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Quaternary Structure and Arrangement of Subunits in Hemocyanin from the Scorpion *Leirus quinquestriatus*†

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ABSTRACT: A study of the quaternary structure of hemocyanin from the scorpion *Leirus quinquestriatus* was carried out. The amino acid composition and the copper content were determined. For the native molecule, a sedimentation coefficient of 36.2 S and a molecular weight of 1.74×10^6 were found. In sodium dodecyl sulfate–polyacrylamide gel electrophoresis, five bands were obtained. Most of the material migrated with a mobility corresponding to a molecular weight of 74×10^3. Dissociation of the molecule at alkaline or acidic pH yielded a 5S component corresponding to individual polypeptide chains. At the transition pH values, intermediate products of dissociation with sedimentation coefficients of 15.4 and 23.9 S, attributed to quarter- and half-molecules respectively, were obtained. Electron micrographs of the native molecule showed two structures, a rectangle and two rhombi diametrically connected together at the corners of the obtuse angle. We propose a model for the molecule consisting of 24 spherical subunits, each subunit representing a 5S particle. Every six subunits are organized in an octahedral arrangement representing a 16S unit, and the 36S molecule is a square-planar structure composed of four 16S units. The square arrangement of the 16S units is consistent with an analysis of the hydrodynamic data by the Kirkwood treatment, and projections of the model agree with particle profiles observed in the electron micrographs. Implications of the proposed structure on the observed heterogeneity of monomeric 5S subunits are discussed.

Hemocyanin is a copper containing respiratory protein found in the hemolymph of molluscs and arthropods. Since the early work of Svedberg and co-workers (Eriksson-Quensel & Svedberg, 1936), it has been realized that the hemocyanins can exist in a number of discrete states of aggregation—100, 60, 20, and 11 S for molluscan and 60, 36, 24, and 16 S for arthropod hemocyanins (Van Holde & Van Bruggen, 1971). Electron microscopy (Mellema & Klug, 1972; Siezen & Van Bruggen, 1974; Van Breemen et al., 1977) has provided what is now a generally accepted model for the assembly of molluscan hemocyanins. Much progress has also been achieved in the elucidation of the structure of hemocyanins from arthropod origin (Van Bruggen, 1968; Van Holde & Van Bruggen, 1971; Lontie & Witters, 1973; Antonini & Chiancone, 1977). Here, ultracentrifugation and electron microscopy have established the 16S aggregation state as the basic structural building block. Electron microscopy has provided, in addition, information on the spatial arrangement of the 16S units in the oligomeric structures. However, the structure of the 16S unit, by itself, is still unresolved. Although it has been established beyond doubt that the 16S unit is a hexamer of 5S subunits—polypeptide chains of about 75 000 daltons that constitute the ultimate dissociation product of all arthropod hemocyanins—the assembly of these subunits remains controversial. No less than five different models have been proposed (Andrews & Jeffrey, 1976).

Hemocyanin from the scorpion *Leirus quinquestriatus* is currently under study in our laboratory. Some of its spectral and functional properties have already been described (Klarman et al., 1977; Klarman & Daniel, 1977). The purpose of the present report is to present the results of a structural study carried out on this arthropod hemocyanin.

Materials and Methods

Preparation of Hemocyanin. Hemocyanin from the scorpion *L. quinquestriatus* was prepared as previously described (Klarman et al., 1977). Unless otherwise stated, hemocyanin solutions were made in buffers devoid of Ca^{2+} and Mg^{2+}. Hemocyanin concentrations were obtained from absorbance measurements at 280 nm. The extinction coefficient at this wavelength was determined by measuring the number of Rayleigh fringes produced in a synthetic boundary experiment in the ultracentrifuge by a sample of known absorbance and taking a value 4.08 fringes/(mg/mL) determined for hemocyanin from *Busycon* (Quitter et al., 1978).

Amino Acid and Copper Analysis. Protein samples were hydrolyzed with constant boiling HCl in sealed and evacuated Pyrex ampules at 110 °C for 24, 48, and 72 h. The amino acid composition was determined by ion-exchange chromatography in a Beckman Unichrom amino acid analyzer (Spackman et al., 1958). Tryptophanyl to tyrosyl ratio was determined spectrophotometrically by the method of Edelhoch (1967). Half-cystine and cysteine were determined as cysteic acid according to Hirs (1956). Copper content was determined by atomic absorption using a Varian Model AA5 spectrophotometer.

