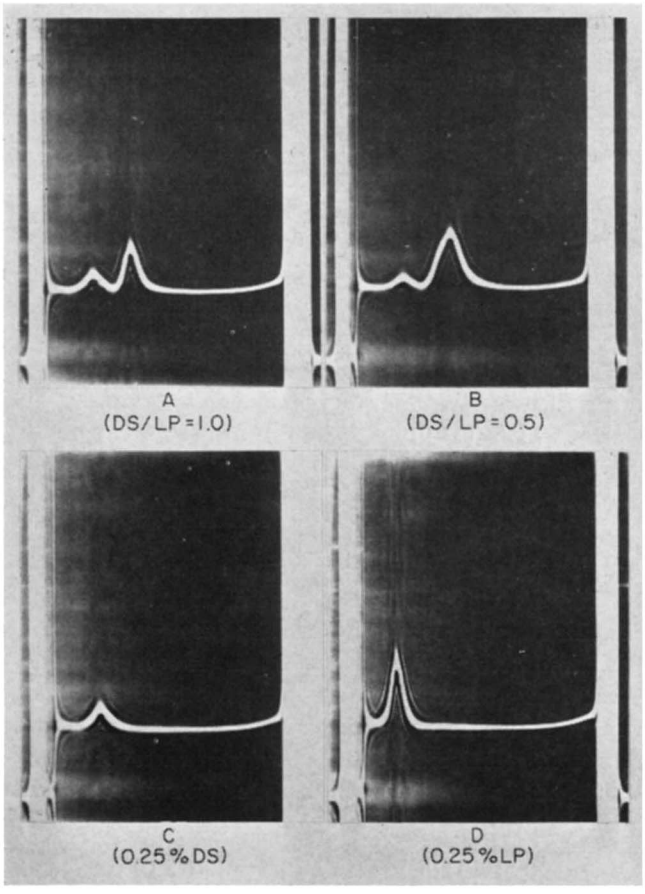

FIG. 1. Sedimentation patterns of dextran sulfate–lipoprotein mixtures in phosphate buffer of pH 7.4 and ionic strength 0.1. *A* and *B* are ultracentrifugal patterns of 0.25% dextran sulfate–0.25% lipoprotein mixture and 0.25% dextran sulfate–0.5% lipoprotein mixture, respectively; *C* is the ultracentrifugal pattern of 0.25% dextran sulfate solution, while *D* shows the pattern of 0.25% lipoprotein solution. In each case, the picture was taken at 22 min at bar angle 55° after a speed of 59,780 rpm with an acceleration time of 6 min had been obtained.

Lipoprotein concentration was estimated from determination of total lipid and protein content as follows. Total lipids of lipoproteins were extracted with chloroform–methanol 2:1 (17), then dissolved in diethyl ether; the solvent was removed, and the residue was dried to constant weight over anhydrous phosphorus pentoxide at 5°. Residual proteins were washed twice with each of three solvents (50% alcohol, absolute alcohol, and diethyl ether) and were then dried and weighed in a similar manner. The lipoprotein sample used in this study contained 79.4% lipids and 20.6% protein. Cholesterol and phosphorus contents as determined by methods described by Sperry and Webb (18) and Fiske and Subbarow (19) were 34.5 and 1.06%, respectively.

The dextran sulfate/lipoprotein weight ratio above which no insoluble complex is formed in phosphate buffer of pH 7.4, $\mu = 0.1$, was determined in the following manner. One-half milliliter samples of the lipoprotein solution (3 mg/ml) in phosphate buffer of pH 7.4, $\mu = 0.1$, were placed in test tubes. Various amounts of dextran sulfate were then added to obtain dextran sulfate/lipoprotein weight ratios ranging from 0.0006 to 2.5, and the total volume was brought to 4 ml with the phosphate buffer. The contents were mixed gently by repeated inversion of the tubes and were allowed to stand for 60 min. Optical density measurements performed with a Cary spectrophotometer at 650 mμ against blank solutions containing the same amount of the lipoproteins indicated that formation of insoluble complex first took place at the dextran sulfate/lipoprotein ratio of 0.002, reached a maximum in the 0.015–0.040 region, and then decreased, becoming completely abolished at the ratio of 0.4.

Ultracentrifugal analyses of the dextran sulfate–lipoprotein mixtures in phosphate buffer of pH 7.4, $\mu = 0.1$, were performed at 20° in a Spinco Model E analytical ultracentrifuge at 59,780 rpm with an acceleration time of 6 min.

