PATTERNS IN THE QUINARY STRUCTURES OF PROTEINS

Plasticity and Inequivalence of Individual Molecules in Helical Arrays of Sickle Cell Hemoglobin and Tubulin

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ABSTRACT The four recognized levels of organization of protein structure (primary through quaternary) are extended to add the designation quinary structure for the interactions within helical arrays, such as found for sickle cell hemoglobin fibers or tubulin units in microtubules. For sickle cell hemoglobin the main quinary structure is a 14-filament fiber, with a number of other minor forms also encountered. Degenerate forms of the 14-filament fibers can be characterized that lack specific pairs of filaments; evidence is presented which suggests an overall organization of the 14 filaments in pairs, with particular pairs aligned in an antiparallel orientation. For tubulin, a range of quinary structures can be detected depending on the number of protofilaments and whether adjacent protofilaments composed of alternating α- and β-subunits are aligned with contacts between like or unlike subunits and with parallel or antiparallel polarity. Thus, in contrast to quarternary structure, which generally involves a fixed number of subunits, the quinary structures of proteins can exhibit marked plasticity and inequivalence in the juxtaposition of constituent molecules.

INTRODUCTION

Early concepts regarding the levels of organization of protein structures included the now familiar designations of primary, secondary, and tertiary structures to specify amino acid sequence, ordered short-range interactions, and three-dimensional folding, respectively (Linderstrom-Lang, 1951). Subsequently the designation quaternary structure was introduced to describe the interactions between individual polypeptide-chain subunits of a protein (Bernal, 1958). While these distinctions for protein structure were being formulated, concepts concerning the assembly of proteins into helical structures were also introduced (Pauling, 1953; Crick and Watson, 1956). The expectation for helical assembly then expressed was that the governing principle would be self-association of protein units at equivalent positions in the helical lattices. This arrangement has been validated for many structures (see reviews by Crowther and Klug, 1975), with tobacco mosaic virus providing a classical example (Bernal and Fankuchen, 1941; Watson, 1954; Franklin and Holmes, 1958) which has now been analyzed to 4-Å resolution (Stubbs et al., 1977). However, certain deviations from the "classical" pattern have recently been reported for helical assemblies of sickle cell hemoglobin and tubulin which involve more dramatic departures from equivalence than occur in the "quasi-equivalence" described for virus structures (Caspar and Klug, 1961; Caspar and Holmes, 1969). Considerations of the architecture of these helical protein arrays have led to the formulation of the quinary level of organization. The need for additional distinction in the levels of organization is especially apparent for hemoglobin, which has a well-defined

quaternary structure (Perutz, 1965) that must be distinguished from the intermolecular interactions between particular subunits occurring in the quinary structure of the sickle cell variant.

GENERAL CONSIDERATIONS

The various levels of organization of protein structure can be readily illustrated for hemoglobin for which the primary to quarternary structures have been fully specified in terms of 141 α-chain residues, 146 β-chain residues, extensive α-helical regions, the characteristic "globin" fold, and the overall $\alpha_2\beta_2$ structure (Perutz, 1965). The next level, quinary structure, would relate to the interactions in helical fibers, as occur in the sickle cell form of hemoglobin (Dykes et al., 1979). Formally, the packing of molecules within crystals, as well as into virus shells, may also be considered examples of quinary structures. Helical quinary structures may arise directly from monomeric units having a characteristic tertiary structure, but no identifiable quaternary structure, as in the case of the G-actin monomer which assembles into F-actin double stranded helix (Moore et al., 1970). Among proteins forming quinary structures which do possess a true quaternary structure, a further distinction can be made as to whether a molecular symmetry axis of the quaternary structure coincides with a helical axis of the quinary structure. Such a coincidence of axes is found for glutamic dehydrogenase tubes (Josephs and Borisy, 1971) or glutamine synthetase cables (Frey et al., 1975),, but not for hemoglobin S. The significance of this point for hemoglobin S fibers is considered in a later section.

Beyond the quinary level of structure, another level, the senary, can be distinquished which involves the interactions between helical fibers. For sickle cell hemoglobin, senary structure involves square or hexagonal arrays of fibers (Crepeau et al., 1978*b* and references cited therein) or a stacking of planes of parallel lines of the fibers, with each plane rotated by 26° degrees due to interdigitation of helical grooves of the fibers in adjacent planes (Edelstein and Crepeau, 1979). For microtubules, the specific architecture of flagella may be regarded as a senary structure, along with a number of the other assemblies of microtubules found in cells (Dustin, 1978).

