The difference CD (also observed with tubulin dimer) resembles that of the protein, with effects due to the aromatic chromophores; thus nucleotide dissociation causes a conformational change in the protein. Formally, it is possible that nucleotide interaction with a rigid site could generate a protein-based difference CD by strong coupling between the transition dipoles of the nucleotide and those of the aromatic residues. The expected exciton effects of such a mechanism are not observed. Additional evidence (proteolytic susceptibility, and protection by pyrophosphate and GDP) argues against a rigid conformation in the nucleotide-depleted protein.

The wavelength-range of the difference CD suggests predominant effects in tyrosine, as opposed to tryptophan residues, and this is borne out by the absence of any change in the fluorescence spectra. The 325 nm emission maximum of tubulin indicates strongly buried sites for probably all the tryptophan residues (four Trp per subunit). This is supported by the very low level of quenching attainable by external quenchers such as iodide. Both of these properties are largely preserved in the nucleotide-depleted protein. The results suggest that Trp-containing domains remain intact, but that tertiary structural changes occur, involving Tyr residues, when GDP dissociates.

Electron microscopy of the nucleotide-depleted MT-protein at 20°C shows the same ring-like oligomers as found in the control (Fig. 2 *a*, *b*). Sepharose-6B chromatography shows a similar distribution of tubulin between oligomer and dimer. At 37°C, amorphous aggregates are formed from nucleotide depleted MT-protein. Inclusion of 3 mM pyrophosphate, without added GTP, induces substantial formation of microtubules of normal morphology but of short length, indicating efficient nucleation (Fig. 2 *c*). These microtubules are cold, dissociable, and contain tubulin >50% depleted in E-site GDP. Under similar assembly conditions, AP-treated tubulin dimer behaves identically with 3 mM pyrophosphate on addition of MAP, and the microtubules formed are >85% depleted in E-site GDP (unpublished results). Thus, although pyrophosphate does not restore the CD typical of tubulin-GTP or tubulin-GDP, it is nonetheless effective in promoting microtubule assembly. This assembly involves incorporation of tubulin-GDP, probably in oligomeric form with MAP, consistent with the interpretation of biphasic kinetics of assembly of MT-protein (2).

DISCUSSION

These results show that whereas no conformational difference can be demonstrated by CD between reconstituted tubulin-(E)-GDP and tubulin-(E)-GTP under nonassembly conditions, removal of the (E)-nucleotide does cause specific protein conformational changes, which appear to involve tyrosine residues and the protein tertiary structure. These changes are not reversed by pyrophosphate ion, and we conclude that they are due to interactions involving the guanine moiety of GTP or GDP.

Nucleotide-depleted tubulin forms morphologically normal oligomeric species with MAP. Most significantly, it forms normal microtubules in the presence of pyrophosphate, and in the complete absence of any added GTP. This effect is apparently specific to pyrophosphate. These results imply that whereas occupancy of the guanine binding site causes conformational effects, binding at this site is not essential for assembly. We infer that the ability of pyrophosphate to promote assembly is due to its occupancy of the sites corresponding to the binding of the β–γ phosphate portion of GTP. Adoption of an assembly-competent state of tubulin dimer might therefore require no more than minor conformational readjustments from local electrostatic charge-neutralization, because of the simultaneous occupancy of two potentially adjacent positively charged sites by a negative bidentate ligand.

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MOLECULAR RECOGNITION IN MACROMOLECULES

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We are developing algorithms to model macromolecular assembly. Examples of assemblages exist at every level of complexity, ranging from individual proteins through protein:DNA complexes to ribosomal particles. Our initial studies have focused on the protein folding problem, but the approach is suited to multimolecular systems as well.

A globular protein is organized as a structural hierarchy, with little self-mixing between individual chain components (1–3). The observed hierarchic organization of these molecules is not intuitively obvious from inspection of physical models because segments of structure are sufficiently well packed in the native state that their individual identity is lost. To illustrate this point, Fig. 1 shows the solvent-accessible surface about two interacting helices from flavodoxin (4). Individual boundaries can no longer be discerned after the helices coalesce because intersegment packing is not distinguishable from intrasegment packing.

Although interacting segments may lose their individual identity, the observation that the native protein is organized as a hierarchy is compatible with folding by step-wise condensation of such segments. In particular, hierarchic organization assures that iterative models, in which segments merge to form successively larger segments, can ultimately result in a complete molecule or macromolecular complex.

The general process of packing three-dimensional segments together so as to optimize their mutual complementarity is termed "docking." Flexible docking exists when interacting segments can enhance complementarity by rotations about single bonds, especially single bonds in side chain residues. Initially, however, our algorithm has been limited to rigid docking, with segments treated as rigid bodies. A planned strategy for flexible docking will use the rigid docking algorithm as an inner loop.

A number of investigators have developed docking algorithms that are based on minimization of interaction potentials between associating units (5–9). However, when Lennard-Jones–type potentials are applied to all-atom representations of units the size of helices, the resultant energy surface is very pitty, reminiscent of the multiple-minimum problem for whole molecules. To circumvent this problem, several groups have resorted to simplified representations of structure (6, 7, 9), with consequent loss of detail.

