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Cockroach Larval-specific Protein, a Tyrosine-rich Serum Protein*

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Larval-specific protein (LSP) is the most abundant protein in the hemolymph of cockroaches shortly before molting, but is rapidly cleared from the hemolymph during the molt (Kunkel, J. G., and Lawler, D. M. (1974) *Comp. Biochem. Physiol.* 47B, 697–710). *Blatta orientalis* LSP was purified by sedimentation in preparative sucrose gradients followed by 2-hydroxypropylamino-cellulose anion-exchange chromatography and gel filtration on a column of Bio-Gel A-1.5m. The amino acid composition of LSP includes 16.3 mol % tyrosine and 4.9 mol % phenylalanine, but virtually no cysteine and little methionine. The following physical properties were determined for LSP: R_s = 68.3 Å, $s_{20,w}$ = 17.8, and $\bar{V}$ = 0.723. From these values an M_r = 507,900 was calculated. In electron micrographs, LSP appears as rectangular particles of 121 by 134 Å. In disc polyacrylamide gel electrophoresis, native LSP exhibits a single band, but in sodium dodecyl sulfate-polyacrylamide gel electrophoresis, LSP is resolved into a doublet of closely spaced bands of M_r = 88,100 and 84,400 present in a ratio of 1.38:1. These data indicate that native *B. orientalis* LSP is a hexamer of subunits averaging approximately M_r = 86,000. Crossed immunoelectrophoresis of *Blattella germanica* larval serum indicates that LSP in that species is a hexamer composed of a random assortment of two subunits of different charge in the ratio 1.25:1. The amino acid composition and physical properties of LSP suggest that LSP may be the hemimetabolous analogue of the tyrosine- and phenylalanine-rich storage proteins of holometabolous insects.

The temporal organization of developmental events during the molting cycle of cockroaches has been studied in synchronously molting cultures (1–3). Among the biochemical changes that proved to be linked to the molting cycle is a dramatic fluctuation in the hemolymph concentration of a protein identified as LSP[1] by Kunkel and Lawler (1). Just before each ecdysis, LSP is the most abundant protein in the hemolymph of *Blatta orientalis*, accounting for 40–50% of the total protein, but by 24 h after each ecdysis LSP is barely detectable (3). Following each larval molt, the accumulation of LSP begins 2 or 3 days after feeding and continues steadily until just before ecdysis. This suggests that LSP is an important protein in the physiology of dictyopterans, possibly serving as a storage protein for the supply of material utilized just prior to and immediately following ecdysis, which are times when the cockroach, a hemimetabolous insect, ceases to feed.

The term "storage protein" has long been applied to members of a class of proteins rich in tyrosine and phenylalanine that accumulate to exceptionally elevated levels in the hemolymph of holometabolous insects during the late larval stages just prior to pupation (reviewed in Refs. 4 and 5). These larval storage proteins are rapidly depleted from the hemolymph during the pupal stage, a nonfeeding stage when the remodeling and synthesis of adult tissues associated with metamorphosis is occurring. The larval storage proteins in several species of the orders Diptera and Lepidoptera have been characterized, and all show striking similarities in structure despite a lack of immunological cross-reactivity across ordinal lines. All are rich in aromatic amino acids and have a hexameric quaternary structure composed of subunits averaging 80,000–90,000 Da. Most exhibit subunit heterogeneity expressed as slight differences in size or charge among the subunits. This striking evolutionary conservation of structure has led to the suggestion that the evolutionary precursors to the larval storage proteins of holometabolous insects may be identifiable among the hemolymph proteins of hemimetabolous insects on the basis of similarities of structure and physiology (5).

Based upon the aforementioned cycles of accumulation and depletion of LSP in larval cockroaches, we suggested that LSP may play a molting-related physiological role in hemimetabolous insects analogous to that of the metamorphosis-related storage proteins of the homometabolous insects (3). If we are to establish a biochemical link between LSP and the holometabolous storage proteins, we must pursue the comparative structural and conformational studies called for by Thomson (5). With that aim in mind, we describe in this report a procedure for the purification of LSP from *B. orientalis* and provide a description of its physical properties and subunit structure.

