The quaternary structures of several monomeric and dimeric kinesin constructs from *Homo sapiens* and *Drosophila melanogaster* were analyzed using small angle x-ray and neutron scattering. The experimental scattering curves of these proteins were compared with simulated scattering curves calculated from available crystallographic coordinates. These comparisons indicate that the overall conformations of the solution structures of *D. melanogaster* and *H. sapiens* kinesin heavy chain dimers are compatible with the crystal structure of dimeric kinesin from *Rattus norvegicus*. This suggests that the unusual asymmetric conformation of dimeric kinesin in the microtubule-independent ADP state is likely to be a general feature of the kinesin heavy chain subfamily. An intermediate length *Drosophila* construct (365 residues) is mostly monomeric at low protein concentration whereas at higher concentrations it is dimeric with a tendency to form higher oligomers.

Motor proteins such as dyneins, myosins, and kinesins convert the energy of ATP hydrolysis into mechanical work. They are involved, among many other processes, in intracellular transport, cell division, and muscle contraction. Kinesins form a superfamily of microtubule-associated motor proteins with about 240 members identified to date.¹

Two of the best described kinesin motors are conventional kinesin (kinesin heavy chain (KHC) subfamily, Ref. 2), the founding member of this superfamily that moves toward the plus end of microtubules, and ncd (non-claret-disjunctional, Ref. 3) that moves toward the minus end (C-terminal subfamily). Conventional kinesin (4) is a heterotetrameric protein composed of two heavy and two light chains. The heavy chains have a three-domain structure; the globular N-terminal motor domain, which contains the ATP- and microtubule-binding sites, is about 340 amino acids long. The heavy chains of kinesin dimerize through an α-helical coiled-coil region, the so-called stalk domain. The third domain consists of a C-terminal globular tail that is thought to be involved in light chain and cargo binding. Several fragments of the kinesin heavy chain from different species have been cloned and characterized. Depending on the length of the coiled-coil region included in these fragments, these constructs preferentially form either monomers or dimers.

Two of the major challenges in kinesin research are to determine the function of these motors in different organisms (5), and to unravel the molecular details of the mechanochemical cycle of a kinesin motor moving along its track. As already suggested in the case of myosin (6), crystal structures of different nucleotide states may represent snapshots of long lived intermediates in the ATP hydrolysis cycle (7–11). Combined with other methods such as electron cryomicroscopy and three-dimensional image reconstruction, x-ray crystallography is a powerful tool to investigate the general mechanism by which chemical energy is converted into mechanical work and movement by the different motor protein superfamilies.

The mechanism of energy conversion in the kinesin superfamily is still incompletely understood. For conventional kinesin, the crystal structures are known for the motor domain itself (12–13) and for one functional dimer (14), both in the microtubule-independent ADP form. The motor domain consists of an eight-stranded mixed β-sheet, flanked on both sides by three α-helices. It contains one tightly bound MgADP molecule in the active site. These crystal structures and those of ncd (15–17) have revealed important features and in particular the very high structural similarity of the motor domains of different members of the kinesin superfamily. Surprisingly, kinesins also have a strong structural similarity with the core of myosin despite the lack of similarity at the amino acid sequence level. The crystal structure of dimeric kinesin revealed an unusual quaternary structure where the two heads of the dimer are related by a rotation of about 120° around an axis that is close to that of the α-helical coiled-coil and not, as expected, by twofold symmetry (Fig. 1a). Apart from the coiled-coil interaction, the two heads have no other areas of direct contact.