Our remedy for the multiple-minimum problem has been to identify the prominent topographic surface features—the bumps and holes—in units of interest (10). Docking can then be reduced to a search for complementarity between features of opposite type, analogous to the assembly of a three-dimensional jigsaw puzzle. Because the number of topographic features is comparatively small for units the size of helices, a docking strategy based upon an exhaustive search becomes feasible.

The algorithm used to identify topographic features consists of the following sequential steps:

Atomic Coordinates

↓

Molecular Surface

↓

Cross-section Perimeters

↓

Two-dimensional Holes

↓

Two-dimensional Bumps

↓

Three-dimensional Bumps

↓

Three-dimensional Holes.

A detailed description of these steps is given elsewhere (10), but the overall ideas are sketched as follows. The molecular surface, as defined by Richards (11), is generated for segments of interest using an algorithm developed by Connolly (12). This surface is then reduced to an ordered set of uniquely defined cross-section perimeters

FIGURE 1 The solvent-accessible surface (12) about two interacting helices (63–75 and 94–106) from flavodoxin (4). Segments of structure are sufficiently well packed in protein molecules that individual segment boundaries are no longer apparent.

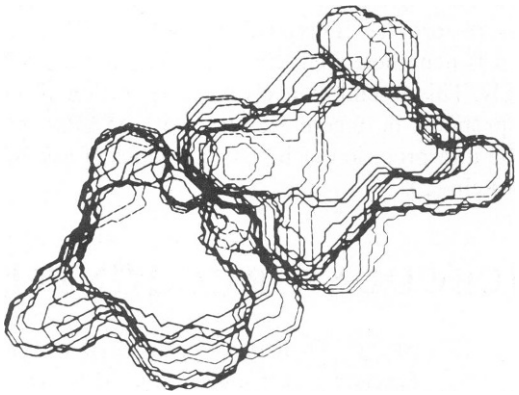

FIGURE 2 Molecular docking between the same two helices shown in Fig. 1. The surface of each helix is represented by an ordered set of cross-section perimeters. Three-dimensional features are then identified within this surface representation. Specificity results from complementarity between features of opposite type.

that well represent the surface topography of the segment. The topographic features of interest (the bumps and holes) are now located within this representation of the surface. Initially, all the two-dimensional bumps and holes are identified within every cross-section. These two-dimensional features are then used in combination to construct their three-dimensional counterparts.

Exquisite specificity is characteristic of molecular docking. The representation of surface topography, as described accurately, conserves such specificity (see Fig. 2). Equally important, the set of discovered three-dimensional features is small enough to permit matching by exhaustive trial between complementary types.

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SIMPLE CHARGE-MEDIUM INTERACTION MODELS OF AMPIPHILIC PROTEINS WITH UNKNOWN TERTIARY STRUCTURE

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Many secreted proteins have unresolved crystallographic structure. Secondary structure can be predicted by several methods, but tertiary organization is unknown. Tertiary organization, with the resultant solvent surface contours, is the most important determinant of function, stability, and bioactivity of proteins. Electrostatic effects dominate the protein surface and its interaction with the surrounding layer of water molecules (1). Double basic residues (R,K) serve as recognition signals for post-translational modification of preproteins and as anchor points for trypsin-like enzymatic cleavage (2).

In a particular protein the pattern of charged, flexible, and polarizable side chains superimposed on secondary structure of polypeptide backbone is unique. Such patterns can be represented by superposition of charge/dipol clusters on the hydropathic profiles (3) (amino acid sequences oriented along a protein's amphiphilic axis). Examples of the cluster patterns for three different globular proteins, shown in Figs. 1–3, reveal substantial differences in their electrostatic environment. Hydrophilic charged microregions are sharp and dense for growth hormone; spotty and diffuse for ribonuclease; and absent for ubiquitin. However the average charge density is similar for all three proteins.

Small globular proteins devoid of quaternary structure show the largest effect of electrostatic interactions on protein conformation. In the early stages of protein folding these interactions can be seen in backbone-side residue interactions (4), and in later stages in solvent-protein surface behavior (aggregation, salt effects)(5). We are attempting to use the charge/dipol protein pattern in computer modeling to predict gross, general features of tertiary structure.

The principal feature of the model consists of substitution of amino acids for atoms in a computer program designed for modeling small molecules with up to 2,000 atoms. The schematic protein model can then be visually manipulated on the screen. The amino acid sequence of globular protein can be represented by a chain of cubic elements (amino acid residues) separated by 4–6 Å (6). The amino acid residue elements are defined as "bulk elastic points" with a short list of parameters (hydrophobicity, fractional charge, pK, side chain flexibility and length, solvent static accesibility, volume, α or β propensity) averaged by the moving window technique. The model is built within known sterical constraints for α-helix, β-sheet, and β-strand, taking advantage of flexibility of