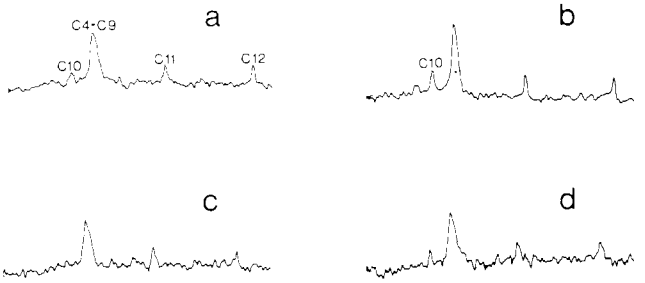

FIGURE 4: (a) Region, between 8.9 and 37.6 ppm, of the natural-abundance ^{13}C NMR spectrum of 0.0079 M 1-dodecanal in 0.1 M potassium phosphate (pH 6.9) and 1% w/v Tween-20 with luciferase added to bring [S]/[E] = 78. Spectral parameters: pulse width = 15 μs; pulse delay = 0.5 s; number of accumulations = 35000; number of data points = 8192; line broadening = 2.0 Hz. (b) Solution (a) after thermal denaturation; number of accumulations = 35000. (c) 1-Dodecanal/luciferase = 42; number of accumulations = 43000; (d) Solution (c) after thermal denaturation; number of accumulations = 60000.

these spectra is poor, the behavior of C(10) is interesting enough to point out a possible selective interaction at the ω-2 position, i.e., very remote from the active center of the molecule.

In order to confirm a selective interaction near the methyl terminal end of the substrate, we are currently elongating the odd-carbon ^{13}C-enriched-dodecanal chain by one carbon. This will yield a 13-carbon straight-chain aldehyde labeled at all of the even carbons. Together with further work planned we hope to completely delineate the interaction and rate of reorientation of long-chain aldehydes in the enzyme active site.

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Hemocyanin from the Australian Freshwater Crayfish *Cherax destructor*. Electron Microscopy of Native and Reassembled Molecules†

P. D. Jeffrey

ABSTRACT: Examination and measurement of electron micrographs of negatively stained hemocyanin molecules from *Cherax destructor* show that the predominant aggregated forms, the 16S and 24S components, are typical structures for arthropod hexamers and dodecamers, respectively. In *Cherax* hemocyanin the hexamers are formed from the monomeric ($M_r \simeq 75000$) subunits, M_1 and M_2, while the dodecamers contain in addition a dimeric ($M_r \simeq 150000$) subunit, M_3'. Studies of the composition of solutions of the subunits M_1 and M_2 to which calcium ions have been added at pH 7.8 show that, under these conditions, reassembly occurs to particles indistinguishable from native hexamers. It is noteworthy that dodecamers are not seen since this confirms the previous suggestion that incorporation of the dimeric subunit in the assembly process is necessary for their formation. The results obtained from *Cherax* hemocyanin are related to those of previous structural studies of arthropod hemocyanins. In particular, the possible controlling role of certain specific subunits in arthropod hemocyanin oligomers containing more than one kind of subunit is illustrated with a model for the *Cherax* dodecamer, in which the dimeric subunit is shared between the two halves of the molecule.

The hemocyanin molecules observed in the hemolymphs of arthropod species belong to several different size classes. They are usually referred to as the 5S, 16S, 24S, 35S, and 60S components and are taken to consist of the equivalent of 1, 6, 12, 24, and 48 monomers, respectively, the monomer being of molecular weight about 75000 and containing two copper atoms. The distribution of the oligomers depends on the biological species. Thus, the higher molecular weight components tend to be emphasized in the hemolymphs of spiders and scorpions, both the 24S and 16S components in crabs and

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0006-2960/79/0418-2508$01.00/0