MATERIALS AND METHODS AND RESULTS[2]

DISCUSSION

There are only three major proteins in the hemolymph of cockroaches: LSP, SP1, and SP2 (1, 3). SP2 is a lipoprotein

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[1] The abbreviations used are: LSP, larval-specific protein; PAGE, polyacrylamide gel electrophoresis; QAE-cellulose, 2-hydroxypropylamino-cellulose; R_s, Stokes' radius; SDS, sodium dodecyl sulfate; SP1, serum protein I; SP2, serum protein II (lipophorin).

[2] Portions of this paper (including "Materials and Methods," "Results," Figs. 1–7, and Tables I and II) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, MD 20814. Request Document No. 83M-1627, cite authors, and include a check or money order for $6.00 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.

of the type presently referred to as lipophorin (14). The purification of LSP, therefore, consists primarily of resolving LSP from SP1 and lipophorin. In this study, LSP was purified by sedimentation in sucrose gradients (Fig. 1) followed by QAE-cellulose chromatography (Fig. 2) and Bio-Gel A-1.5m chromatography (Fig. 3).

The physical properties of LSP are listed in Table II. The Stokes' radius of LSP as estimated by gel filtration on Bio-Gel A-1.5m is 68.3 Å. This value is consistent with the linear dimensions of 121 × 134 Å obtained for negatively stained LSP particles in electron micrographs (Fig. 6). The $s_{20,w}$ of LSP monomer as estimated in isokinetic sucrose gradients (Fig. 5) is 17.8. A value of 0.723 for $\bar{V}$ was calculated from the amino acid composition (Table I). Using these values, the M_r of native LSP was calculated to be 507,900.

LSP is resolved as a single band by disc PAGE (Fig. 4*A*), but as a closely spaced doublet of bands by SDS-PAGE (Fig. 4*B*). The M_r values of the two subunits determined by SDS-PAGE (Fig. 4*B*) are 84,400 and 88,100, respectively. Densitometry of the gel bands indicates that the ratio of the heavier subunit to the lighter subunit is 1.38:1. A hexamer of these two subunits in this proportion should have a native M_r = 519,300, in good agreement with the 507,900 figure calculated from R_s, $s_{20,w}$, and f/f_0. Additional evidence for the hexameric structure of LSP was obtained by crossed immunoelectrophoresis of *B. germanica* larval serum (Fig. 7). Seven isozyme-like peaks were obtained, compatible with a random series of hexamers composed of two subunits of differing charge in the proportions represented by the binomial expansion of (4/9 fast + 5/9 slow)^6; the ratio of the slow subunit to the fast subunit is therefore 1.25:1. In addition, the negatively stained image of the LSP particle is consistent with the two-dimensional projection of an octahedral arrangement of a hexamer with D_3 dihedral symmetry (15). In conclusion, a hexameric quaternary structure for LSP is clearly indicated.

The most noteworthy feature of LSP, however, is its amino acid composition (Table I). LSP is exceptionally rich in tyrosine (16.33 mol %) and ranks among the most tyrosine-rich of proteins. For example, among the 314 families of sequences listed by Dayhoff *et al.* (16), only six families exceeded 15 mol % tyrosine. Other noteworthy features of the amino acid composition of LSP are the virtual absence of cysteine (0.03 mol %), the low level of methionine (0.28 mol %), and the relatively high phenylalanine and tryptophan contents (4.90 and 3.89 mol %, respectively). The lack of cysteine is consistent with the lack of reducible intersubunit disulfide bonds detectable by SDS-PAGE (not shown).

The amino acid composition, molecular weight, and subunit composition of LSP are remarkably similar to those of the storage proteins of holometabolous insects. Calliphorin, isolated from the blowfly, *Calliphora erythrocephala*, was the first of the holometabolous storage proteins to be characterized and serves as the prototype for such proteins (17, 18). There are striking similarities between calliphorin and LSP. Like LSP, calliphorin is rich in tyrosine, containing 10.5 mol % (18). The M_r of native LSP and calliphorin are similar, 517,000 and 528,000, respectively. Both proteins are hexamers, although calliphorin dissociates into subunits above pH 6.5. The size ranges of the LSP and calliphorin subunits are similar, 84,400–88,100 and 87,000, respectively. Also, the subunits of both proteins differ in charge so that the native hexamers display an isozyme-like pattern of heteropolymers.