RESULTS

Sickle Cell Hemoglobin

Structural studies on the fibers of hemoglobin S responsible for sickle cell disease had been hampered by instability of the fibers in solutions at concentrations below ~25% and by indications of considerable polymorphism, but in the last few years studies have yielded a fairly consistent interpretation of the quinary structure and the significance of various forms. Our laboratory has emphasized the 21 nm-diameter form of the fibers which has been found by electron microscopy and three-dimensional computer reconstructions to be composed of 14 strands or filaments of hemoglobin S (Dykes et al., 1978, 1979). The 14 filaments are arranged with a central core of 4 filaments surrounded by an outer sheath of 10 filaments. A typical fiber and a representation of its structure in a ball model are presented in Fig. 1; for the cross sectional arrangement see Fig. 2. On the basis of an agreement between the diameter of these fibers measured from flattening-corrected single images and the diameter of fibers observed in embedded and sectioned sickle cells, we have concluded that the 14-filament fibers are the predominant form in sickled cells (Crepeau et al., 1978*b*). Earlier measurements

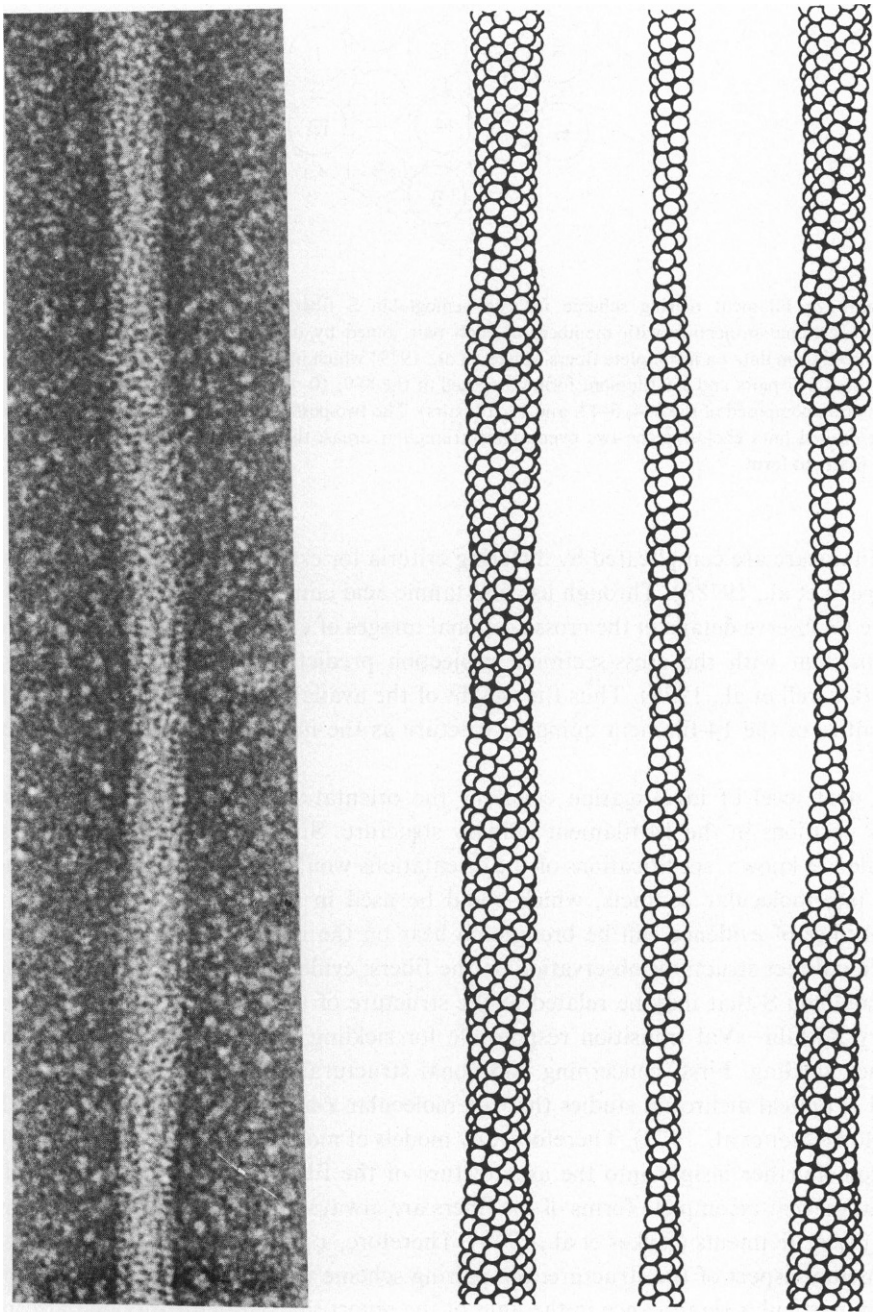

Figure 1 Electron micrograph of negatively stained fiber of hemoglobin S and structure deduced by three-dimensional image reconstruction. The reconstructed structure is presented as ball models, where each ball represents a hemoglobin S tetramer and the three representations correspond to the outer sheath (left), the inner core (center), and a combination of both inner and outer elements (right). Details of the reconstruction procedure are presented in Dykes et al., 1979.