Polyacrylamide Gel Electrophoresis. Sodium dodecyl sulfate gel electrophoresis was performed in vertical gel slabs (7.5% acrylamide, 0.2% bis(acryloylamido)methane, 0.035% *N,N,N′,N′*-tetramethylenediamine, 0.03% ammonium peroxodisulfate), using a Hoefer Scientific Instruments apparatus, Model SE 500. Samples for electrophoresis were incubated in Tris-HCl buffer containing 2% sodium dodecyl sulfate and 1% 2-mercaptoethanol for 2 min at 100 °C. Tris–glycine

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Table I: Amino Acid Composition of *L. quinquestriatus* Hemocyanin[a]

amino acid	wt %	amino acid residue/75 000 mol wt
Lys	5.9	34.6
His	6.2	34.2
Arg	8.5	40.9
Asp	11.5	75.0
Thr	4.6	34.2
Ser	4.2	36.2
Glu	12.6	73.3
Pro	4.1	31.7
Gly	3.0	39.5
Ala	3.1	32.7
$^1/_2$-cystine	0.7	5.1
Val	5.6	42.4
Met	2.3	13.2
Ile	5.6	37.2
Leu	8.2	54.4
Tyr	5.7	26.2
Phe	6.7	34.2
Trp	1.5	6.0

[a] The data for threonine, serine, and tyrosine were extrapolated to zero time of hydrolysis and those for valine and isoleucine to infinite time of hydrolysis. Tryptophan was determined spectrophotometrically. Half-cystine was determined as cysteic acid.

buffer containing 0.1% sodium dodecyl sulfate, pH 8.6, was used as the electrode buffer. A constant voltage (150 V) was applied for 2 h. Gels were calibrated with protein markers of known molecular weight according to the standard procedure (Weber & Osborn, 1969).

Ultracentrifugation was performed with a Beckman Model E ultracentrifuge. Schlieren optics were used to locate the position of the boundary in sedimentation velocity runs and interference optics were used to determine the concentration distribution in sedimentation equilibrium experiments. Interference patterns were read using a Nikon Model 6 C microcomparator. Calculation of the molecular weight was made using the equation $M = [2RT/(1 - \bar{v}\rho)\omega^2]\mathrm{d} \ln c/\mathrm{d}r^2$, where R is the gas constant, T is the absolute temperature, ω is the angular velocity, $\bar{v}$ is the partial specific volume, ρ is the density of the solution, c is the protein concentration, and r is the distance from the axis of rotation. A value $\bar{v} = 0.727$ mL/g (Quitter et al., 1978) was used.

Electron microscopy was carried out using the technique of negative staining (1% uranyl acetate). Observations were made with a Jeol-Jem-100B electron microscope.

Results

Characterization of Native Hemocyanin. Purified scorpion hemocyanin sediments as a single sharp boundary in the ultracentrifuge. The sedimentation coefficient extrapolated to zero concentration is $s^0_{20,w} = 36.2$ S. The molecular weight of the 36S molecule was determined by sedimentation equilibrium. A meniscus depletion experiment (Yphantis, 1964) in 0.1 M Tris-HCl buffer, pH 7.6, containing 10^{-2} M Ca^{2+} yielded a linear ln c vs. r^2 plot. From the slope, a molecular weight of 1.74×10^6 was calculated.

The amino acid composition of *L. quinquestriatus* hemocyanin is given in Table I. A copper content of 0.176% was determined, corresponding to 2 atoms of copper per 72 700 daltons. At 280 nm, in 0.1 M Tris-HCl buffer, pH 7.6, a value $E_{1cm}^{1\%} = 14.7$ was found. For oxyhemocyanin, the ratio of the absorbance at 340 to that at 280 nm is 1:4.0.

