The Molecular Structure of Lubricating Glycoprotein-I, the Boundary Lubricant for Articular Cartilage*

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Lubricating glycoprotein-I (LGP-I) was prepared from bovine synovial fluid by density gradient sedimentation and gel-permeation chromatography. The LGP-I sample obtained was able to lubricate articular cartilage in a manner equivalent to that of whole synovial fluid. Chemical, physical, and electron microscope measurements were carried out to determine the structure of the LGP-I molecules. The molecular weight calculated from sedimentation equilibrium measurements was \(2 \times 10^6\), and the solute distribution obtained indicated that LGP-I was relatively monodisperse. The \(s_{20,w}\) value was 4.84, and the intrinsic viscosity was 92 ml/g. The molecular weight and diffusion coefficient calculated from later light-scattering measurements was \(2.06 \times 10^6\) and \(1.10 \times 10^{-7}\) cm/s, respectively. The electron microscope measurements showed that the LGP-I molecules had a number average length of 204 nm, a weight average length of 222 nm (with a standard deviation of 54 nm), and a width of 1-2 nm.

These data and the kinked appearance of the molecules indicate that LGP-I is a partially extended flexible rod. The hydrodynamic measurements also indicate that LGP-I has the same structure in solution, although the apparently high \(s_{20,w}\) value, compared to other rod-like molecules, suggests that due to its flexibility LGP-I can occupy a more compact domain than would be expected based on its extended dimensions. The name of "lubricin" is suggested for this lubricating glycoprotein.

Previous studies (1-3) have shown that the boundary lubrication property of whole synovial fluid is quantitatively recovered in the protein fraction following density gradient sedimentation and that, following further fractionation procedures, the lubricating activity is present in a glycoprotein fraction. Later studies demonstrated the presence of two glycoproteins, LGP-I1 (4) and LGP-I2 (5), in this lubricating fraction and that only LGP-I possesses lubricating activity (6, 7). Purified LGP-I samples, however, exhibit a lower lubricating activity than whole synovial fluid with the result that it was not known if LGP-I is the articular lubricant per se or whether other constituents also contributed to the function of this molecule. These questions were resolved when it was demonstrated (8) that fractions containing only LGP-I were able to lubricate articular cartilage in a manner equivalent to that of whole synovial fluid at concentrations similar to those that are thought to occur in vivo. It was concluded, therefore, that LGP-I is the molecule responsible for the boundary lubricating properties of synovial fluid and that it can perform this function in the absence of other constituents.

Chemical and physical measurements (4) have shown that LGP-I is composed of approximately equal quantities of peptide and carbohydrate constituents and has a molecular weight of 227,500. Threonine, proline, glutamic acid, and lysine (224, 242, 127, and 128 residues/1000 residues, respectively) account for 75% of the amino acids in the protein core of the molecule and GalNAc, Gal, and NeuAc account for 98% of the total carbohydrate constituents. Degradation experiments carried out by treatment with alkali, periodate, and enzymes (9) demonstrated the presence of threonine (serine)-\(O\)-GalNAc linkages and that the major oligosaccharide side chain in the molecule is \(\alpha\)-NeuAc-(2\(\rightarrow\)3)-\(\beta\)-D-Galp-(1\(\rightarrow\)3)-\(\beta\)-D-GalNAcp. Small quantities of Man and GlcNAc, however, were consistently observed in LGP-I samples (4, 7, 8). This indicated that N-linked oligosaccharides are also present in LGP-I. Based on these properties a model for the molecular structure of LGP-I was proposed (7). In the present study we have carried out electron microscope, light scattering, sedimentation, and viscosity measurements on a preparation of LGP-I that has the same lubricating properties as whole synovial fluid in order to determine the molecular structure of this glycoprotein.