Table I: Sedimenting Components of Arthropod Hemocyanins Observed at or near Physiological pH

species	approximate sedimentation coefficient[a] 5 S	16 S	24 S	35 S	60 S	pH	ref
Arachnida							
Dugsiella californica				●		7.2	Markl et al. (1976)
Dugsiella helluo				●		7.2	Markl et al. (1976)
Cupiennius salei		●	●			7.2	Markl et al. (1976)
Androctonus australis				●		7.0	Feytmans et al. (1966)
Buthus occitanus				●		7.0	Feytmans et al. (1966)
Merostomata							
Limulus polyphemus	○	○		○	●	7.6	Erikson-Quensel & Svedberg (1936)
Crustacea							
Callianassa californiensis		○		●		7.65	Roxby et al. (1974)
Astacus fluviatilis		○	●			7.7	Erikson-Quensel & Svedberg (1936)
Cherax destructor	○	●	● 30 S?			7.8	Murray & Jeffrey (1974)
Cancer magister		○	●			7.8	Ellerton et al. (1970)
Cancer pagurus		○	●			7.5	Erikson-Quensel & Svedberg (1936)
Carcinus moenas		○	●			7.4	Erikson-Quensel & Svedberg (1936)
Eriphia spinifrons		●	●			7.1	Di Giamberadino (1967)
Homarus americanus			●			7.8	Morimoto & Kegeles (1971)
Homarus vulgaris		○	●			7.0	van Bruggen et al. (1963)
Jasus edwardsii		●				7.0	Ellerton et al. (1976)
Panulirus interruptus		●				8.0	Kuiper et al. (1975)
Panulirus vulgaris		●				7.0	van Bruggen et al. (1963)
Pandalus borealis		●				7.4	Erikson-Quensel & Svedberg (1936)

[a] The sedimentation coefficients are not $s^0_{20,w}$ values but those commonly used to label aggregates of 1, 6, 12, 24, and 48 subunits of molecular weight 75 000, respectively. Filled circles indicate the major components.

freshwater crayfish, the 24S component in lobsters, and the 16S component in spiny lobsters (Table I). A number of the structures from several species have been examined in the electron microscope [for example, van Holde & van Bruggen (1971), Schepman (1975), Loewe et al. (1977), and Lamy et al. (1977b)], and models have been proposed for some of the aggregated forms.

Cherax destructor hemolymph contains mainly 24S and 16S forms of hemocyanin, and one purpose of the present work was to compare the appearances of the corresponding structures with those of other arthropod hemocyanins which have been studied by electron microscopy. A second aim was to compare with the native structures those formed by reassembly of the *Cherax* monomers M_1 and M_2 in the presence of calcium ions. Here, it was particularly relevant to verify by direct observation that the association does not proceed beyond hexamers under these conditions. Correlation of the information with existing knowledge might then be of use in commenting on the possible mode of assembly of the subunits in arthropod hexamers and dodecamers.

Experimental Section

Preparation of Hemocyanin Components. Serum was prepared from *C. destructor* hemolymph as described previously (Murray & Jeffrey, 1974). All of the hemocyanin components used were isolated from serum pooled from several animals. The 24S and 16S fractions were prepared by electrophoresis of serum on columns of 4% polyacrylamide gel in 0.025 M Tris[1] and 0.1 mM EGTA buffer of pH 7.8, in an LKB 7900 Uniphor apparatus. The monomers M_1 and M_2 were separated in the same manner, but the polyacrylamide concentration was 5% and the buffer was 0.025 M glycine and 0.1 mM EGTA, pH 10.1. Further details may be found in a previous publication (Jeffrey et al., 1978). After separation, all fractions were dialyzed against 0.025 M Tris buffer, pH 7.8, containing 0.01% sodium azide. The samples were sent by air at 0 °C from Australia to the University of Groningen, The Netherlands, where the experiments described in this report were performed.