Dependence of the Sedimentation Coefficient on pH. Figure 1 presents a plot of $s_{20,w}$ vs. pH for *L. quinquestriatus* hemocyanin in solutions devoid of Ca^{2+} and Mg^{2+}. It is seen

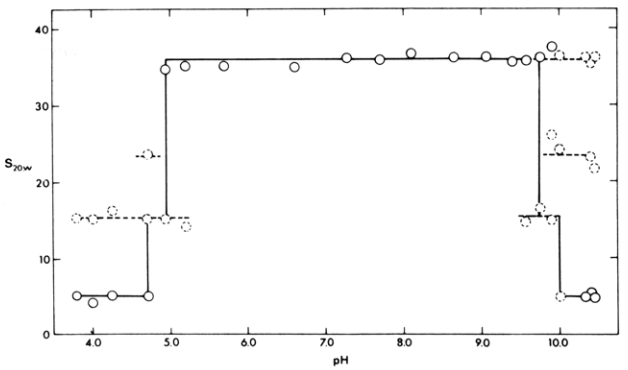


FIGURE 1: The effect of pH on the sedimentation coefficient of *L. quinquestriatus* hemocyanin. Protein concentration was about 1 mg/mL. Buffers (Ca^{2+},Mg^{2+}-free) were used in the pH ranges shown: 0.1 M acetate (3.8–6.0), 0.1 M cacodylate (6.0–7.0), 0.1 M Tris-HCl (7.2–8.5), and 0.1 M glycine–NaOH (8.5–10.5). Full circles indicate that the corresponding species represents 90% or more of the sedimenting material.

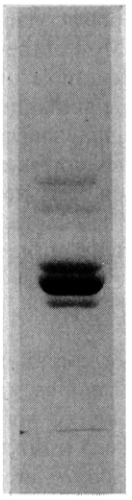


FIGURE 2: Sodium dodecyl sulfate–polyacrylamide gel electrophoresis of *L. quinquestriatus* hemocyanin. Electrophoresis is from top to bottom.

that the native structure is stable over a wide range of pH. At pH values near 9.5 on the alkaline side, and near 5.2 on the acidic side, dissociation of the 36S particle begins and two components appear with sedimentation coefficients ~24 and ~16 S. At pH 10, and pH 4.8, a species with sedimentation coefficient ~5 S appears. As the pH is further increased, or decreased, the 5S species becomes the preponderant one (>90% of the sedimenting material).

Polyacrylamide Gel Electrophoresis. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis in the presence of 2-mercaptoethanol yielded five bands (Figure 2). The bulk of the material migrated in three bands with mobilities corresponding to molecular weights of 70, 74 (major band), and 82×10^3. Two faint bands of lower mobilities were consistently observed. The electrophoretic pattern was unchanged when mercaptoethanol was omitted.

Electron Microscopy. Figure 3 is an electron micrograph of *L. quinquestriatus* hemocyanin. The molecule is seen in a variety of projections, out of which two have particularly well-defined delineations. In one, the molecule is seen as a square (245 × 240 Å). Parallel to the one side of the square a gap about 20 Å wide may be discerned. In the second, the molecule has the shape of two identical rhombi (angles 77° and 104°, side 113 Å) diametrically connected together at the corners of the obtuse angles.

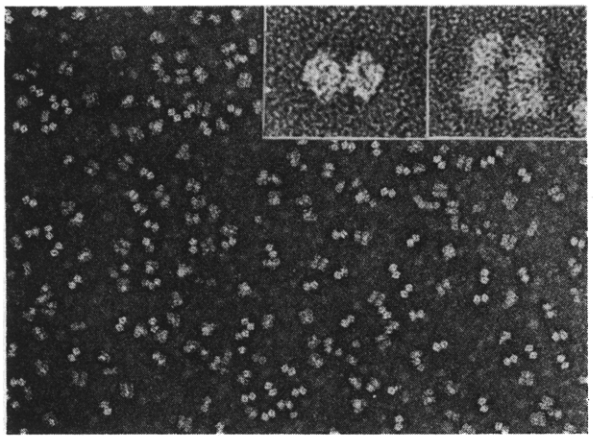


FIGURE 3: Electron micrograph (×73 800) of negatively stained *L. quinquestriatus* hemocyanin. Inset: Magnified views of the square and rhombus-pair projections seen in the micrograph.

A marked difference between the two projections can be clearly seen in the contrast they offer against the background. The contrast of the rhombus pairs is consistently more conspicuous than that of the squares.