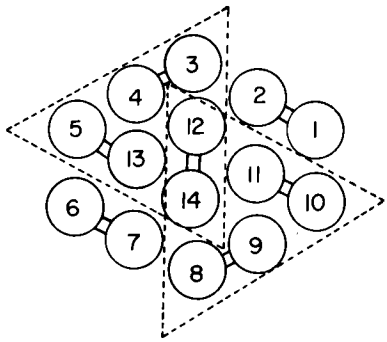

Figure 2 Filament pairing scheme for the hemoglobin S fibers. The 14 filaments are shown in cross-sectional projection with members of each pair joined by double lines. This pairing scheme is deduced from data on incomplete fibers (Dykes et al., 1979) which include a 10-filament form lacking the 1–2 and 6–7 pairs and a 6-filament form composed of the 8–9, 10–11, and 12–14 pairs (or the eqivalent structure composed of the 3–4, 5–13, and 12–14 pairs). The two possible 6-filament units are indicated by the dashed lines enclosing the two overlapping triangular areas; the combined areas also delineate the 10-filament form.

in the literature are complicated by differing criteria for estimating diameters (see discussion in Crepeau et al., 1978*b*). Through use of a tannic acid embedding procedure it has also been possible to observe details in the cross-sectional images of embedded and sectioned fibers that are consistent with the cross-sectional projection predicted by the 14-filament structural model (Garrell et al., 1979). Thus the weight of the available evidence is strongly in favor of the identity of the 14-filament quinary structure as the major fiber of interest in sickle cell disease.

The next level of investigation concerns the orientation of individual molecules at the various positions in the 14-filament quinary structure. Since the atomic coordinates of the molecule are known, specifications of the orientations would also establish the sterochemistry of the intermolecular contacts, which could be used in the design of antisickling agents. Several lines of evidence can be brought to bear on the molecular orientation in the fibers including: direct structural observation on the fibers; evidence from certain crystalline forms of hemoglobin S that may be related to the structure of the fibers; and the locations of the primary $\beta 6$ Glu→Val transition responsible for sickling and various other mutations that influence sickling. First, concerning additional structural information, it has been demonstrated in optical dichroism studies that the molecular x-axis must lie within 22° of the fiber axis (Hofrichter et al., 1973). Therefore, any models of molecular orientation must satisfy this condition. Further insight into the architecture of the fibers has also been provided by the observation that incomplete forms of the fibers are always missing certain filaments in sets of two adjacent filaments (Dykes et al., 1979). Therefore, a pairing of filaments appears to be a fundamental aspect of the structure; the pairing scheme is summarized in Fig. 2. This point takes on particular significance in the light of the report that x-ray diffraction patterns of the fibers display a close correspondence to a crystalline form of hemoglobin S that also involves a pairing of strands of molecules (Magdoff-Fairchild and Chiu, 1979).

The second line of evidence regarding molecular orientation is provided principally by the crystal structure described by Wishner et al., (1975). In this structure the hemoglobin S molecules occur in pairs of half-staggered strands, with the molecular x-axis close to the strand axis and members of each strand of a pair related by a twofold screw axis. The other

important feature of the structure is the location of the primary $\beta 6$ site (as well as a number of other sites on the surface of the molecule that have been implicated in sickling) at inter molecular contacts. Thus the orientation of the molecules in these crystals may have some correspondence to the orientation in the fibers, although perturbations would obviously be required to convert linear strands of the crystals into the helical spirals of the fibers.

The third line of evidence concerns the information obtained from examining the influence of various modified forms of hemoglobin on the sickling process. One important observation in this regard in that only one β-6 Val site is needed per hemoglobin tetramer, since asymmetric hybrids composed of one sickle-type β-chain and one normal β-chain readily form fibers (Bookchin and Nagel, 1971; Goldberg et al., 1977; Bookchin et al., 1977; Benesch et al., 1978). This observation strengthens the argument that the crystal structure of Wishner et al. (1975) is relevant to the fiber structure, since in crystalline structure as well, only one β-6 site per tetramer participates in the contacts between molecules in the pairs of strands. As noted above, many other mutations known to influence sickling occur at positions which have been identified at intermolecular contacts between molecules in the strand-pairs of the crystals (Nagel and Bookchin, 1978). Moreover, a large body of data is accumulating on additonal sites that influence sickling particularly in the α-chains, which have been investigated in symmetric double mutants (Benesch et al., 1976, 1977, 1979) and in mixtures (Nagel and Bookchin, 1978). The homogeneous double mutants studied by Benesch et al can reveal cis as well as trans effects, while the mixtures studied by Nagel and Bookchin can reveal exclusively trans effects (where cis and trans refer to the occurrence of the variant α chain in the same (cis) or opposite (trans) $\alpha\beta$-dimer with respect to the $\beta 6$ Val site that participates in the interstrand contact). Ultimately, all of this information should be accommodated by any comprehensive structural model for the detailed molecular structure of hemglobin S fibers.

Presently it is possible to formulate a plausible molecular model for the fibers of hemoglobin S which incorporates much of the available data. The key step in this process is relating the paired strands observed in the crystals to the 14 filaments determined for the fibers. As already noted, the incomplete forms of the fibers suggest a pairing of filaments, such that the 14 filaments can be represented as 7 specific pairs (Fig. 2). Moreover, each of the 7 pairs of filaments deduced in this way possesses the property that the individual filaments of each pair are close to half-staggered. Thus a correspondence between the fiber filament pairs and the crystal strand pairs is suggested, but one additional factor must be considered before this connection can be applied. The pairs of strands in the crystals actually occur in sets of two pairs, with each pair in the set having opposite polarity (Wishner et al., 1975). In applying the strand pairs of the crystal to the filament pairs of the fibers several alternative models can be considered. All of the pairs of filaments could have the same polarity, the polarity of the pairs of filaments could be random, or there could be a specific distribution of filament pairs of opposite polarity in the ratios 6:1, 5:2, or 4:3. At the present levels of resolution in the three-dimensional reconstructions an overall polarity to the fibers can be detected, although we have not been able to assign polarity to individual filaments. However, from the location of individual molecules along the 14 filaments there are clues which may provide an answer to the question of protofilament polarity. The relevant arguments are as follows.