To assess the relevance of the crystal structure of dimeric kinesin in the microtubule-independent ADP state, it is important to establish if this asymmetric structure also exists in solution or results from crystal packing effects. Fitting crystal structures of dimeric motor proteins into three-dimensional image reconstructions of microtubules decorated with these motors is obviously only legitimate if these structures are undistorted. In contrast, if the asymmetric conformation found in the crystal also exists in solution, there would be at least a hint as to whether or not kinesin changes its conformation upon binding to microtubules.
We have chosen to work with conventional kinesins from *Drosophila melanogaster* and *Homo sapiens* because they have been well studied biochemically and enzymatically. The *Drosophila* full-length kinesin heavy chain (18–19) and its light chains (20–22) are well characterized, and several *in vivo* mutants have been described (23). Expression clones coding for kinesin heavy chain fragments of nearly any desired length exist (24–29). Monomeric constructs of about 340 residues covering the motor domain or short dimeric ones including the motor domain, linker, and dimerization domain have been comprehensively studied by biochemical methods, and their ATPase properties and oligomeric characteristics are known. These protein fragments, in the stable ADP-bound form, can be obtained quickly and in large amounts. Both fruit fly and human conventional kinesins share a high sequence similarity with their rat homolog. The size of the DKH381\(^2\) (28) and HK379 fragments (30–31) is in the same range as that of the rat kinesin dimer RK379 used for structure determination, thus justifying a direct comparison between crystal and solution structures.

Small angle scattering bridges the gap between structural and hydrodynamic methods and between crystallography and electron microscopy. The technique is sensitive to the scale of conformational changes that often occur as a result of substrate or effector binding (32–33) or as a consequence of crystal packing forces (34). Furthermore, solution scattering data can also be usefully be incorporated into contrast transfer function correction (CTF) procedures for electron microscope image reconstruction (35) and can complement low angle diffraction and electron microscopy in the study of large structures (36).

The aim of the present work was to use small-angle x-ray and neutron scattering first, to describe and quantify the self-association behavior of monomeric and dimeric kinesin fragments from human, and *D. melanogaster*. Further, we aimed to compare the solution structures of human and *Drosophila* conventional kinesin in the microtubule-independent ADP state to that of the crystal structure of rat kinesin to verify its asymmetric structure. Comparison of conventional kinesins from different sources should allow more general conclusions to be drawn concerning the overall conformation in this important kinesin subfamily. We show that the resolution obtained by scattering methods should be sufficient to detect nucleotide-dependent conformational changes of dimeric kinesin motors in solution.

**EXPERIMENTAL PROCEDURES**

**Purification of Recombinant Proteins**—The plasmids pDKH365, pDKH381, and pDRK392 were a kind gift from David Hackney (University of Pittsburgh), plasmid pDKH337 was kindly provided by Sharon Endow (Duke Medical Center), plasmid pHKH379 was a generous present from Ronald Vale (University of California). All protein purifi-
cation steps were performed at 4 °C. Escherichia coli cells were always disrupted twice with a French pressure cell and the resulting crude extract was cleared by centrifugation at 15,000 × g for 1 h to remove insoluble material.

The purification of the kinesins used in this work was performed as follows. DKH337 was purified as described previously for DKH340 (24), DKH365, DKH381, and DKH392 were purified according to Jiang and Hackney (28) with the following modifications. As an additional step, the proteins were concentrated using a Centricon YM-30 (Amicon) and loaded onto a gel filtration column (Sephacryl S-300, Amersham Pharmacia Biotech), previously equilibrated in buffer A (20 mM Pipes pH 7.3, 150 mM NaCl, 1 mM MgCl₂, 1 mM dithioreitol, 1 mM EGTA). The eluted peak fractions were reconcentrated, and fractions of 150 μl/tube in the range of 1–32 mg/ml were frozen in liquid nitrogen, and stored at −80 °C until use.

For molecular weight determination of proteins by gel filtration chromatography, we used a self-packed Sephacryl 300 S column for preparative work and a Superose 12 column for analytical studies. Albumin (67 kDa), ovalbumin (43 kDa), chymotrypsinogen (25 kDa), and ribonuclease A (13.7 kDa) were used as standards. HK379 was purified according to Ma and Taylor (30), introducing the following modifications. As an additional step, the cells were grown overnight at 20 °C, instead of 33 °C. As a final step, the protein was loaded onto a gel filtration column, as described above for Drosophila kinesin fragments.