Discussion

At pH 7.0, the physiological pH, scorpion hemocyanin from *L. quinquestriatus* exists exclusively as 36S molecules. The molecular weight of 1.74×10^6 determined here is close to the value 1.70×10^6 determined for 39S *Callianassa californiensis* (Roxby et al., 1974) and that of 1.69×10^6 found for the 36S component of *Limulus polyphemus* (Johnson & Yphantis, 1978) hemocyanins. *L. quinquestriatus* hemocyanin retains its native structure over a wide range of pH. Outside the stability range, the molecule dissociates into subunits of sedimentation coefficient about 5 S. Around the transition pHs, intermediate species with sedimentation coefficients about 24 and 16 S are observed. The fact that the latter species are found always as minor components in a mixture precluded a direct determination of their molecular weights. However, there exists little doubt, if any, that the 24 and 16S components represent half and quarter 36S molecules (Van Holde & Van Bruggen, 1971). The 5S component represents isolated polypeptide chains. The pattern observed in sodium dodecyl sulfate gel electrophoresis clearly brings out the fact that the 5S species is not homogeneous. The heterogeneous character of the constituent polypeptide chains in arthropod hemocyanin has been amply demonstrated in many laboratories (references cited in Table III below). For scorpion hemocyanin, six (Lamy et al., 1974, 1977a,b) and five (Sugita & Sekiguchi, 1975) kinds of subunits were found.

The problem of the structure of scorpion hemocyanin may be approached by first examining the way in which the four 16S units are associated together to give the 36S molecule. To this end, we carried out a Kirkwood analysis of the hydrodynamic data, as applied by Van Holde (1975) to protein multisubunit structures. Table II presents calculated values of the ratio $s_4°:s_1°$; for four spherical particles in a linear, square planar, or tetrahedral arrangement, where $s_4°$ and $s_1°$ stand for the intrinsic sedimentation coefficients of the tetramer and monomer, respectively. A comparison of the experimentally determined ratio of the sedimentation coefficients with the values expected for the three tetrameric models shows that the structure of the native 36S molecule may best be approximated by a square planar arrangement of 16S units.

The choice of a model for the arrangement of the 5S subunits within the 36S molecule needs some assumptions to be made regarding the way in which these subunits are associated within the 16S unit. Over the years, a number of different models for the structure of the 16S component of arthropod hemocyanin have been proposed: 12 units in a truncated tetrahedron (Levin, 1963a), 8 units in a cube (Van Bruggen et al., 1963), 6 units in a trigonal antiprism (Wibo, 1966), 12 units in a hexagonal prism (Di Giamberardino & De Haen, 1965), 12 units arranged in pairs in a distorted octahedron (Lontie & Witters, 1973), 6 units arranged into two triangles that lie in parallel planes rotated by about 25° around the common threefold symmetry axis (Schepman, 1975, quoted by Antonini & Chiancone, 1977), and 6 elongated units in a cyclic structure (Andrews & Jeffrey, 1976). The establishment of the 16S unit as a hexamer of 5S subunits (Van Holde & Van Bruggen, 1971) evidently rules out the acceptability of some of the early models. The opinion has

Table II: Relationships between the Sedimentation Coefficients of the Different Aggregation States in *L. quinquestriatus* Hemocyanin

$s_{20,w}$	n^c	$(s_n/s_1)_{obsd}$	$(s_n°/s_1°)_{calcd}{}^d$ linear	square planar	tetrahedral
15.4[a]	1				
23.9[a]	2	1.55	1.50		
36.2[b]	4	2.35	2.08	2.353	2.500

[a] Average sedimentation coefficients obtained at a total concentration of 1 mg/mL protein (Figure 1). [b] Extrapolated value at zero concentration. [c] State of aggregation of oligomer relative to 16S component taken as monomer. [d] Calculated according to the relation (Van Holde, 1975) $s_n°/s_1° = 1 + (1/n)\Sigma_i\Sigma_j(1/\alpha_{ij})$, where $s_n°$ is the intrinsic sedimentation coefficient of an assembly of n spherical subunits of radius R and intrinsic sedimentation coefficient $s_1°$ and $\alpha_{ij} = R_{ij}/R$, R_{ij} being the center-to-center distance between subunits i and j.