Despite the many questions that remain about the functions of LSP and calliphorin, the biochemical similarities reported here and the previously recognized physiological similarities (3) strongly suggest that LSP and calliphorin are evolutionarily related and that LSP is a hemimetabolous analogue to the holometabolous storage proteins. If so, LSP may more closely resemble the ancestral insect storage protein since molting cycle physiology is ancestral to the development of complete metamorphosis.

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COCKROACH LARVAL-SPECIFIC PROTEIN (LSP), A TYROSINE-RICH SERUM PROTEIN

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MATERIALS AND METHODS

Materials

QAE-cellulose, Bio-Gel A-1.5m and all reagents for electrophoresis were obtained from Bio-Rad Laboratories. Blue Dextran 2000 was from Pharmacia Fine Chemicals. Protein standards were obtained from Sigma Chemical Co.

Animals and Hemolymph Collection

Synchronously-molting cultures of the oriental cockroach, B. orientalis, were maintained as described previously (1,6). Larval hemolymph was collected 2 or 3 days before the mean ecdysis time, which is the time when the LSP concentration is at its peak (3). The mean ecdysis time from day of feeding was determined to be 9.8±0.4 (S.D.) days in the sixth molting cycle, and 14.2±0.4 and 16.3±0.4 days for females and males, respectively, in the seventh cycle.

Animals were placed under CO_2 anesthesia for hemolymph collection. Bulk harvesting of hemolymph was accomplished by flushing the abdominal cavity with 1 ml of PBS. Buffer was introduced through a needle inserted through an intersegmental membrane in the posterior region of the abdomen and collected from an opening produced by cutting off one of the anterior legs. The diluted hemolymph was cleared of cells by centrifugation and the serum proteins concentrated by ultrafiltration on a 30,000 molecular weight exclusion-limit membrane (XM30 Diaflo membrane, Amicon Corp.).

Purification by Sedimentation in Linear Sucrose Gradients

Linear gradients of 5 to 30% sucrose (w/v) in PBS were prepared over a 6 ml layer of 40% sucrose in 3.5 x 1 in. cellulose nitrate tubes. A volume of 2.4 ml of dialysed larval serum adjusted to an absorbance of 10.0 at 280 nm in a 1 cm cuvette was layered on top of the gradient. The tubes were centrifuged at 26,400 rpm for 20 h at 4°C in an SW27 rotor (Model L-2 ultracentrifuge, Beckman Instruments Co.). The gradients were eluted with continuous recording of absorbance at 254 nm. The 18S peak containing both LSP and SP1 was concentrated by ultrafiltration and dialysed against the starting buffer for QAE chromatography.

Purification by QAE-Cellulose Chromatography

The QAE-cellulose column (2.5 cm x 26.0 cm) was equilibrated with 0.01 M Tris-citrate (pH 7.0). Samples were applied in the same buffer and eluted with a linear 600 ml gradient to a limit of 0.2 M NaCl at a flow rate of 90 ml/h. The Oudin assay was used to assay the column fractions for LSP and SP1 (see below). The fractions from the forward region of the LSP peak that contained no detectable SP1 were pooled and concentrated by ultrafiltration.

Purification and Stokes' Radius Determination by Bio-Gel A-1.5m Chromatography

A column of Bio-Gel A-1.5 m (1.0 cm x 117 cm) was used for both the preparative separation of native LSP from denatured LSP and to determine the R_S of native LSP. The column was eluted with 0.01 M sodium phosphate buffer (pH 7.4), 0.15 M NaCl at 5.9 ml/h and monitored at 280 nm. A standard curve relating R_S to Ve/Vo was obtained using Blue Dextran 2000 and the following standards: bovine thyroglobulin (R_S = 86 Å), E. coli -galactosidase (R_S = 69 Å), ferritin (R_S = 57.9 Å) and catalase (R_S = 52 Å).

Preparation of Specific Antisera

Young male Dutch belted rabbits were immunized with the 18S fraction of larval B. orientalis serum. Crude antiserum was made specific for LSP by absorbing with old adult B. orientalis hemolymph, which contains SP1, but no LSP. The specificities of the antisera were verified by immunodiffusion and immunoelectrophoresis (1).