For data collection, the freshly thawed proteins were either directly measured in 100-μl quartz cuvettes, centrifuged at 10,000 × g during 30 min at 4 °C, or subjected to an additional gel filtration. This procedure allowed the protein solutions to be studied under the best possible monodisperse conditions.

X-ray Scattering Experiments and Data Processing—Synchrotron radiation x-ray scattering data were collected following standard procedures on the X33 camera (37–39) of the EMBL on the storage ring DORIS III of the Deutsches Elektronen Synchrotron (DESY) and multiwired proportional chambers with delay line readout (40). The lower concentration solutions (from 1 to 16 mg/ml) were measured at a sample-detector distance of 4.0 m covering the range of momentum transfer 0.1 nm⁻² < s < 2.0 nm⁻¹ (s = 4πsinθ/λ, where θ is the scattering angle and λ a of 0.15 nm is the wavelength). The more concentrated solutions (20–35 mg/ml) were recorded at sample-detector distances of 2.0 or 1.4 m to cover the ranges 0.2 nm⁻² < s < 3.5 nm⁻¹ or 0.3 nm⁻² < s < 5.0 nm⁻¹, respectively. The data were normalized to the intensity of the incident beam and corrected for the detector response. The scattering of the buffer was subtracted and the difference curves were scaled for concentration. All procedures involved statistical error propagation using the program SAPOKO. The scattering patterns recorded in the range 0.1 nm⁻² < s < 2.0 nm⁻¹ were extrapolated to zero concentration following standard procedures (42) and merged with the higher angle data to yield the final composite scattering curves.

The maximum dimensions of the particles in solution were estimated using the orthogonal expansion program ORTOGNOM (43). The forward scattering I(0), distance distribution functions π(r) and radii of gyration Rg were evaluated with the indirect transform package GNOM (44–45). The molar masses (MM) of the solutes were calculated by comparison with the forward scattering from reference solutions of bovine serum albumin (MM = 66 kDa).

Data Analysis—The coordinates for the construction of atomic models for monomeric and dimeric kinesins were taken from the Protein Data Bank (Ref. 46, accession number 3KN1). The amino acid sequences of the rat, fruit fly, and human conventional kinesin were aligned including the first 392 residues of Drosophila kinesin, the longest construct used in this study. The model structure used to compare the simulated solution scattering curves was based on the atomic model of rat kinesin where identical and highly similar residues were kept, whereas those with a low similarity or different ones were replaced by alanines. Two regions in the rat kinesin crystal structure were not resolved probably because of their flexibility: the first one is loop LI (Ser⁴⁰⁰–Asn⁴⁰⁵), which is absent in all conventional kinesin structures solved so far. The second missing region is at the end of the polypeptide chain starting from residue Arg³⁷⁰. In the shorter kinesin fragments (DKH381, DKH392), the excess residues at the C terminus were added at the C terminus.

The scattering curves were calculated from the atomic models using the program CRYSOL, an improved version of the programs CRYSOl (48) and CRYSON (49), which take the scattering from the solvation shell into account. In this model, the macromolecule is surrounded by a 0.3-nm thick hydration layer with an adjustable density ρh that may differ from that of the bulk solvent ρ. The scattering from the particle in solution is,

\[ I(s) = \frac{1}{N-1} \sum_{i=1}^{N} \left[ \frac{I_\text{exp}(s_i) - I(s)}{\sigma(s)} \right]^2 \]  

where N is the number of experimental points, and I(s), I_\text{exp}(s), and σ(s) denote the calculated intensity, the experimental intensity and its standard deviation, respectively.

The volume fractions of monomers and dimers for DKH365 solutions at different concentrations were calculated using the program Oligomer.