For the reassembly experiments, 1 mL of a solution of concentration 1 mg/mL of monomer M_1 in the pH 7.8 Tris buffer was dialyzed for 48 h at room temperature vs. 2 250-mL volumes of the same buffer, 0.03 M with respect to calcium. After storage at pH 7.8 it was found that, even in the absence of calcium, monomer M_2 was mostly in the aggregated hexameric form. Reassembly experiments with this monomer were therefore conducted by first dialyzing a 1-mL volume of concentration 1 mg/mL to pH 10 in the glycine buffer to dissociate the protein and then dialyzing back to pH 7.8 in 0.025 M Tris buffer, 0.03 M with respect to calcium, by using the same procedure that was used for M_1.

Polyacrylamide Disc Gel Electrophoresis. The experiments were carried out on 7-cm gels, of acrylamide concentration 4%, at a current of 5 mA per tube. The electrode buffer was 0.025 M Tris, pH 7.8, and protein solutions were made 10% in glycerol before loading. Electrophoresis was allowed to continue for 2 h, after which gels were stained for 0.5 h in 0.05% Coomassie brilliant blue in 25% isopropyl alcohol–10% acetic acid. Excess stain was removed by continuous elution overnight with 7% acetic acid.

Electron Microscopy. The protein solutions at pH 7.8 and of concentration 0.02–0.1 mg/mL were sprayed onto 400-mesh copper grids covered with thin carbon film. Negative staining was carried out with 1% uranyl acetate solution, a drop of which was left in contact with the protein solution on the grid for 0.5–1 min. The procedure was performed with cold (about 4 °C) solutions and freshly picked up carbon films, at a constant relative humidity of 65%. A Philips EM 201 and a JEOL JEM 100B electron microscope were used. Each instrument was provided with an anticontamination device cooled by liquid nitrogen and was operated at 80 kV. The double-condenser lens system was used with apertures of 200 and 100 μm, respectively, and a spot size of 5–10 μm. The objective apertures were 40 and 30μm, respectively. Photographs were taken on 35- or 70-mm film at electron optical mag-

[1] Abbreviations used: Tris, tris(hydroxymethyl)aminomethane; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid.

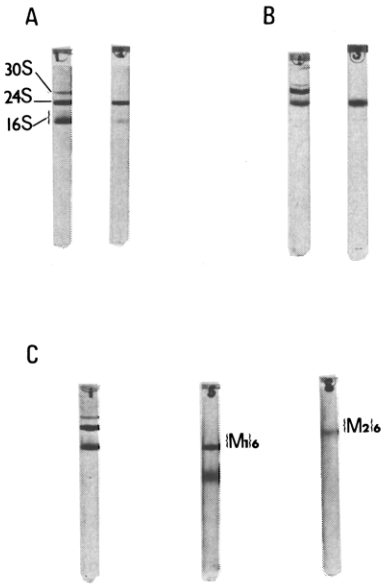

FIGURE 1: Polyacrylamide gel electrophoresis patterns of *Cherax destructor* hemocyanin fractions. Protein components in the fractions may be identified by their relative mobilities with respect to those in the gel on their left which is of a hemolymph sample run at the same time. The buffer was 0.025 M Tris, pH 7.8, and the cathode is at the top. (A) The 24S fraction. (B) The 16S fraction. (C) Reassembly of hexamers from monomers M_1 and M_2 by the addition of calcium ions.

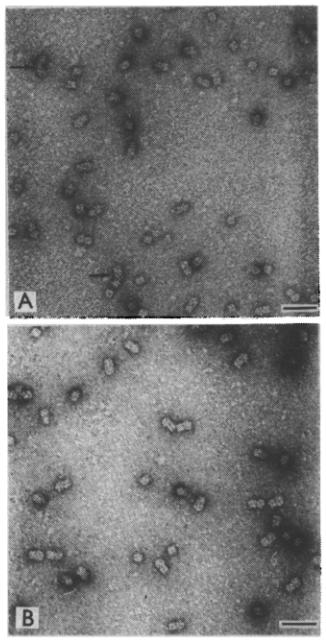

FIGURE 2: Negatively stained molecules in *Cherax destructor* hemolymph and in the 24S fraction. (A) Hemolymph; the arrowed molecules may represent 30S species (see text), and the other profiles are of hexamers and dodecamers. (B) The 24S fraction whose gel electrophoresis pattern is given in Figure 1A. The bars represent 50 nm