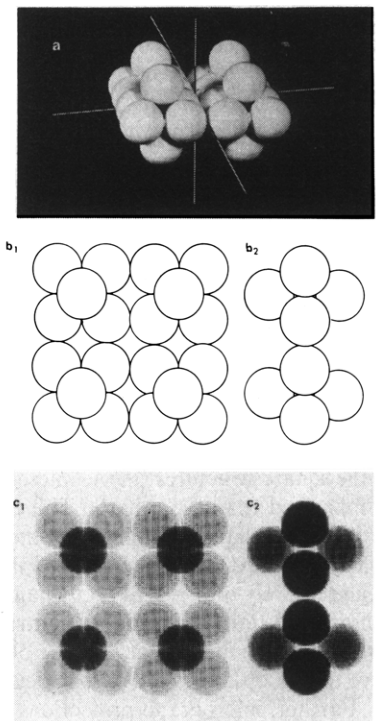


FIGURE 4: Proposed model for scorpion hemocyanin. (a) Photograph of a model made up of 24 identical spheres occupying the corners of four regular octahedra situated in a square planar arrangement. (b_1, b_2) Views of the model along two axes, one (front view) normal to and one (end-on view) contained in the plane formed by the centers of the four octahedra. (c_1, c_2) Projections of the density on planes normal to the two axes, drawn from computed analogues.

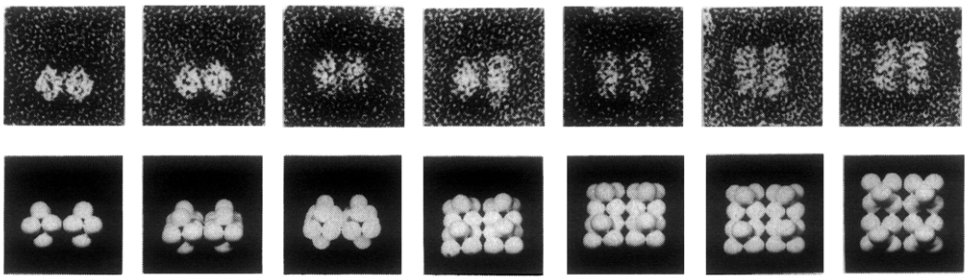


FIGURE 5: (Upper row) Magnified projections selected from the electron micrograph of scorpion hemocyanin (Figure 3), showing the transition from the rhombus pair (left) to the square (right) projections. (Lower row) Photographs of the model shown in Figure 4 in orientations intermediate between the ones corresponding to the end-on (left) and the front (right) views.

been expressed that, whatever the exact details of the correct model turn out to be, it should have some kind of prismatic structure (Van Holde & Van Bruggen, 1971).

Figure 4 presents a model for the arrangement of subunits in *L. quinquestriatus* hemocyanin, based on the evidence from electron microscopy. The model is composed of 24 spherical subunits, each representing a 5S particle, organized in four groups, the 16S units. The six subunits of each group occupy the corners of a regular octahedron. The four 16S units are arranged in a square planar arrangement to give the 36S molecule. The front and end-on views of the model bring forward the characteristic features seen in the electron micrographs. The front view clearly shows the square structure, and the end-on view has the appearance of a pair of rhombi with angles of 108° and 72°. Computed projections of the model along the two viewing directions (Figure 4) make it possible to account for additional features observed in the electron micrographs. The front projection shows two perpendicular strips of low-density running parallel to, and equidistant from, each pair of opposite sides corresponding to the gaps seen in the square structures (in the micrographs, one strip is more emphasized than the other). The end-on projection reveals a larger overlap of subunits than the front one, indicating that a larger fraction of the path of the electron beam goes through relatively transparent protein and a smaller fraction through opaque stain. The higher contrast against background observed in the end-on view of the electron micrographs, relative to that observed in the front view, may thus be rationalized. Further, micrograph projections other than the square and rhombus pair can be associated, in the light of the proposed model, with views of the molecule other than the front and end-on views. This interpretation is favored by a comparison (Figure 5) of some projections appearing in the electron micrograph of Figure 3 with photographs of the model in particular orientations, intermediate between the ones considered in Figure 4.

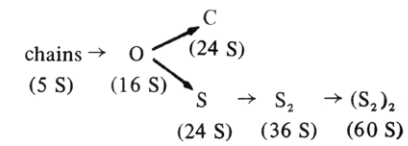
It has been indicated before that the 16S unit is universally accepted as the structural building block of arthropod hemocyanins. Consequently, an important criterion for the acceptability of a model for the 16S unit is its ability to account for the structure of the diverse states of aggregation of the molecule found in nature. The fact that the octahedral model can account for the square, rectangular, and hexagonal projections seen in electron micrgraphs of 16S arthropod hemocyanin (Figure 12 in Van Holde & Van Bruggen, 1971) is demonstrated in Figure 6. Also, it has long been realized (Levin, 1963b; Van Holde & Van Bruggen, 1971) that a satisfactory model for the 24S crustacean hemocyanin is provided by a combination of two basic 16S units rotated 90° to each other. Figure 6 shows that such a combination of two octahedral units is expected to yield hexagon–rectangle projections like the ones actually observed in electron micrographs of 24S crustacean hemocyanin (Figure 13 in Van Holde & Van Bruggen, 1971). The adequacy of a square planar arrangement of four octahedral 16S units to describe the structure of 36S scorpion hemocyanin has been demonstrated in the present study. Finally, an octamer of the 16S unit, obtained by stacking two 36S tetrameric structures of the type proposed for scorpion hemocyanin is expected to yield projections of the type seen in electron micrographs of 60S arthropod hemocyanin (Figure 15 in Van Holde & Van Bruggen, 1971; Schutter et al., 1977).

