photosphere of a massive superstar[17] or from very-high-density broad-line-region gas close to the central object[18].

Observations of time variability of the continuum spectral shape of Seyferts and quasars are going to be important in understanding the origin of the continuum and the observations by Ulrich *et al.*[1] and others[19,20] have shown interesting changes in the ultraviolet continuum of NGC4151 and other Seyferts. IUE observations alone, however, just do not provide enough wavelength coverage and the changes reported are not, I believe, true changes in the continuum shape but rather changes in the relative intensity of the Fe II emission at 3,000 Å (that is, the 3,000 Å bump). This is probably the cause of the otherwise puzzling result of Ulrich *et al.*[1] that the C IV 1,549 Å emission-line variability is better correlated with the continuum emission at 2,500 Å than with the continuum further into the ultraviolet (that is, nearer to the relevant ionization edges), and other inconsistencies which they note. To measure the true continuum one needs to cover many octaves in frequency and it is to be strongly hoped that future IUE observations of NGC4151 (and other Seyferts) will be scheduled with simultaneous longer-wavelength optical and infrared observations (and for that matter X-ray observations as well).

Although our picture of NGC4151 and other active galactic nuclei is still very fuzzy and little has actually been proved, it must be said that everything is still consistent with such objects having at least one massive black hole in their centres (and some may have two[21]). Similarly there is no evidence so far that quasars do not have accretion discs powering them, but it would be nice to have proof that they do. □

C. Martin Gaskell is on the faculty of the Astronomy Department, University of Texas, Austin, Texas 78712.

Protein structure

Oxygen carriers of a third kind

from K.E. van Holde

HAEMOGLOBIN is not the only way to transport oxygen in a circulatory system. Some invertebrate phyla use altogether different proteins to serve this function. These include the haemerythrins, utilized by sipunculids, brachiopods and a few other invertebrates, and the haemocyanins, found in a wide variety of molluscs and arthropods. The structures of many haemoglobins, and even one haemerythrin[1], have been determined to high resolution by diffraction techniques, but until the paper of Gaykema *et al.*[2] on page 23, such information has been wholly lacking for the haemocyanins. This hiatus has seemed almost paradoxical, for the haemocyanins were the first proteins to be studied in detail by biophysical techniques, notably in some of the earliest ultracentrifuge studies of Svedberg and his collaborators[3].

The problem of how to bind oxygen in a way that is reversible receives a radically different solution in haemocyanins than it does in haemoglobins and haemerythrins. Rather than using iron as an oxygen ligand, haemocyanins employ pairs of antiferromagnetically coupled copper ions[4,5]. Such copper pairs are found in a number of other proteins, but for none of these is the detailed structure known. Thus the determination, to a resolution of 3.2 Å, of the structure of the haemocyanin of the spiny lobster, *Panulirus interruptus*, is of major importance.

The native molecule, which is one of the simpler haemocyanins, exists as a hexamer of two kinds of very similar subunits, each of molecular weight (MW) about 75,000. The entire hexameric structure has now been determined by Gaykema *et al.*[2], making it one of the largest proteins to be analysed by X-ray diffraction. The subunits are found to be arrayed with 32 symmetry; two trimers, related by 2-fold axes, are stacked face to face. Each subunit consists of three domains of approximately equal size. The second of these domains carries the copper pair. Each copper ion is bound by three residues, two of which are definitely histidines. The third ligand is probably also a histidine, but confirmation will require further sequencing.

The structure determined by Gaykema *et al.* seems to be that of deoxyhaemocyanin but studies of a subunit of *Limulus* oxyhaemocyanin are under way in the laboratory of W. Love, at Johns Hopkins University[6]. It will be of considerable interest to see how the oxy- and deoxy-structures compare, for haemocyanins are allosteric proteins, exhibiting both cooperativity in oxygen binding and heterotropic allostery, including a pronounced Bohr effect. So far, our only model for these functions has been haemoglobin (haemerythrin appears to be non-cooperative[7]); it is conceivable that similar effects will be found to be produced by quite dissimilar mechanisms in a protein as different as haemocyanin. This would represent an interesting example of convergent evolution of protein function.

The structure found for the haemocyanin seems wholly unrelated to that of haemerythrin or any haemoglobin, so haemocynanin must have a different ancestry. Is it shared with tyrosinases, ceruloplasmin and laccase, all proteins with a similar binuclear copper centre? Although the three-dimensional structures of these proteins are unknown, their amino acid sequences have certain motifs in common, suggesting similar modes of copper binding. In all three proteins there exist pairs of histidines separated by three amino acids, resembling those now identified among the copper ligands of haemocyanin. Confirmation of the hypothetical ancestry may be difficult, however, for the haemocyanins have presumably been evolving independently for something on the order of 500 million years. Indeed, a comparison of the complete sequence of a spider haemocyanin with *Neurospora* tyrosinase gave no evidence of homology[8].

Gaykema *et al.* point out a peculiar aspect of the *Panulirus* haemocyanin structure. Its carboxy-terminal domain contains a seven-stranded antiparallel β-barrel, remarkably similar in topology to the β-barrels in (Cu–Zn) superoxide dismutase and immunoglobulins[9]. Since the amino acid sequences are entirely different, they argue that this may simply be a very stable structure which has evolved independently in different proteins.

A final puzzle, which may be clarified by further structural studies, is the relationship between arthropod and molluscan haemocyanins. The oxygen-binding site of both types seems quite similar but their gross molecular architecture is entirely different. All known arthropod haemocyanins are built of 75,000-MW chains, arranged in hexamers, as in *Panulirus*. These hexamers are often combined to form even larger structures. On the other hand, the polypeptide chains of molluscan haemocyanins are much larger; units of MW about 400,000, containing eight globular domains and eight oxygen-binding sites, are commonly observed[4,5]. Gaykema *et al.* suggest that a molluscan domain of ~50,000 MW may correspond to two of the three 25,000-MW domains observed in the *Panulirus* structure, one carrying a binuclear copper site. Thus, it is conceivable that molluscan and arthropod haemocyanins arose via separate evolutionary events from some common copper-protein ancestor.

The new structural studies, combined with a number of sequence determinations now underway, promise to broaden our perspectives concerning the mechanisms that organisms have evolved to transport oxygen. □

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K.E. van Holde is in the Department of Biochemistry and Biophysics, Oregon State University, Corvallis, Oregon 97331.