nifications between 30 000× and 60 000×. The magnification for photographs which were to be measured was calibrated with photographs, taken at the same time as the specimen, of a replica of a 2160 lines per millimeter diffraction grating. Measurements were made on photographic prints accurately enlarged from the 35-mm negatives, with a Zeiss TGZ 3 Teilchengrössenanalysator.

Results

Gel Electrophoresis. The compositions of the samples as revealed by disc gel electrophoresis are shown in Figure 1. Unfractionated *Cherax* hemocyanin has the usual distribution of components: a sharp band, usually seen as a doublet, of sedimentation coefficient about 30 S, a dense band of 24S material, and a rather broad band representing a series of hexamers (16 S). In this particular sample the hexamers richer in monomer M_1 are more heavily represented than usual. The 24S fraction contains a trace of hexameric material, but the 16S fraction is apparently free of any other component. Dialysis of monomer M_1 against pH 7.8 Tris buffer containing calcium ions produces $(M_1)_6$, as expected, but some protein remains in monomeric form. This is a not uncommon observation, and there is some evidence that the unconverted material is a degradation product of monomer M_1, of molecular weight about 65 000. In the presence of calcium ions the other monomer, M_2, is completely converted into $(M_2)_6$ as judged from its electrophoretic mobility relative to the components of the hemolymph sample.

Electron Microscopy. Electron micrographs of the *Cherax* hemocyanin components, whose electrophoretic patterns are given above, are presented in Figures 2–4. The 24S fraction (Figure 2B) consists mainly of structures showing the combination of a hexagonal to round profile in combination with a square or rectangle, while the 16S fraction (Figure 3A) shows round to hexagonal and square to rectangular profiles. A few such profiles were also seen in the 24S specimens, reflecting, no doubt, the slight contamination with 16S material visible in the gel pattern. The shapes seen in the 24S and 16S specimens account for most of the images visible in whole hemolymph preparations (Figure 2A) as expected from the gel electrophoresis of these three solutions. Structures of the same appearance as those reported here for *Cherax destructor* hemocyanin are also described in previous electron microscope studies of the 24S and 16S hemocyanins of arthropod species. These include the horseshoe crab *Limulus polyphemus* (Schutter et al., 1977), the scorpion *Androctonus australis garzonii* (Lamy et al., 1977b), the tarantula *Dugsiella californica* (Loewe et al., 1977), the spiny lobster *Panulirus interruptus* (Schepman, 1975), and the American lobster *Homarus americanus* (Fernandez-Moran et al., 1966).

Measurements of the largest diameter of the hexagons seen in photographs of the *Cherax* hemolymph, 24S, and 16S samples showed that all had the same dimension, namely, 135 ± 10 Å (SD). This result may be compared with reported values ranging from 90 Å [*Dugsiella*, Loewe et al. (1977)] to 130 ± 10 Å [*Panulirus*, Schepman (1975)]. The rectangular profiles seen in *Cherax* specimens containing hexamers were 135 ± 10 by 110 ± 10 Å and thus apparently represent a projection at right angles to that displaying the hexagonal profile. The largest diameter of the hexagon seen in combination with a square or rectangular projection, in the 24S particles, was also 135 ± 10 Å. The width of the square in the 24S particles was 110 ± 10 Å, while the total length of the particles was 230 ± 15 Å. These dimensions are comparable with those given for the 24S structure by other workers, for example, *Cupiennius salei*, 90 × 190 Å (Loewe et al., 1977), and *Homarus vulgaris*, 100 × 235 Å (van Holde & van Bruggen, 1971). Thus, both in appearance and in dimensions *Cherax destructor* hexamers and dodecamers are quite typical arthropod structures. Although not too much weight should be placed on measurements of electron micrographs, there is a suggestion of some variation between arthropod species in the sizes of the 24S and 16S components, those in the spiders *Dugsiella* and *Cupiennius* (Loewe et al., 1977), for example, appearing to be smaller than the corre-