Quantitative Oudin Assay

The single-immunodiffusion assay of Oudin (7) was performed as described earlier (1). The migration distance of the precipitin front is measured at various times using a dissecting scope with a filar ocular micrometer, and the K value is calculated ($K = d \times t^{-0.5}$). It was convenient to express d in cm and t in sec x 10^{-6} so that the usual range of K was from 0 to 2.5. Standard curves were prepared by plotting K against the log of the antigen concentration. It was determined that 1 Oudin unit of LSP in our assay corresponds to 0.5 μg of protein and 1 Oudin unit of SP1 corresponds to 0.67 μg protein.

Disc PAGE

Electrophoresis of proteins under non-denaturing conditions was performed according to Davis (8), but with the following modifications: The separating gel, stacking gel and sample buffers were prepared without SDS, and the electrode buffer was diluted 10-fold. The separating gel was of 5% acrylamide with an acrylamide/bis-acrylamide ratio of 37.5. The gels were stained in 0.025% Coomassie Blue R-250 in 45% methanol, 10% acetic acid for 16 h and destained in 7.5% acetic acid. Slab gels were scanned in a Quick-Scan^R densitometer (Helena Laboratories).

SDS-PAGE

Samples denatured by heating in SDS were subjected to electrophoresis in gels of 7% acrylamide with an acrylamide/bis-acrylamide ratio of 37.5 in the discontinuous buffer system of Laemmli (9). The gels were stained and scanned as described above for disc gels. The standards used for M_r estimation were myosin (197,000), β-galactosidase (116,200), phosphorylase b (97,400), bovine serum albumin (66,200), fibrin β chain (55,000) and ovalbumin (44,600).

Determination of Sedimentation Coefficients in Isokinetic Sucrose Gradients

The sedimentation coefficient of LSP was determined by comparison to known standards in isokinetic sucrose gradients as described by Martin and Ames (10). Isokinetic gradients (ISCO tables, p. 29, 8th ed., ISCO, Inc.) were centrifuged at 34,500 rpm for 16 h at 6°C. Elution of the gradients was monitored at 280 nm. The following standards were used: bovine thyroglobulin (19.3 S), β-galactosidase (16.1 S), catalase (11.3 S) and aldolase (7.35 S).

Electron Microscopy

Electron microscopy was generously performed by Dr. C. F. Woodcock of the Zoology Department, University of Massachusetts. QAE-purified LSP was diluted in 0.05 M NaCl, 0.01 M phosphate buffer (pH 7.0), deposited on coated grids, negatively-stained in dilute buffered saline and uranyl acetate, and examined at 50,000-fold magnification in a Siemens electron microscope.

Amino Acid Analysis

Lyophilized samples were hydrolyzed in constant boiling HCl containing 0.25% phenol for 24 h at 115°C under a N_2 atmosphere. To determine the half-cysteine content and to eliminate cysteine from the proline peak, cysteine was oxidized to cystine (11). Amino acid analysis was performed on a single column of Beckman W-1 resin using a four-buffer system (12) in a Beckman model 121 amino acid analyzer.

Tryptophan Analysis and Determination of Absorbance

The tryptophan content was determined from the absorption spectrum of LSP obtained at a concentration of 0.5 mg/ml in 0.1 N NaOH (13). The specific absorbance at 280nm and pH 7.0 was also obtained. Solutions were prepared gravimetrically using LSP that had been extensively dialyzed against distilled water and lyophilized.

Crossed Immunoelectrophoresis

The first dimension was performed in a 1.5 mm gel of 0.8% agarose in 0.05 M Tris-citrate buffer, pH 8.6. Fresh 4 μl samples of hemolymph from individual animals were diluted 1:4 with buffer supplemented with 5% glycerol and bromophenol blue tracking dye and loaded on the gel. Power was applied at low amperage until the dye entirely entered the gel. The current was then raised to 20 mA and the tracking dye was plotted against time and allowed to run off the gel until LSP was predicted by experience to be approaching the end of the gel. A track from the first dimension gel was then transferred to a second dimension gel of dilute antiserum-agarose for crossed immunoelectrophoresis. After electrophoresis the gel was dried and stained with 0.05% Coomassie Blue-R250 in 7% acetic acid for 1 hour. Quantitation was based on the heights of the precipitin peaks.