The pairing scheme deduced for the 14 filaments involves a central pair surrounded by six outer pairs (Fig. 2). Five of the pairs can be viewed as forming the sides of the overlapping triangular units, with the central pair contributing to both triangular sets. On the basis of conventional helical lattice building, one would expect a regular, third-staggering of the

molecules along the fiber axis for the pairs in each triangular unit. The staggering is not regular, however, but discontinuous in an interesting way. The molecules at the contacts between the central pair and its four neighbors in the two overlapping triangles are quarter-staggered, whereas the molecules at the contacts between the noncentral pairs in the two triangles are half-staggered. An attractive explanation for this difference between quarter staggered and half-staggered contacts is that the former involves pairs of the same polarity and the latter involves pairs of opposite polarity. This scheme is supported by the fact that, in the crystals, the sets of pairs of opposite polarity occur with quarter-staggering between pairs. If an analogous pattern is assumed to carry over to the fibers, the central pair would be assigned an opposite polarity to the other four pairs that participate in the triangular units. No strong arguments can be made for the polarity relationship of the remaining two pairs present in the full 14 filament structure which have been ignored in the above arguments. If a relative balance between pairs of opposite polarity is favored by the mechanism of assembly (which

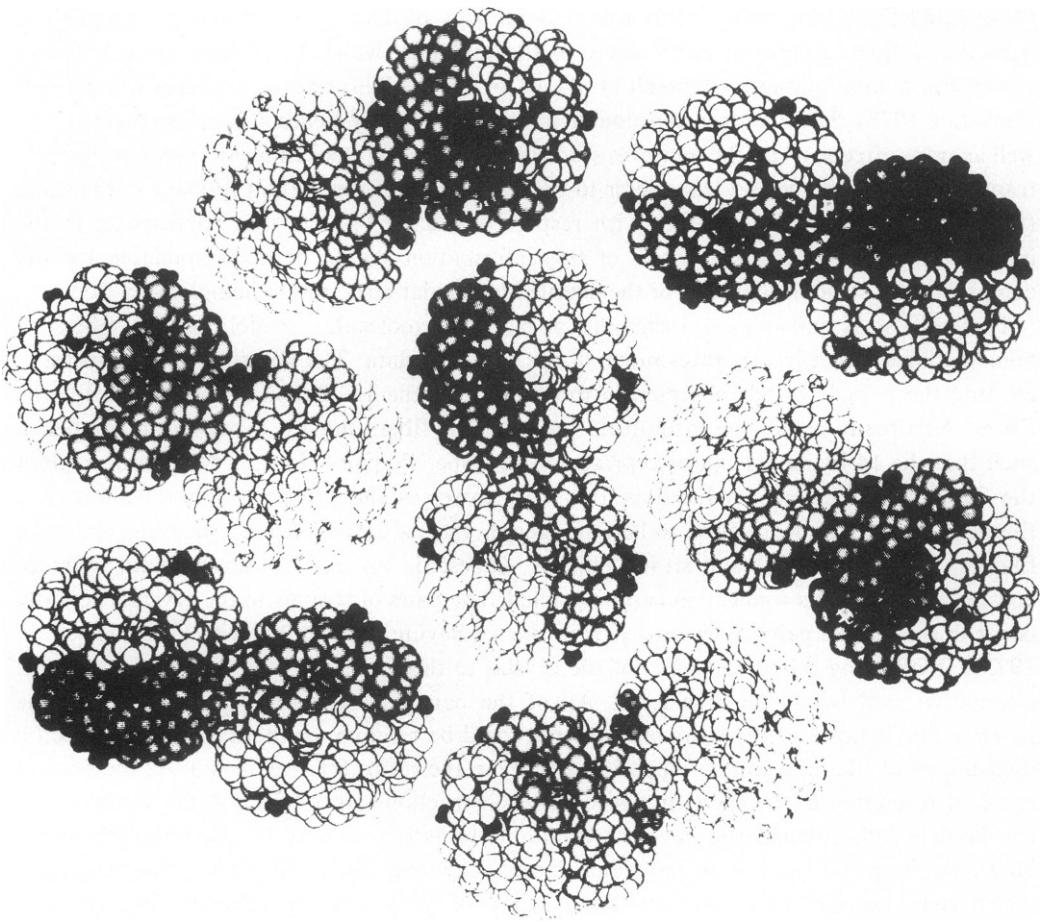

Figure 3 Molecular model of the hemoglobin S fibers in cross-sectional projection. Hemoglobin molecules are represented with a sphere for each amino acid residue, with darker spheres denoting residues of the β-chains. The 14 molecules are placed in the positions of pairs summarizd in Fig. 2, with pairs 1–2, 6–7, and 12–14 represented in opposite polarity to the remaining 4 pairs. In the projection the distances between the seven pairs are exaggerated to avoid overlap; in the fibers adjacent molecules are not in the same plane, permitting closer packing than in this planar projection.

could involve filament-pair intermediates), the 4:3 ratio for the polarity of pairs would prevail and this arrangement is summarized in Fig. 3.