sponding structures in the lobsters *Homarus* (van Holde & van Bruggen, 1971) and *Cherax* (present work). A large part of such a difference could be attributable to differences in size between the subunits comprising the aggregate form. Lamy et al. (1977b), for instance, report a size difference in scorpion hemocyanin hexamers assembled from different subunits.

In most of the *Cherax* hemolymph specimens examined in the electron microscope, a few molecules larger than dodecamers were noticed. They usually have the appearance of 24S particles with another hexamer attached at an angle, as may be seen in the marked examples in Figure 2A. These may represent the material, of sedimentation coefficient about 30 S, which is visible in disc gel electrophoresis (Figure 1) and sedimentation velocity experiments (Murray & Jeffrey, 1974) of *Cherax* hemolymph. If so, they represent structures of a size hitherto unreported for hemocyanins, but confirmation of this idea awaits isolation of the component in question.

The results of electron microscopy of specimens obtained by reassembly of monomers M_1 and M_2 in the presence of calcium ions are presented in Figures 3 and 4. Images indistinguishable in appearance or dimensions from those of the hexameric molecules in the hemolymph and the 16S fraction were observed. Similar experiments with other hemocyanins have also been reported as showing no measurable differences in reassembled and native structures (Schutter et al., 1977; Loewe et al., 1977). In the reassembly experiments with the *Cherax* monomers, it is noteworthy that no 24S structures were observed in the electron microscope since this confirms the result of gel electrophoresis experiments that, in *Cherax*, the presence of a dimeric subunit is necessary to the formation of the dodecamer.

Discussion

Previous studies, to which reference has already been made, are in agreement that the images of negatively stained 16S hemocyanin molecules seen in the electron microscope are qualitatively the same in all arthropod species. The same can be said of the 24S molecules. It is reassuring that the observation of the corresponding components of *Cherax destructor* hemocyanin does nothing to upset this conclusion. The implication is that, although there may be small differences in size, the arrangement of the subunits in these aggregated structures is fundamentally similar throughout the arthropods utilizing hemocyanin. This is not necessarily true of other aggregated forms; for example, van Holde et al. (1977) report that the 34S component of *Callianassa* hemocyanin is tetrahedral in form, whereas in other species it presents a square-planar appearance.

Numerous models have been proposed for the arrangement of the subunits in the 16S oligomer [see, for example, van Holde & van Bruggen (1971)], but most of these attempts were handicapped by uncertainty about the number of subunits involved. The best suggestion currently is that the 16S structure, a hexamer of six 5S particles, is very probably a trigonal antiprism (Schutter et al., 1977). This conclusion is based partly upon the appearance of the profiles visible in the electron microscope and partly upon the determination of a point-group symmetry of 32 for *Panulirus interruptus* hemocyanin by X-ray crystallography (Schepman, 1975). A structure with this symmetry would be expected to dissociate via dimers or trimers, but the failure, so far, to observe such intermediate species may be due to selection of too powerful dissociating conditions. It is worth noting that the sedimentation ratio (Andrews & Jeffrey, 1976) calculated for a trigonal antiprismatic arrangement of six spherical subunits is 3.31. This result predicts a value of 17.5 S for $s^0_{20,w}$ for the hexamer when $s^0_{20,w}$ for the monomer is taken as 5.3 S, in exact agreement with the experimental value measured for the *Cherax* 16S component. This approach, admittedly approximate, does indicate that the proposed structure is also hydrodynamically plausible. It should be pointed out that an earlier attempt to define a model for the hemocyanin hexamer by the calculation of sedimentation ratios (Andrews & Jeffrey, 1976) was based on the assumption that the nonspherical subunits occupied equivalent positions and thus did not include the structure under discussion.