RESULTS

Purification in Linear Sucrose Gradients

Three peaks are resolved by the sedimentation of larval B. orientalis serum in a 5-25% linear sucrose gradient (Fig. 1A). Analysis of the protein content of the three peaks by disc PAGE indicates that the main peak, which sediments with a sedimentation coefficient of 18S, consists of both LSP and SP1 (Fig. 1B), while the more buoyant peak consists of the lipoprotein, SP2 (Fig. 1C), and the minor peak that sediments faster than the 18S peak consists of a mixture of SP2-like protein, LSP and SP1 (Fig. 1D). Only the 18S peak was used for subsequent purification of LSP by QAE-cellulose chromatography.

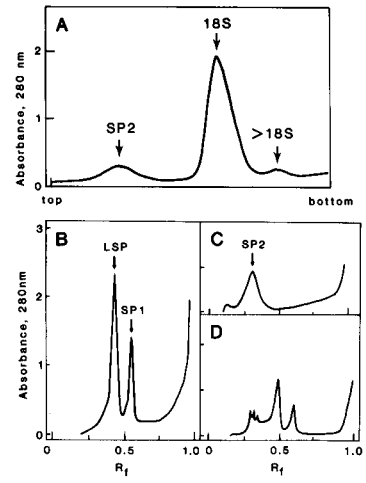

Fig. 1. A. Sedimentation pattern of B. orientalis larval serum in 5-30% linear sucrose gradient centrifuged in the SW-27 rotor for 16 h at 26,400 rpm. B. C. and D. Analytical 4% disc gels of the protein peaks from the sucrose gradient shown in panel A: 18S peak (B), SP2 peak (C) and 18S peak (D). The gels were scanned at 280 nm immediately after the termination of electrophoresis. The protein bands are identified according to the terminology of Duhamel and Kunkel (3).

Purification by QAE-Cellulose Ion-Exchange Chromatography

The 18S peak from the preparative sucrose gradients was loaded on the QAE-cellulose column and eluted with a gradient of NaCl. Elution of LSP and SP1 was monitored by quantitative immunodiffusion in Oudin tubes. The elution pattern (Fig. 2) demonstrates that although the LSP and SP1 peaks overlap, the forward region of the LSP peak, which elutes first, is free of SP1. The early-eluting, SP1-free LSP fractions from several column runs were collected and concentrated by ultrafiltration in preparation for gel filtration.

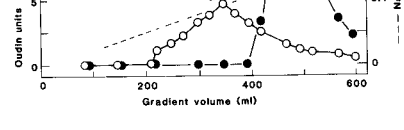

Fig. 2. QAE-cellulose chromatography of the 18S peak from the sucrose gradient shown in Fig. 1. The sample was loaded on the column in 0.01 M Tris-citrate buffer (pH 7.0) and washed for 1 column volume before initiation of the NaCl gradient. See text for details of the quantitative Oudin assay used to determine the concentrations of LSP and SP1. One Oudin unit of LSP and SP1 correspond to approximately 1.0 and 0.67 μg of protein, respectively.

Purification and Stokes' Radius Determination by Bio-Gel A-1.5m Chromatography

Gel permeation chromatography of QAE-purified LSP on Bio-Gel A-1.5m was used both preparatively, for the isolation of LSP, and analytically, for the estimation of its Stokes' radius. The elution pattern is shown in Fig. 3. Both the void volume peak and the main peak at V_e/V_o = 1.4 consist exclusively of LSP, as indicated by SDS-PAGE, which suggests that the void volume peak consists of denatured or unfolded LSP. The LSP preparation was subjected to long-term storage at 4°C in distilled water prior to Bio-Gel A-1.5m chromatography, and denaturation presumably occurred during this time. This denaturation appears to be irreversible since native LSP was not obtained upon re-chromatography of the void volume material. The only non-LSP contaminants were minor peaks eluting at or near the column volume, which were not characterized further.

Only native LSP was used in subsequent structural studies. The calibration curve for the determination of the R_s of native LSP on the Bio-Gel A-1.5m column is shown as an inset in Fig. 3. The R_s of LSP was determined to be 68.3 Å by linear regression.