RESULTS AND DISCUSSION

Ultracentrifugal analysis of the 0.25% dextran sulfate–0.25% low-density lipoprotein mixture in phosphate buffer of pH 7.4 and ionic strength 0.1 revealed the presence of two independent peaks (Fig. 1, *A*). The sedimentation rate of the slower moving boundary was approximately the same as that of 0.25% dextran sulfate solution (Table 1). On the other hand, the fast-moving boundary had a sedimentation rate of 14.0, far greater than that of either dextran sulfate (7.8) or the lipoproteins (5.9). When the lipoprotein concentration in 0.25% dextran sulfate solution was increased, the area under the faster moving peak also increased; a less pronounced but significant decrease in the area under the slower moving peak occurred, as can be seen by

TABLE 1 OBSERVED SEDIMENTATION RATES OF THE COMPONENTS IN DEXTRAN SULFATE–LIPOPROTEIN MIXTURES IN PHOSPHATE BUFFER OF pH 7.4 AND IONIC STRENGTH 0.1

Materials*	Observed Sedimentation Rate	
0.25% DS–0.25% LP mixture	(Slow)†	8.0
	(Fast)	14.0
0.25% DS–0.50% LP mixture	(Slow)	8.2
	(Fast)	16.0
0.25% DS	—	7.8
0.25% LP	—	5.9
0.50% LP	—	5.8

* DS and LP indicate dextran sulfate and low-density lipoproteins, respectively.

† (Slow) and (Fast) designate the slower and faster moving boundaries, respectively.

TABLE 2 SEDIMENTATION RATES OF THE COMPONENTS IN DEXTRAN SULFATE–LIPOPROTEIN MIXTURE IN THE PRESENCE OF 0.5 M SODIUM CHLORIDE*

Materials	Observed Sedimentation Rate	
0.45% DS–0.45% LP mixture	(DS)†	11.0
	(LP)	2.2
0.45% DS	—	11.3‡
0.45% LP	—	2.3‡

* Ultracentrifugal analysis was performed in the medium containing 0.5 M sodium chloride and phosphate buffer of pH 7.4 and ionic strength 0.1.

† (DS) and (LP) indicate the boundaries of dextran sulfate and of low-density lipoproteins, respectively.

‡ The difference in sedimentation rate of low-density lipoproteins reported in Tables 1 and 2 may be due to difference in solvent density. The higher sedimentation rate of dextran sulfate in Table 2 may be caused by the change in the configuration of dextran sulfate from an expanded form to a more compact form in the medium of high ionic strength.

comparison of the sedimentation patterns of the mixtures of the ratios 1.0 and 0.5 (Fig. 1). Therefore, the slower and faster moving boundaries apparently corresponded to free dextran sulfate and soluble dextran sulfate–lipoprotein complex, respectively. The large sedimentation rates of the soluble complexes as compared to those of both dextran sulfate and the lipoproteins can be explained by a drastic change in the molecular size of the lipoprotein molecules in the presence of dextran sulfate. It seemed that the soluble complex formed under the present conditions is a large polymer of lipoprotein molecules linked by dextran sulfate bridges.

The presence of 0.5 M sodium chloride abolished the interaction between dextran sulfate and low-density lipoproteins in phosphate buffer of pH 7.4, $\mu = 0.1$; the dextran sulfate–lipoprotein mixture did not present any faster moving boundary. Two components in the mixture migrated independently with the sedimentation rates of dextran sulfate and of the lipoproteins (Table 2).

Comparison of the peak area of the slower moving boundary of the 0.25% dextran sulfate–0.25% lipoprotein mixture with that of the 0.25% dextran sulfate boundary (Fig. 1) indicated that 75% of the added dextran sulfate was present in free form. Thus 25% of the added dextran sulfate was involved in the complex formation, giving 0.25/1 as the weight ratio of dextran sulfate to the lipoproteins in the soluble complex. Furthermore, in the 0.25% dextran sulfate–0.5% lipoprotein mixture, 55% of the added dextran sulfate was in free form, indicating a weight ratio of 0.23/1 for the components of the soluble complex. Since the Johnston-Ogston effect is operative in a two-component system, the amount of dextran sulfate participating in the complex formation as estimated from peak areas might be slightly reduced.

It must be noted that mixing of dextran sulfate and low-density lipoproteins at the ratio of 0.23–0.25 caused the formation of some insoluble complex, as manifested by the development of some turbidity. Thus, the conversion of the soluble complex with the dextran sulfate/lipoprotein ratio of 0.23–0.25 into an insoluble molecular aggregate seemed to be prevented by high concentrations of unbound dextran sulfate present in the system. We have also noted that as dextran sulfate/lipoprotein weight ratios were made progressively lower than 0.4 there was a concomitant decrease in the concentration of unbound dextran sulfate, accompanied by increasing formation of insoluble complex and by decreasing concentration of soluble complex. These soluble complexes exhibited heterogeneous sedimentation boundaries and seemed to represent various transition states of polymers from disintegrated units to large insoluble molecular aggregates. Furthermore, in the region where maximum formation of insoluble complex was observed, the concentration of soluble complexes was minimized, and the free dextran sulfate boundary disappeared as a free lipoprotein boundary became observable. Thus, it is apparent that the amount of free dextran sulfate in dextran sulfate–lipoprotein mixtures is one of the decisive factors in the distribution of various molecular species formed by the interaction of dextran sulfate with low-density lipoproteins. The mode of association between the components at various dextran sulfate/lipoprotein weight ratios is currently a subject of further study at this laboratory.

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