The 24S structure is often referred to in terms that imply it is assembled from two hexamers. The usage arises naturally from its size and from the projections—combinations of hexagon and rectangle—that it presents in the electron microscope. While the implication may sometimes be correct, there are certainly cases where it is not. The former contention is supported by the observations made of a reversible equilibrium between hexamers and dodecamers in *Homarus americanus* (Morimoto & Kegeles, 1971) and in *Callianassa californiensis* (van Holde et al., 1977) hemocyanins. The latter contention is supported by reassembly experiments with *Limulus* (Schutter et al., 1977), *Androctonus* (Lamy et al., 1977b), *Dugsiella* (Linzen et al., personal communication), and *Cherax* (Jeffrey et al., 1978), which show that association stops at the hexamer unless certain subunits, usually dimeric, are present in the mixture before reassembly starts. Thus, while the 24S structure may have the appearance of two hexamers juxtaposed with some rotation of one with respect to the other, in many arthropods this notion may be misleading. This is not to say that the subunits in such 24S structures could not be disposed in the same way as that which would result from the direct aggregation of two hexamers.

Figure 5 shows a model of a dodecamer assembled from two hexamers, each made from spherical subunits arranged in a trigonal antiprism. One hexamer is rotated through 90° with respect to the other in the direction of the long axis of the structure and then through a small angle about its own threefold axis to obtain a close fit of the two hexamers. This orientation is consistent with the usual description of the 24S structure and with the dimensions given earlier. That is, the length of about 230 Å is regarded as the sum of the diameter of a hexamer, ~135 Å, and its depth, ~110 Å, less some small amount to allow for packing the two units together. Comparison of the appearance of the model with the electron microscope image shows that, when due allowance is made for the approximation of subunits by smooth spheres, the arrangement in the model is a feasible one. Moreover the structure could be formed either by the aggregation of two hexamers or by the assembly from subunits, even if it is required that one of the subunits be dimeric.

There is good evidence that the dodecameric 24S structure in both *Cherax* (Jeffrey et al., 1978) and *Androctonus* (Lamy et al., 1977a) is composed of one dimeric and ten monomeric subunits. A possible way in which a dimeric subunit might link the two halves of the dodecamer is indicated in Figure 5. As pointed out by Lamy and colleagues (Lamy et al., 1977a), a dodecamer linked in this way would be expected to "explode" directly into the monomeric and dimeric subunits upon dissociation, exactly the behavior observed with *Cherax* and *Androctonus* dodecamers. The species variation in the composition of arthropod bloods with respect to the aggregated forms they contain is one of the central observations in the hemocyanin literature (Table I). With the recent findings, for several species, that the formation of particular oligomers depends on the presence of specific subunits, it is becoming

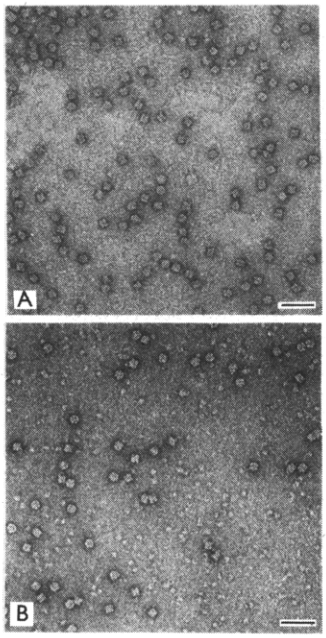

FIGURE 3: Negatively stained hexamers of *Cherax destructor* hemocyanin. (A) The native 16S fraction whose gel electrophoresis pattern is given in Figure 1B. (B) Hexamers reconstituted from monomer M_1 whose gel electrophoresis pattern is shown in Figure 1C. The bars represent 50 nm.