The octahedral structure proposed here for the 16S unit is similar to the model of Wibo (1966). Like the Schepman model (Schepman, 1975), it assumes the 16S unit to consist of six subunits arranged in two parallel layers that have a common threefold axis, the difference being confined to the orientation of the two layers relative to each other. However, the model proposed by us for the 36S scorpion hemocyanin is not a combination of two 24S structures of the type found in crustacean hemocyanin as has often been suggested (Van Holde & Van Bruggen, 1971; Schutter et al., 1977). The differences in the mode of assembly of the 16S units might explain why 24S crustacean hemocyanin shows little tendency to self-aggregate beyond the dimeric stage.[1]

The structures of 36 and 60S hemocyanins have often been described as a dimer and tetramer (dimeric dimer) of the 24S structure. Such a description tacitly assumes what may be termed a "linear" development of structure

chains	→	O	→	C	→	C_2	→	$(C_2)_2$
(5 S)		(16 S)		(24 S)		(36 S)		(60 S)

where C stands for a crustacean-type dimer composed of two octahedral 16S units, O, rotated 90° with respect to each other, C_2 is a dimer of the 24S structure, and $(C_2)_2$ is a dimer of the 36S structure. That two structures are possible for the 24S dimer (Figure 6)—a dimer of the crustacean type, C, and a dimer of the type involved in scorpion hemocyanin, S—suggests that the development of higher structures in arthropod hemocyanin follows a "bifurcated" path.



[1] Crustacean hemocyanin from *Callianassa californiensis* can exist in a stable state of aggregation characterized by $s^0_{20,w}$ = 38.8 S (Roxby et al., 1974). However, this exception to the rule is more apparent than real since (a) the 39S molecule is stable only in the presence of Mg^{2+} and, unlike scorpion hemocyanin, undergoes dissociation in solutions devoid of this ion (Roxby et al., 1974; Van Holde et al., 1977) and (b) electron micrographs of the 39S molecule seem to indicate a tetrahedral arrangement of 16S units, which is different from the square planar arrangement characteristic of scorpion hemocyanin (Van Holde et al., 1977).

Table III: Heterogeneity of Constituent Polypeptide Chains in Arthropod Hemocyanins

max state of aggregation of the molecule	no. of types of equiv positions[a] in model	no. of types of polypeptide chains found	hemocyanin	reference
16 S	1	3	*Panulirus interruptus*	Kuiper et al., 1975; Van den Berg et al., 1977
		3	*Jassus edwardsii*	Robinson & Ellerton, 1977
24 S	3	2	*Cancer magister*	Loehr & Mason, 1973; Carpenter & Van Holde, 1973
		3	*Cherax destructor*	Murray & Jeffrey, 1974
		3	*Cupiennius salei*	Markl et al., 1976
		2	*Callinectes sapidus*	Hamlin & Fish, 1977
36 S	4	6	*Androctonus australis*	Lamy et al., 1974
		5	*Heterometrus* sp.	Sugita & Sekiguchi, 1975
		6	*Eurypelma californicum*	Schneider et al., 1977
		5	*Leirus quinquestriatus*	this work
60 S	5	5	*Tachypleus tridentatus*	Sugita & Sekiguchi, 1975
		>5	*Limulus polyphemus*	Sullivan et al., 1976

[a] The number of types of equivalent positions may be readily found by examining the various models for hemocyanin structure presented in Figures 4 and 6. The 16S structure has six equivalent positions. In a 24S dimer of both the S and C types (Figure 6) the six positions of each 16S monomer are "split" into three different classes: two positions occupied by subunits which make connections with the other 16S unit, their nearest neighbors (two positions), and their next to nearest neighbors (two positions). Formation of the 36 and 60S structures introduces further splitting, producing one additional type at each step.