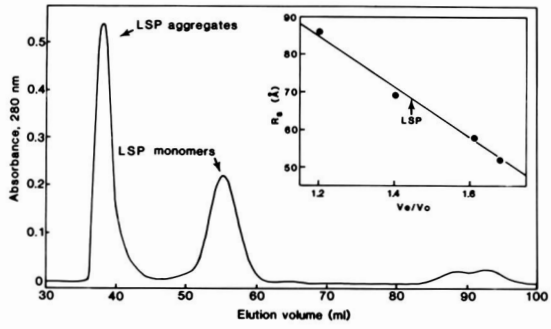

Fig. 3. Bio-Gel A-1.5m gel filtration of the SP1-free region (210-390 ml elution volume) from the LSP peak of the QAE-cellulose column shown in Fig. 2. Both the void volume peak (denatured) and the peak at V_e/V_o = 1.4 (native) consist exclusively of LSP as judged by disc and SDS-PAGE. Inset is the calibration curve used to estimate the R_s of LSP on the Bio-Gel A-1.5m column. The standards used were bovine thyroglobulin (86 Å), β-galactosidase (69 Å), ferritin (57.9 Å) and catalase (52 Å). Linear regression yields a value of 68.3 Å for the R_s of LSP.

Disc PAGE

LSP was subjected to electrophoresis in 4% disc gels (8). Densitometry of the Coomassie Blue-stained gel indicates the presence of a single component (Fig. 4A).

SDS-PAGE

LSP was denatured in SDS and subjected to SDS-PAGE. Two bands were obtained differing in M_r by less than 5% (Fig. 4B). Based on a standard curve, the M_r of the LSP subunits were estimated at 84,400 and 88,100, respectively. Densitomery of the two bands indicated a ratio of the heavier band to the lighter band of approximately 1.4:1. The same gel pattern was obtained whether or not the sample was treated with 2-mercaptoethanol, indicating the absence of intermolecular disulfide bonds.

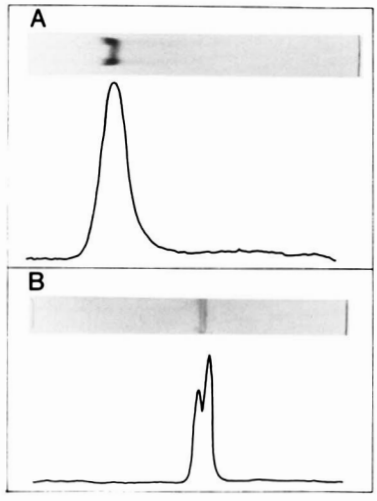

Fig. 4. A. 5% disc gel of purified LSP. B. 7% SDS-PAGE of purified LSP. Both gels were stained with Coomassie Brilliant Blue R-250. The gels were scanned in a densitometer.

Analytical Sedimentation in Isokinetic Sucrose Gradients

The $S_{20,w}$ value of LSP was determined in isokinetic sucrose gradients by comparison against a series of standards. The calibration curve is shown in Fig. 5. The $S_{20,w}$ of LSP was determined to be 17.8 by linear regression.

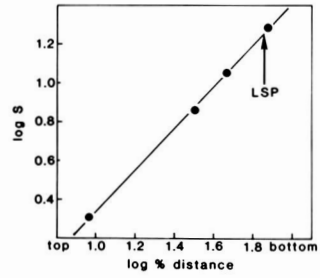

Fig. 5. Calibration curve for estimating the sedimentation coefficient of LSP. Isokinetic sucrose gradients were centrifuged in the SW 50.1 rotor at 34,500 rpm for 16 h at 6°C. The standards used were bovine thyroglobulin (19.3S), β-galactosidase (16.1S), catalase (11.3S) and aldolase (7.35S). Linear regression yields a value of 17.8S for LSP.

Electron Microscopy

Negatively-stained LSP appears as almost-square rectangular particles in electron micrographs (Fig. 6). Measurements of 50 particles from an electron micrograph yielded a mean width of 121.3 ± 14.7 Å and a mean length of 134.0 ± 16.0 Å.

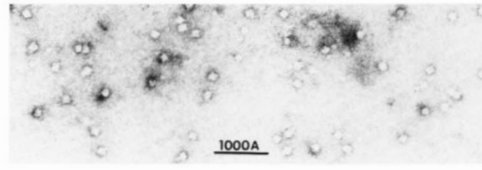

Fig. 6. Electron micrograph of negatively-stained LSP monomer. LSP was deposited on a carbon film and stained with uranyl acetate. 90,000 x magnification.