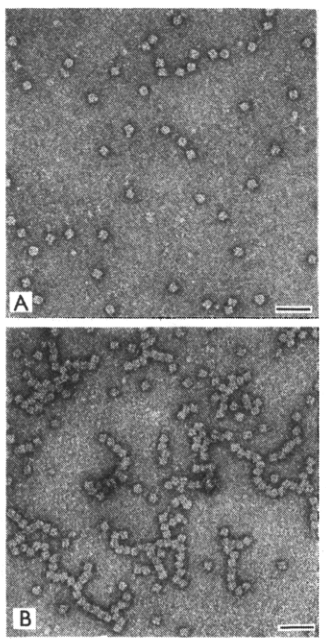

FIGURE 4: Negatively stained hexamers of *Cherax destructor* hemocyanin. Both photographs are of hexamers reconstituted from monomer M_2 whose gel electrophoresis pattern is given in Figure 1C. (A) A typical specimen. (B) A single example observed of a tendency to linear aggregation. This photograph is very similar to one of a specimen of *Homarus vulgaris* hemocyanin presented by Fernandez-Moran et al. (1966). The bars represent 50 nm.

clear that this variability is closely related to the equally common subunit heterogeneity of the hemocyanins of these animals. The observations made above with reference to the formation of dodecamers are indicative and, together with the other results cited, encourage the belief that the rather bewildering species diversity in the distribution of aggregated forms in arthropod hemocyanins will soon be seen in detail as variation, controlled by the subunits present, on a single theme of organization.

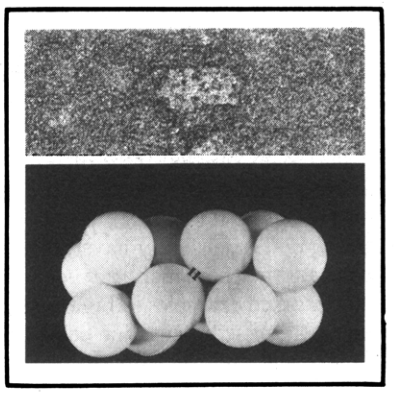

FIGURE 5: A model for the dodecamer of arthropod hemocyanin constructed as described in the text. A dimeric subunit is represented by the two spheres joined by bars to indicate how, in some species, such a subunit could act as a link. An electron micrograph of a negatively stained 24S molecule of *Cherax destructor* hemocyanin, magnified about 259 200×, is included for comparison.

Acknowledgments

I thank Professor E. F. J. van Bruggen and the members of the electron microscopy unit for their hospitality and assistance. In particular I thank Wilma Schutter for help with the electron micrographs.

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Proton Nuclear Magnetic Resonance Studies of Bence-Jones Proteins†