The schematic molecular model for the hemoglobin S fibers summarized in Fig. 3 is a plausible synthesis of the available data. It now remains to be seen whether the various contacts predicted by the model (especially the new contacts between strand pairs which do not occur in the crystals) can be reconciled with data on mutant hemoglobin sites which influence sickling. Work on this issue is underway.

Tubulin

Microtubules, composed principally of the protein tubulin, are ubiquitous structures in eucaryotic cells. In sectioned cells microtubules can be resolved as hollow structures containing in most instances 13 protofilaments. On the basis of similarities to flagellar outer doublet A-tubules and the notion of tubulin as an $\alpha\beta$-heterodimer, microtubules are generally regarded as possessing a structure with the 13 protofilaments composed of alternating α-and β-units staggered slightly to give a 3-start helix with contact between protofilaments involving unlike subunits; (Amos and Klug, 1975); Erickson, 1975). This juxtaposition of protofilaments is summarized in Fig. 4 *A*. However, recent results suggest that the structures of microtubules and related arrays are considerably more complex, in several respects: (*a*) The number of protofilaments in microtubules reassembled in vitro can assume a range of values which can be influenced by solution varibles (Pierson et al, 1978; McEwen and Edelstein, 1979). In a typical experiment in our laboratory, end-on views of sectioned microtubules reveal a mixture of 13- and 14-protofilament structures (Fig. 5). Thus, apart from specific

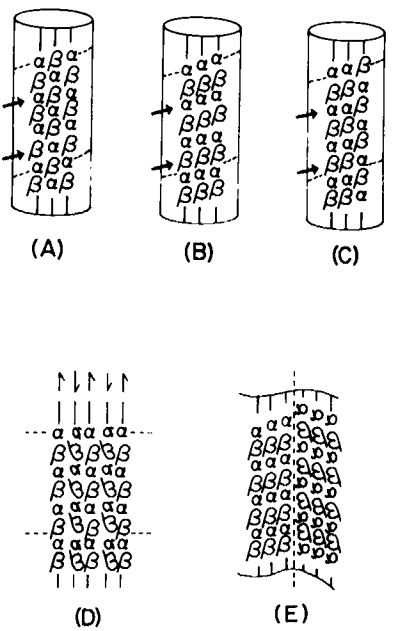

Figure 4 Alignment schemes of tubulin protofilaments. (*A*) Contacts between unlike subunits of adjacent protofilaments. (*B*) Contacts between like subunits of adjacent protofilaments. (*C*) Mixed contacts between subunits of adjacent protofilaments. (*D*) Antiparallel alignment of protofilaments related by a twofold screw axis as occurs in zinc induced sheets. (*E*) parallel rotation of adjacent parallel protofilaments as occurs at the inflection plane (dashed line) of S-sheets. For *A-C* the arrows indicate the progress of one of the shallow helical lines corresponding to a 3-start helix.