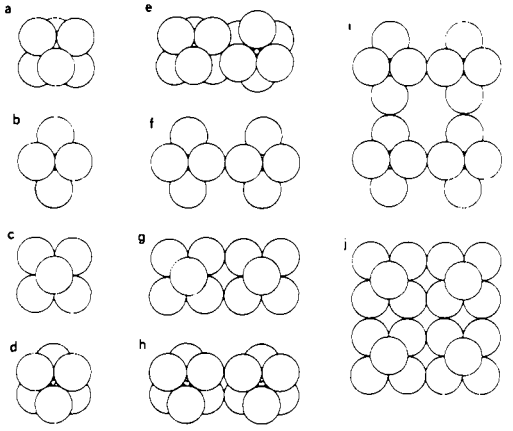


FIGURE 6: Projections of different combinations of the octahedral basic unit, representing 16, 24, and 60S aggregation states of arthropod hemocyanins. (a–d) Four projections of an octahedral unit, showing rhombus, square, rectangular, and hexagonal profiles. (e) Projection of a combination of two octahedral units rotated 90° to each other (C-type dimer). (f–h) Projections of a combination of two octahedral units in the same orientation (S-type dimer). (i, j) Two projections of an octamer of the octahedral unit composed of two tetramers of the type proposed for scorpion hemocyanin (Figure 4), stacked in an eclipsed arrangement.

It is interesting to note that this scheme places crustacea on one branch and merostomata and arachnida on another, in accord with their accepted phylogenetic relationship (Sharov, 1966; Ghiretti-Magaldi et al., 1975; Ghiretti-Magaldi & Tamino, 1977).

A remaining question concerns the heterogeneity of the 5S subunits. Sullivan et al. (1974, 1976) have attributed a functional role for the polypeptide chain heterogeneity in *L. polyphemus* hemocyanin. That the differences in the 5S subunits have a structural significance as well is evidenced by reconstitution experiments of arthropod hemocyanins which show that most, if not all, components are needed for reassembly (Schutter et al., 1977; Lamy et al., 1977a,b). Let us examine this problem in connection with the models proposed for arthropod hemocyanin structures. Table III presents a listing of the types of environmentally equivalent positions in the 16, 24, 36, and 60S structures and a corresponding enumeration of the types of subunits actually found in arthropod hemocyanins. If one assumes that identical subunits occupy structurally equivalent positions (Klotz et al., 1970; Mathews & Bernhard, 1973), then the number of types of the latter gives the minimal number of subunit types structurally required. Inspection of Table III shows that, but for one or two exceptions, the observed number of subunit types is equal to or greater than the number of types of structurally equivalent positions. Thus, the heterogeneity of the 5S subunits can be partly accounted for. The fact that the number of types of 5S subunits generally exceeds the number of types of equivalent positions suggests deviations of actual structures of hemocyanins from idealized models[2] or the existence of subunit heterogeneity not related to structural requirements of the kind discussed here. The latter interpretation is supported by the report (Van den Berg et al., 1977) that *Panulirus interruptus* hemocyanin shows heterogeneity on the level of the native 16S molecule. The finding (Roxby et al., 1974) that hemocyanin from *Callianassa californiensis* exists in the hemolymph in two forms, a 39S form and a 17S form incompetent to associate to the 39S form, represents in fact an extreme case of heterogeneity. It is clear that a test of the ideas advanced here would be much more conclusive if carried out on hemocyanin preparations which have been demonstrated to be truly homogeneous in the native aggregation state. The possibility of carrying out reassociation studies with purified polypeptide chains (Schutter et al., 1977; Lamy et al., 1977a,b) is certainly a way to circumvent the problem. As such, annealing experiments seem promising in the effort to get a better understanding of the role of multiplicity of subunits in the determination of structure in arthropod hemocyanins.

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[2] We have in mind deviations like the one introduced in the tetrahedral model by the nonidentical subunits of human hemoglobin. Attention has already been drawn to the fact that two low-density strips of equal intensity are expected in the front view projection of the model of scorpion hemocyanin, whereas in the electron micrographs of the molecule one strip is much more emphasized than the other. This evidence suggests that a 16S unit is not symmetrically situated with respect to its two adjacent neighbors in the square planar arrangement.

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