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ABSTRACT: ^{1}H nuclear magnetic resonance (NMR) studies of λ- and κ-type Bence-Jones proteins are reported. Fifteen λ-type Bence-Jones proteins, including proteins Blo, Mcg, Sh, Vi, and Weir, were used to examine the pH titration curves of the two constant domain histidines (His-189 and His-198). The His-189 titration curves were all quite similar, with a pK_a of 7.3 ± 0.1. On the other hand, there is significant heterogeneity in the His-198 titration curves; the pK_a values are in no case greater than 4.5. The line width of the His-198 peak is much broader than that of the His-189 peak. In addition, the C(2)–H proton of His-189 incorporates deuterium from D_2O rapidly, whereas virtually no exchange occurs at His-198. The NMR results are compared with X-ray crystallographic studies of the λ-type Bence-Jones dimer Mcg [Edmundsen, A. B., Ely, K. R., Abola, E. E., Schiffer, M., & Panagiotopoulos, N. (1975) *Biochemistry 14*, 3953] which shows that His-189 belongs to an exposed loop, whereas His-198 is oriented toward the interior. The resonances of His-189 and His-198 reflect well the environments expected from the tertiary structure, and the heterogeneity observed for the His-198 resonances probably reflects the Mcg isotypic substitutions. The constant fragment (C_L) obtained by limited tryptic digestion of the λ-type Bence-Jones protein Nag was also examined, and the results were compared with those for the intact λ-type Bence-Jones proteins. It was concluded that the tertiary structure of the immunoglobulin fold is well preserved even in the C_L fragment. ^{1}H NMR spectra of three kinds of κ-type Bence-Jones proteins were examined. There is a difference between λ- and κ-type Bence-Jones proteins in the His-189, and more significantly in the His-198, titration curves. We suggest that the difference in the chemical shift of the His-189 resonances makes it possible to quantitate the λ/κ ratio for the normal light chain. It was shown that His-198 in the κ-type Bence-Jones proteins is much more difficult to protonate; the His-198 peak begins to shift downfield only below pH 4, where the proteins begin to denature. We conclude that λ- and κ-type Bence-Jones proteins are basically similar in conformation in the constant domain. However, the constant domain of the κ-type proteins appears to be more compact than that of λ-type proteins.

Bence-Jones proteins, which are excreted into the urine of patients with multiple myeloma, are dimers of homogeneous light chains of immunoglobulins. The light chains are divided into two homology units (domains) of about 110 amino acid residues. The variable, amino-terminal domain differs markedly from one Bence-Jones protein to another, whereas the constant, carboxy-terminal domain has essentially an invariant sequence. The light chains exist in two types, κ and λ, the structural differences of which are reflected in antigenic differences.

The crystal structure of a λ-type Bence-Jones dimer designated Mcg has been analyzed at 2.3 Å by Edmundsen & co-workers (1975). X-ray studies of the dimer of the variable domain of the κ-type Bence-Jones protein REI has also been reported (Epp et al., 1974). It has been demonstrated by Edmundsen et al. (1975) that, in the Mcg dimer, each of the two light chains is different in conformation and forms an architecture which is quite similar to that of the Fab fragment of the immunoglobulin molecules; three- and four-chain layers of antiparallel β-pleated sheets constitute the basic *immunoglobulin fold*. They also reported binding studies on Mcg crystals that demonstrated that the Mcg dimer binds a variety of small molecules at three different binding sites whose structure has been elucidated in detail on the basis of the X-ray crystallographic data (Edmundsen et al., 1974).

The λ-type Bence-Jones proteins have in common two histidine residues (His-189 and His-198) in the constant domain of each of the light chain.[1] In a previous paper (Arata et al., 1978), we have reported a ^{1}H NMR spectrum of a λ-type Bence-Jones dimer Ak which contains no histidine residue in the variable domain; assignments of the C(2)–H proton signals of His-189 and His-198 have been accomplished by using a deuterium-labeling technique which is similar to that used by Markley & Kato (1975).

In the present work, we have examined by ^{1}H NMR more than 20 Bence-Jones proteins.[2] These include λ-type Bence-Jones dimers Mcg and Sh with known amino acid sequences. In addition to three Mcg(+) proteins, Mcg, Hu,

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[1] The numbering system used in the present paper is based on protein Sh (λ-type) and protein Ag (κ-type). See Putnam (1969).

[2] The authors are indebted to the following for samples of Bence-Jones proteins: Dr. T. Azuma and Dr. K. Hamaguchi, Osaka University; Dr. H. F. Deutsch, University of Wisconsin; Dr. F. W. Putnam, Indiana University; Dr. N. Hilschmann, Max-Planck-Institute; Dr. T. Ikenaka, Osaka University; Dr. S. Migita, Kanazawa University; Dr. C. Milstein, MRC Laboratory of Molecular Biology; Dr. T. Shinoda, Tokyo Metropolitan University.

0006-2960/79/0418-2513$01.00/0