**EXPERIMENTAL PROCEDURES**

Preparation and Purification of LGP-I—Pooled adult bovine synovial fluid was obtained from the slaughterhouse and fractionated essentially as described by Swann et al. (4). Following three sequential CsCl density gradient procedures, the glycoprotein Fraction II (Fig. 1c: Swann et al. (4) was fractionated by gel-permeation chromatography (Bio-Gel A-5m column (200 x 2 cm) column eluted with 1.0 M GdmCl, 0.01 M Tris/HCl, pH 7.9). In the earlier study (4) the LGP-I fraction obtained by Bio-Gel A-5m chromatography was reduced and alkylated and then refractionated on a Bio-Gel A-15m column. This reduction/alkylation step was used in order to separate LGP-I from high molecular weight aggregates eluted at or near the column void volume. It was subsequently observed that reduction/alkylation decreased the lubricating activity of LGP-I (8) and that aggregate formation was eliminated or minimized by decreasing the time involved in the preparation and maintaining LGP-I containing fractions in dissociative solvents. The reduction/alkylation step, therefore, was omitted and the Bio-Gel A-5m LGP-I fraction was refractionated by chromatography on a column (100 x 2.5 cm) packed with Sephacryl CL-2B and eluted with 3 M GdmCl, 0.05 M Tris/HCl, pH 7.2. The purified LGP-I eluting at a \(K_v\) value of 0.7 was then refractionated in a fourth CsCl density gradient (Gradient IV: 200,000 \(\times\) for 64 h at...
Analytical Procedures—Amino acid and hexosamine analyses were performed using a semiautomatic microamino acid analyzer (4, 5). N-Acetylenuraminic acid was determined by gas-liquid chromatography after methanalysis and preparation of the permethylsilyl derivatives (10). A manual resorcinol method (11) was used to determine the sialic acid content of column fractions. The hexosamine content of the Gradient-IV fractions was determined using a modified manual Elson-Morgan method following hydrolysis with 8 M HCl at 105 °C for 3 h (12).

Sedimentation Measurements—Sedimentation velocity measurements were performed on solutions containing 2.4 mg of LGP-I that had been dialyzed against 0.5 M sodium acetate, 0.01 M Tris/HCl, pH 7.2. Sedimentation equilibrium measurements were performed in a Beckman model E analytical ultracentrifuge on LGP-I samples made in the viscometer by addition of known volumes of dialysate and the relative viscosity was determined at each dilution. The refractive index increment (dn/dc) was determined using a Cannon-Ubbelohde semimicro dilution viscometer at 4 °C using the light scattered at 4° to the transmitted beam.

Light-scattering Measurements—The diffusion coefficient of LGP-I solutions (0.1 to 0.8 M) of LGP-I was performed using a Cannon-Ubbelohde semimicro dilution viscometer on solutions containing 2.4 mg of LGP-I that had been dialyzed against 0.5 M sodium acetate, 0.01 M Tris/HCl, pH 7.0. Dilutions were made in the viscometer by addition of known volumes of dialysate and the relative viscosity was determined at each dilution. The intrinsic viscosity was calculated from a plot of the reduced viscosity (ηm - 1) versus the LGP-I concentration (where c is the LGP-I concentration in grams/ml at each dilution) by extrapolation to zero concentration.

Viscosity Measurements—Intrinsic viscosity measurements were performed using a Cannon-Ubbelohde semimicro dilution viscometer on solutions containing 2.4 mg of LGP-I that had been dialyzed against 0.5 M sodium acetate, 0.01 M Tris/HCl, pH 7.0. An An/D rotor operated at a speed of 12,272 rpm at 20 °C and the short column, multichannel cell described by Yphantis (13) was used.

Sedimentation Measurements—Sedimentation velocity measurements were performed on solutions containing 2.0-8.0 mg LGP-I/ml after extensive dialysis against 0.5 M sodium acetate, 0.01 M Tris/HCl, pH 7.2. Sedimentation equilibrium measurements were performed in a Beckman model E analytical ultracentrifuge on LGP-I samples (100 to 500 µg/ml) after dialysis against 0.5 M sodium acetate, 0.01 M Tris/HCl, pH 7.0. An An/D rotor operated at a speed of 12,272 rpm at 20 °C and the short column, multichannel cell described by Yphantis (13) was used.

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Electron Microscopy—Solutions of LGP-I (100 µg/ml in 0.15 M ammonium acetate, pH 7.0) were prepared and photographed as previously described (16) using rotary shadow casting with platinum for contrast enhancement. Measurements were made from the micrographs and several fields were examined to reduce selectivity. A discussion of the factors that influence the accuracy of the length and width measurements has been published (17).

Lubricating Activity—Friction measurements were performed using a cartilage on glass apparatus (8) on samples that had been extensively dialyzed against 0.15 M veronal buffer, pH 7.2. The relative lubricating ability of samples was then calculated by comparing the friction versus speed curves with the curves obtained with veronal buffer and bovine synovial fluid used as negative and positive controls (8).