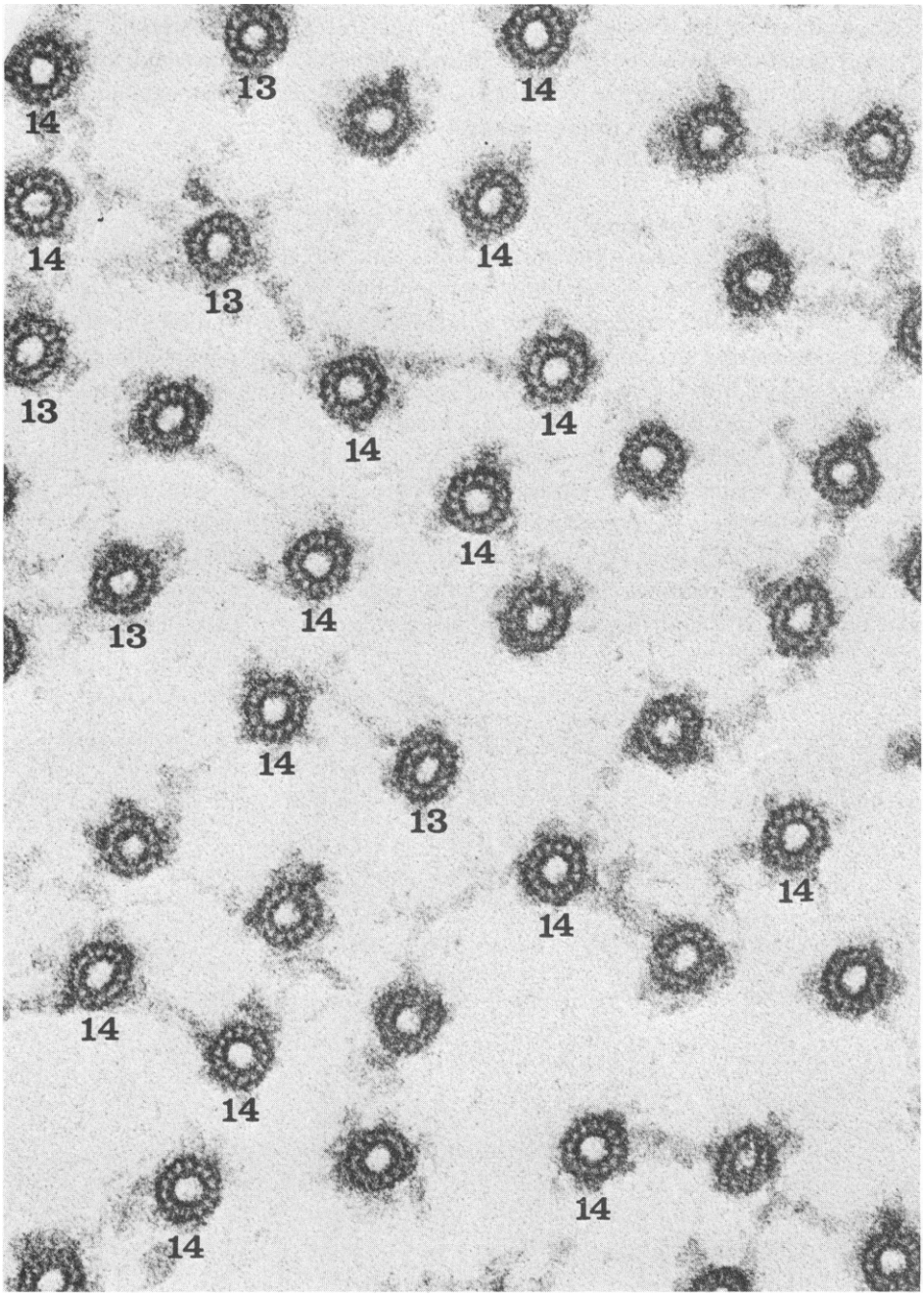

Figure 5 Cross sections of in vitro reassembled microtubules. The number of protofilaments in the examples that could be readily enumerated are presented below each image. Other details are presented in McEwen and Edelstein (1979).

considerations regarding the alignment of protofilaments (see below), there is clearly a plasticity that permits formation of a range of quinary structures with different inter-protofilament angles. For example, the difference between 13- and 14-protofilament structures involves a 2° difference in the angle relating adjacent protofilaments. Since structures in the range from 11–17 have been reported (Pierson et al., 1978; McEwen and Edelstein, 1979), the maximum values of the angular differences in orientation is over 10° which corresponds to considerable alteration in the structures of the various forms, approaching the range observed for the tomato bushy stunt virus protein with a specific hinge region connecting two rigidly folded domains (Harrison et al., 1978).

(*b*) Within the range of microtubule quinary structures containing from 11–17 protofilaments, it is not possible to account for the forms as variations on a single helical lattice theme. For example, the predominant form observed in in vitro reassembled microtubules is the 14-protofilament structure (Pierson et al., 1978; McEwen and Edelstein, 1979; Kim et al., 1979) which is incompatible with a continuous 3-start helical lattice. If the protofilaments are all aligned with contacts between unlike subunits (Fig. 4 *A*), then an odd number of protofilaments is required to form a continuous helical structure. If the protofilaments are all aligned with contacts between like subunits as suggested by Crepeau et al. (1978*a*), a continuous helix could be formed with any number of protofilaments, but only with an even-start helix (see Fig. 4 *B*). Since a 3-start helix is maintained for the 14-protofilament structures, (McEwen and Edelstein, 1979) the even-start possibility can also be eliminated. Thus we are forced to introduce another level of plasticity involving the alignment of adjacent protofilaments in two inequivalent modes, involving contacts between both like and unlike subunits. For the 14-protofilament structures, both types of contacts must occur in the same microtubule (Fig. 4 *C*) and a similar situation is likely to prevail in microtubules with other numbers of protofilaments.

(*c*) More extreme forms of plasticity can also be observed for tubulin arrays, with the best studied being the planar arrays formed in the presence of zinc (Larsson et al., 1976). In this case three-dimensional reconstructions have been completed (Tamm et al., 1979; Amos and Baker, 1979) and reveal a structure based on protofilaments with alternating α- and β-subunits, as believed to occur for microtubules, but with the protofilaments in the zinc-induced sheets in antiparallel alignment, as summarized in Fig. 4 *D*. Yet another alignment pattern for microtubules has also been observed, in this case involving parallel rotation of protofilaments at an inflection point to give S-shaped sheets (Mandelkow and Mandelkow, 1979; D. Pantaloni, B. McEwen, and S. Edelstein, manuscript in preparation). This arrangement is summarized in Fig. 4 *E*.

On the basis of these various structures we can conclude that tubulin-containing protofilaments can exhibit a remarkable degree of plasticity of quinary structure permitting alternate modes of bonding involving: the angle of adjacent protofilaments leading to the range of microtubules with 11–17 protofilaments; the nature of the contact between adjacent protofilaments filaments leading to interactions between like or unlike subunits; and the polarity and rotational orientation of the subunits leading to the planar zinc-induced sheets and the S-sheets. The functional significance (if any) of this versatility of assembly modes remains to be determined. However, from the structural point of view it is clear that we must begin to appreciate the wide range of assembly possibilities inherent in a single protein such as tubulin. Moreover, cellular factors yet to be defined must exist to constrain assembly to 13 protofilaments in vivo.