Amino Acid Analysis

The amino acid composition of LSP is listed in Table 1. The most noteworthy feature of the composition is the extraordinarily high tyrosine content (16.33 mol %). Also noteworthy are the virtual lack of cysteine and the low methionine content. The lack of cysteine was corroborated by the inability to incorporate radiolabeled N-ethylmaleimide into LSP (not shown). Other noteworthy features are the relatively high phenylalanine and tryptophan contents.

TABLE 1. Amino acid composition of LSP[a]

Amino Acid	mol %[b]
Asp	13.03
Thr	4.26
Ser	2.69
Glu	9.46
Pro	6.40
Gly	5.65
Ala	5.08
Cys/2	0.03
Val	5.78
Met	0.28
Ile	3.80
Leu	6.63
Tyr	16.33
Phe	4.90
His	1.00
Lys	4.43
Arg	6.37
Trp[c]	3.89

[a]Mean of two 24 h hydrolysates.
[b]Mol % = (molar fraction of each amino acid) x 100
[c]Trp was calculated from the tyr content and the tyr/trp molar ratio determined from the absorption spectrum (13).

Physical Properties of LSP

The physical properties determined as described above are listed in Table II, along with the values calculated for $\bar{V}$, M_r of the native molecule, and f/fo. A comparison of the M_r of the native particle with the M_r of the two subunits suggests that native LSP is a hexamer of subunits averaging 85,450 M_r. The hexameric subunit size, predicted from the native M_r of LSP is 84,650, in close agreement with the mean M_r of 86,250 for the two subunits obtained by SDS-PAGE.

TABLE II. Physical properties of LSP

Stokes' radius (Å)	$S_{20,w}$	$\bar{V}$	Native M_r (x 10^{-3})	f/f_o	Subunit M_r (x 10^{-3})	A_{280}
68.3[a]	17.8[b]	0.723[c]	507.9[d]	1.298[e]	84.4[f] 88.1	17.9[g]

[a]Mean of three replicates by gel permeation chromatography on Bio-Gel A-1.5m.
[b]Mean of three replicates in isokinetic sucrose gradients (10).
[c]Calculated from the amino acid composition in Table I (19).
[d]Calculated from Stokes' radius, $S_{20,w}$ and $\bar{V}$ (20).
[e]Calculated from Stokes' radius, M_r and $\bar{V}$ (21).
[f]Mean of five replicates by SDS-PAGE (9).
[g]Determined at pH 7.0 from a solution prepared gravimetrically. Corrected to 10 mg/ml.

Crossed Immunoelectrophoresis of B. germanica LSP

Additional evidence for the hexameric structure of LSP was obtained by crossed immunoelectrophoresis of B. germanica larval serum as shown in Fig. 7. The LSP antigen produced 7 fused peaks in the second dimension which were quantitated as in conventional rocket electrophoresis. Assuming that the peaks represent an isozyme-like series of hexamers composed of varying proportions of two subunits, fast (f) and slow (s), the appropriate values of f and s that fit the binomial expansion $(f + s)^6$ drawn from the peaks in Fig. 7 are f = 5/9 and s = 4/9. The ratio of major subunit to minor subunit is therefore 1.25:1 for the putative B. germanica subunits. Electrophoresis of B. orientalis LSP on horizontal agarose gel electrophoresis similar to the first dimension used for B. germanica yielded a similar separation into isozyme-like bands, but the resolution was not sufficient to give discrete peaks in the second dimension (not shown).

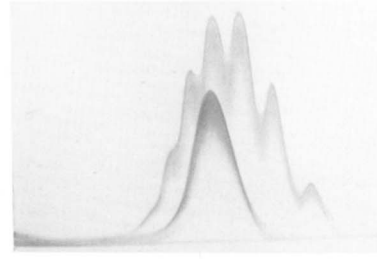

Fig. 7. Crossed immunoelectrophoresis of B. germanica larval hemolymph. First-dimension electrophoresis was carried out at pH 5.5 for 8 h at 4°C. Twenty-fold diluted antiserum against B. germanica larval serum was present in the second-dimension agarose (pH 8.3). The slide was washed, dried and stained with Coomassie Blue R-250. The antiserum contained antibodies reactive with both LSP and SP1. The upper precipitin pattern consists of the 7 LSP isomorphs with the leftmost recognizable as a shoulder rather than a distinct peak. The lower precipitin pattern is formed by SP1.