RESULTS

Fractionation of the pooled synovial fluid in three sequential cesium chloride gradients (4) and gel chromatography using a column (200 x 2 cm) packed with Bio-Gel A-5m and eluted with 1.0 M GdmCl, 0.01 M Tris/HCl, pH 7.5, produced a series of LGP-I subfractions with different buoyant densities (Fig. 2). Hexosamine analysis and absorbance measurements at 230 nm showed that LGP-I was distributed over a range of densities (1.39-1.56 g/ml) with a peak concentration at 1.42 g/ml. Friction measurements carried out on equal aliquots of the gradient fractions, however, showed a peak of lubricating activity at a density of 1.45 g/ml. Those fractions (7-12) with the highest activity were then pooled and, after dialysis against distilled water, 1 M NaCl, and distilled water, were freeze-dried. This sample accounted for 48.4% of the total freeze-dried LGP-I recovered from the Gradient IV fractions and was used for all the further studies.

The chemical and physical properties of this LGP-I sample are given in Table I. The amino acid and carbohydrate compositions are similar to those reported previously (4, 6, 7) for LGP-I purified by procedures involving reduction and alkylation. The intrinsic viscosity was 92 ml/g (Fig. 3a) and the s20w value at zero concentration calculated from a reciprocal plot of the s values determined at different concentrations (Fig. 3b) was 4.84. The same s20w value within experimental error was obtained when measurements were performed after dialysis against 0.5 M sodium acetate, 0.01 M Tris/HCl, pH 7.0, buffer. Sedimentation equilibrium measurements carried out using dilute solutions of LGP-I showed that there was an essentially linear distribution of solute when fringe displacement was plotted against r²/2 (Fig. 4). The effective reduced molecular weights (κν) calculated from the friction displacement curves at initial loading concentrations of 100, 200, and 400 µg/ml were 3.30, 3.21, and 3.30, respectively. The molecular weight of LGP-I (1.96 x 10⁶) was calculated using the formula

\[ M_c = \frac{RT}{(1 - \frac{V_p}{V})}, \]

and values of 3.27 for κν, 0.69 for the partial specific volume (\(\bar{b}\)), and 1.86 for the solvent density (\(\rho_s\)).

The D0.6w value at zero concentration calculated from the light scattering data obtained at a clocktime of 5 ms was 1.10 ± 0.06 x 10⁻² cm²/s. The Mc calculated from the inverse of KC/R0 (where R0 is the Rayleight ratio) after extrapolation to zero concentration was 2.06 x 10².

A photograph of LGP-I molecules viewed using the electron microscope is shown in Fig. 5. Measurements from the electron micrography (Fig. 6) showed that LGP-I had a most frequent length of approximately 200 nm. The number-average length was 204 nm, compared to a weight average length of 222 nm. The standard deviation was 54 nm. After correction for the replica thickness (16) the particle width was estimated to vary between 1 and 2 nm. In general appearance LGP-I occurred as a rod-like molecule, but kinks were observed along the axis (Fig. 5), indicating that the molecule possessed a certain degree of flexibility. LGP-I, therefore, appears to be a flexible rod.
FIG. 5. Electron micrograph of LGP-I molecules prepared and photographed as described by Slayter (15). Magnification, \( \times 61,000 \).

Fig. 6. A frequency histogram of the length of LGP-I molecules measured from electron micrographs.

DISCUSSION

The present study was carried out using a sample of LGP-I that possessed high lubricating activity. The chemical properties of this LGP-I sample, however, were similar to those reported previously (4, 6, 7) for LGP-I purified by a procedure involving reduction and alkylation. It can be concluded, therefore, that LGP-I occurs as such in synovial fluid and, although it is associated with other constituents, it is not linked to these constituents by reducible bonds. Similarly the ability of LGP-I to act as a boundary lubricant in an equivalent manner to whole synovial fluid in the absence of other constituents and at concentrations in which it is thought to occur in vivo indicates that this molecule is the boundary lubricant (7, 8).