DISCUSSION

Quinary interactions in helical arrays have been described for hemoglobin S fibers and tubulin arrays which exhibit considerable plasticity and inequivalence in the locations of individual protein units. Similar features have been recognized in crystal polymorphism, presumably due to the relative weak nature of the interactions involved. Plasticity is observed in the locations of hemoglobin S molecules at various positions in the fibers in different environments, including the probable juxtaposition of certain filament pairs in antiparallel alignment. Similar manifestations of plasticity in quinary structure are observed for tubulin arrays which can vary in the number of protofilaments in microtubules and hence the bonding angle between adjacent protofilaments. In addition, protofilaments can align with contacts between either like or unlike subunits and in various orientations, including an antiparallel arrangement in the zinc-induced sheets. What emerges from these considerations is an appreciation of the complexities peculiar to the higher levels of protein structure. In particular, the features of quinary interactions are clearly important to keep in mind in structural studies of helical arrays and may be of special significance in the explanation of the functional properties of helical arrays such as microtubules, with roles in the cellular life cycle involving a multiplicity of interactions.

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Note added in proof: After this paper was written, it was discovered that use of the term *quinary structure* had been made earlier by B. K. Vainshtein (1973. Three-dimensional electron microscopy of biological macromolecules. *Sov. Usp. Phys.* **16**:185–206).

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DISCUSSION

Session Chairman: Alan Schechter *Scribe*: Martin Potschka

ERICKSON: I would like to describe protein–protein interaction at the microscopic level as bonding between highly specific patches on the surfaces of adjacent subunits, and to suggest mechanisms at this level that might explain the range of structures observed. Within any system the strongest interactions, e.g. the longitudinal bonds joining hemoglobin molecules in the filament or those connecting tubulin subunits in a protofilament, are probably identical in all structures. The range of structures you have described as *quinary* may then be attributed to the formation of new bonds, often weak, between subunits in adjacent filaments. In the case of tubulin these bonds may be of several different types, between unlike or like subunits and with protofilaments parallel or antiparallel, generating a multiplicity of structures.

In every case a major factor in determining the range of structures is the inherent flexibility of the protein. It seems from the structures described here, as well as in other systems, that small deformations corresponding to a strain of a few percent (or a bend of 2°–5°/50Å subunit) can be accommodated by this flexibility. The helical curvature of the hemoglobin filaments in the fibers and the difference in diameter of 13 and 15 filament microtubules are within this range. The free energy required to change the curvature may be easily compensated by the formation of new bonds between filaments. Deformation outside this range, such as the two different arrangements of subunits in TBSV, would require a conformational change in the bonding interface, the structure of the subunit, or both.)

Do you think that the range of structures observed can be explained by these factors? If so, I wonder if it is really necessary to introduce a new term, quinary structure, to describe what is really a set of quaternary structures, related in having some common strong interaction (the basic quaternary structure), and differing in the weak interactions or bonds between these basic quaternary structures and in the small deformations required to for these new bonds.

EDELSTEIN: I certainly agree that some of the effects that I am talking about would be accommodated by the flexibility of the type you refer to. This includes the difference in the number of protofilaments. At that level I agree that flexibility is all that we have to invoke, perhaps extending it to a conformational change. But we do have to keep in mind that there are very dramatic differences within parallel microtubule filaments. There are shifts to give contacts of the type $\alpha\beta$ or $\alpha\alpha$ and $\beta\beta$. These subunits are estimated to be only 50% homologous. We would not expect this degree of plasticity in a quarternary structure, like Hb, where α or β align in a very specific manner.

The antiparallel relationships in the sickle cell fiber, and between protofilaments in the Zn tubulin sheets indicate dramatically different bonding domains upon 180° rotation. This seems sufficient qualification to recognize it as a different kind of structure from the type the term quaternary represents.

CASPAR: Polyethylene in the solid state is a one-stranded structure. For hemoglobin crystals Wishner observed that the aggregates of molecules were arranged in a chain related by a twofold screw. In the crystal there are chains that point up and chains that point down. He suggested that these chains were the basic aggregate in Hb-S. Magdoff-Fairchild has established that this is indeed the case. Four years ago she showed me diffraction patterns from gels that had crystallized and which were remarkably similar to those of Wishner. There were slight intensity differences at smaller angles, but there was no doubt that Wishner's supposition was correct. Since then she has crystallized another form of Hb-S which is essentially the same except that the antiparallel chains are slightly shifted against each other (Magdoff-Fairchilf and Chiu, this volume). The intensities she observed are combinations of those two crystal forms. Furthermore, there is one crystal form where chains point in only one direction and the intensity distribution in this pattern is entirely different. These observations establish as fact that Hb-S polymers consist of pairs of Hb-S aggregates connected together in these chains.

The observations of Dr. Edelstein with electron microscopy are suggestive. There are questions that could be raised about the detailed significance of his three-dimensional image reconstruction. But what is clearly going on there is that there is a twisted crystal. The twofold screw axis is supercoiled instead of straight. If you take a crystal Hb-S fiber and twist it, the chains will be twisted more and more as you go outward. There will be a natural limitation to the growth of such a structure. This sort of problem has been considered in the formation of collagen fibers and other fibrous structures as a kind of quasicrystalline structure where there is a variation in packing relations in going out from the center of the structure. It is obvious that you can account for your data if you use these established facts as constraints in your reconstruction.