The physical measurements carried out to determine the molecular weight of LGP-I agree closely. Light scattering measurements gave a value of \( 2.06 \times 10^7 \) for the weight average molecular weight compared to a value of \( 1.996 \times 10^7 \) obtained from sedimentation equilibrium measurements. The solute distribution obtained in the sedimentation equilibrium analysis also indicate that the LGP-I sample used in this study was relatively monodisperse (Fig. 4). The electron microscope measurements (Fig. 6), however, showed that the length of the LGP-I molecules varied over a wide range. Based on the determined amino acid content of 43.5% (w/w), a \( M_r \) of \( 2 \times 10^7 \) and an average amino acid residue weight of 109, the peptide chain of LGP-I has a \( M_r \) of \( 8.7 \times 10^4 \) and contains 800 residues. The extreme length value of a single polypeptide of this size would be approximately 290 nm, if it had an extended configuration (3.64 Å/amino acid (18)) compared to a value of approximately 120 nm if it had an \( \alpha \)-helical configuration. The average length observed (222 nm; Fig. 6) which is toward the middle of the range of values indicates that the major portion of the molecules has a partially extended structure. Using this length the average axial spacing/amino acid is 2.8 Å, very
close to the value for collagen (2.866 Å; (19)) which is known to have a rigid rod configuration. It is clear, however, from the electron microscope pictures (Fig. 5) that LGP-I is not a rigid molecule. Frequent kinks were observed along the axis of the molecule. It is concluded, therefore, the LGP-I is primarily a flexible rod and that the variation in the lengths of the molecule is due to different degrees of extension.

Although it cannot be assumed that LGP-I in solution will have the same configuration as that calculated from electron microscope measurements, it is possible to calculate the diffusion coefficient from the molecular dimensions and compare this value to the one determined by the light scattering measurements. Using values of 222 nm for the length and 1.5 nm for the diameter (Table I), the calculated translational diffusion coefficient using the equation

$$D_{\text{ton}} = 2.15 \times 10^{-11} \text{ cm}^2/\text{s} \left( \frac{\ln 2a - \ln b}{a} \right)$$

where $2a$ is the length and $b$ is the radius, is $1.11 \times 10^{-7} \text{ cm}^2/\text{s}$. This equation is derived (14) by combining the Stokes-Einstein equation (15) and the Perrin equation (20) for a prolate ellipsoid. The similarity of the experimentally determined and calculated values for $D_{\text{ton}}$ indicate that LGP-I has the same dimensions in solution as those determined by electron microscopy measurement. The determined value for the $s_{\text{ton}}^2$ (4.84, Table I), however, is significantly larger than the value of 2.50 calculated from the Svedberg equation

$$S = D \times M \left( \frac{1 - \epsilon_0}{RT} \right)$$

using the experimentally determined values of $1.1 \times 10^{-7} \text{ cm}^2/\text{s}$ for the diffusion coefficient and $2 \times 10^5$ for the molecular weight. This difference is thought to be due to the flexibility of the LGP-I molecules which under the experimental conditions used to measure the sedimentation coefficient results in the molecules occupying a more compact domain than would be expected based on the extended dimensions. Thus, the $s_{\text{ton}}^2$ of collagen (2.70 (21)), which is known to be a rigid rod, is similar to the calculated value for LGP-I. In addition, the intrinsic viscosity value obtained for LGP-I (Table I) is much lower than that reported for collagen (22).

In conclusion, the electron microscope measurements have shown that LGP-I is an asymmetric molecule with a weight average length of 222 nm and a diameter of 1-2 nm. The molecular model that best fits these dimensions and the kinked appearance is that of a partially extended, flexible rod. The hydrodynamic measurements are consistent with this model and indicate that LGP-I also has a flexible rod structure in solution. The apparently high $s_{\text{ton}}^2$ value and low intrinsic viscosity, however, suggest that due to its flexibility LGP-I can occupy a more compacted domain than other rod-like molecules.

The present concept of boundary lubrication of articular cartilage by LGP-I is that this molecule binds to the cartilage and is able to act as a lubricant via its ability to stabilize a layer of water at the tissue surface (7, 8). Now that the properties and molecular structure of this glycoprotein have been partially characterized, it should be possible to examine the mechanisms involved in lubrication and the molecular basis for this important joint function.

The name LGP-I was given to this glycoprotein because it was one of the two glycoproteins present in the lubricating fraction isolated from bovine synovial fluid (2, 4). Now it is known (8) that LGP-I is the boundary lubricant for articular cartilage and that lubricating glycoprotein-II does not possess lubricating activity it would seem appropriate to rename these glycoproteins. Based on the functions of LGP-I, a name of "lubricin" is suggested for this glycoprotein.

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