EDELSTEIN: I am not sure what the point was you were trying to make. I emphasized in the text of the paper that I am relating to the work on the crystals. We see filament pairs in the fiber and I have tried to relate the pairs in the fibers to those in the Hb-S crystal.

CASPAR: My point is that you have to have an even number of these two-fold screw axes. You have 7 in your 14 strand structure.

EDELSTEIN: I disagree with you. I think there the analogy has to break down between crystals and fibers.

CASPAR: One point you make is that there is variation in these fibers in the micrographs. But the packing relations you put into your three-dimensional image are not constrained by the observed packing relation of the crystal. This of course fits the idea of a twisted crystal structure. As far as I understand, there are no threefold translational relations in the crystal of the antiparallel twofold screw structures.

EDELSTEIN: Of course not. We can argue by analogy to a certain point, but there is clearly a difference between a crystal and a fiber. At that point, the analogy breaks down.

CASPAR: The fiber is obviously not a crystal. Once the structure of the fiber is clear, we will have the answer. But it is certainly not clear at this point.

MAGDOFF-FAIRCHILD: I thank Don Caspar for having told half of my story and just want to add more points. It seems to me that if we can match the diffraction pattern of the fiber to a combination of the transforms of two crystals out to 0.5 nm resolution (we have a 13th order on some of the patterns) then the correspondence of these structures is indisputable. Our diffraction pattern suggests that there are either 12 or 16 individual filaments, that is, six or eight pairs. That will account for the fiber pattern diffraction to fairly high resolution and for the crystalline patterns as well.

My other point about the fiber and about your model is that in your model you have several pairs that are translated with respect to each other by a quarter of a molecular diameter. The Wishner crystal has this quarter translation. We do not know what it is in the second crystalline form, but it cannot be one-quarter. In this regard your proposed fiber structure does not match our pattern. How often do you see fibers that have lost 2 or 4 or 6 strands?

EDELSTEIN: We find them rarely, but when we find them they sometimes are continuous from the 14-filament fiber which makes us believe that they are degenerate forms of the 14 fiber. There are indications that the crystal contains pairs of pairs that are antiparallel. That's why people here suggested that there should be a multiple of 4 in the number of filaments. That seems a good starting point. But I think that the fiber and the crystal are actually different in that we have a 4 and 3 relationship rather than 4 and 4. If you feel you can interpret the detailed structures of the fiber into 12 or 16, I am happy to hear you. But I think on the basis of our evidence we have to accommodate the crystal information into a 14-filament structure.

MAGDOFF-FAIRCHILD: Eventually the transform of the 14-filaments and their arrangement must correspond to the x-ray diffraction pattern. That would check the validity of your structure.

EDELSTEIN: The detailed positioning of the atoms in our model is probably not sufficient to calculate the diffraction pattern since a small difference from quarter staggering would probably greatly influence the reflections you are talking about.

STERNLICHT: Fig. 4 of your paper implies that microtubule ensembles *in vitro* can have considerable disorder in terms of $\alpha\,\beta$. How much is this disorder? I was under the impression that optical diffraction shows extensive order out to 96 nm. How did you obtain insight into this disorder?

EDELSTEIN: This is done by computer transforms. There are two types of arrangements which will give reflections in different positions. The fact that we find 14 protofilaments means that you can not have a lattice in which all sides are α-β since that requires 13 or any odd number. That alone tells us that we must have a mixed lattice. We were in fact able to analyze the detailed computer transform and the predominant type of interaction was α-α or α-β, with about 20% α-β, β-α.

STERNLICHT: We have found that antimicrotubular drugs like colchicine will bind to subunit tubulin and copolymerize (Sternlicht et al., this volume). Is it possible that these drugs will go in and increase this disorder, thereby leading to changes in kinetics and equilibrium constants?

EDELSTEIN: There is no indication that this disorder is necessarily bad. It is just something we have observed in vitro. It is very possible that drugs could push the distribution we see in one or the other direction. I have given some thought to that, but not specifically to colchicine.

ENGLANDER: I gather that the fundamental double-strand structures you have are the same as the Wishner-Love crystals and presumably have the same contacts between the pairs. When you pack the doubles together, 7 at a time, they pack in a heterogeneous way. You must have many different sets of contacts that pack particular doublets together with other particular doublets. What is the minimum number of different areas that then have to be invoked to give you that structure?

EDELSTEIN: It is on the order of ten.

SCHECHTER: Would you like to comment why the structure punctuates and does not continue into a crystal?

EDELSTEIN: It is kind of a hexagonal lattice in cross-section that could keep extending. One thought was that putting certain protofilament pairs antiparallel would have an effect of punctuating it. A more general argument may simply be that in building a helix of this type, the further the filaments are from the center the more strain they are under in order to complete the helical twist. That may simply provide an energy barrier that limits expansion.

JOHNSON: It would seem that once a given lattice is established, it will be propagated so that a given polymer will be homogeneous along its length. Do you have any evidence that this is the case and that differences in polymers in a given preparation are a function of heterogeneity in the nucleation step?

EDELSTEIN: We know that if we initiate with a population of one type, then it propagates through. This concerns also the size. In the cell there seems to be only one kind of view of nucleation mechanism which restrains microtubules to the 13 protofilament form.