Neutron Scattering Experiments—Neutron scattering experiments were performed on the D22 small angle instrument at the Institut Laue-Langevin in Grenoble (51). Samples were contained in quartz cells (Helma) of 1.00-mm optical path length. The sample temperature was 6 °C during experimental runs. The sample concentrations were between 1 and 10 mg/ml. Data were collected using a neutron wavelength, λ = 0.6 nm with a spectral width of 8% and a sample detector distance of 4.0 m, to cover the range of the momentum transfer, s (0.1 nm⁻² < s < 2.3 nm⁻¹). Data were corrected for buffer scattering and normalized to the scattering of 1.0 mm of H₂O in the standard manner. The scattered intensity, I(Q), in the small angle range (s < 0.4 nm⁻¹), was analyzed according to the Guinier approximation as described (52),

\[ \ln I(s) = \ln I(0) - 1/3 R_g^2 s^2 \]  

where I(0) is the forward scattered intensity from which the molar mass of the scattering particle can be calculated (53), and Rg is the radius of gyration of contrast in the particle. The scattering curves in the full s range were analyzed using the GNOM program (44–45).

RESULTS

The aligned sequences of conventional kinesin heavy chains from rat (residues 2–379), human (residues 2–379), and fruit fly (residues 1–392) are shown in Fig. 2. The amino acid identity between rat and human kinesin throughout the motor domain, linker, and part of the first coiled-coil is 86%, whereas between rat and fruit fly kinesin it is 74%. In comparison with the other two species, Drosophila kinesin displays a slightly longer N-terminal region with an additional 5 residues (because of N-terminal excision in E. coli the first Met is missing in rat as well as in the human kinesin, Ref. 54) and a two-residue insertion inside the small three-stranded antiparallel β-sheet between β1a and β1b. Both rat and fruit fly kinesin have two additional one-residue insertions. The first occurs in the second loop region of the three-stranded β-sheet flanked by β1b and β1c and the second one in loop L12 between α4 and α5 (see Fig. 1b). All together, the sequences are highly similar, making direct structural comparisons possible.

Drosophila Kinesin DKH337—Two similar, slightly longer Drosophila constructs DKH340 and K341 have been studied extensively (24–25, 28, 55). It has been shown that DKH340 contains a stoichiometric amount of tightly bound ADP. The
protein is monomeric, as shown by sucrose gradient velocity centrifugation ($S_{20,w}$ value of 3.3 S) and gel filtration (39 kDa). These results are in general agreement with work showing that the K341 construct is a monomer in solution up to at least 10 mM, as established by sedimentation velocity and sedimentation equilibrium methods (55). At higher protein concentrations, the data suggest that there is a slight irreversible aggregation in solution.

DKH337 is equivalent to a rat protein construct of 331 residues (Figs. 1b and 2) including the complete motor domain and part of the linker region up to b9. The helix a7, responsible for dimerization by forming an a-helical coiled-coil, is absent. Gel filtration chromatography of DKH337 yields a molar mass of 38 kDa (data not shown).

The composite x-ray scattering curve from DKH337 in Fig. 3 yields a molar mass of $39 \pm 4$ kDa for the construct in good agreement with the value estimated from the primary sequence of the monomeric protein (36 kDa). The maximum dimension of the particle and the radius of gyration are $7.0 \pm 0.5$ nm and $2.15 \pm 0.02$ nm, respectively. These parameters are close to those evaluated from the atomic model of the monomer displayed in Fig. 1b, and the scattering curve computed from the atomic model is in excellent agreement with the experimental data (Fig. 3, $\chi = 0.67$ for an excluded volume $V = 48.2$ nm$^3$ and a contrast in the hydration shell $\rho_{hy} = 42$ e/nm$^3$). It can thus be concluded that DKH337 is monomeric in solution and that there are no significant differences between the overall structure of the monomer in the crystal and in solution.

Drosophila Kinesin DKH365—This kinesin construct was previously shown to be monomeric in solution at concentrations between 0.01–0.03 mM used for ATPase assays (28). Sucrose density centrifugation yields a $S_{20,w}$ value of 3.5 at concentrations up to 1 mM and of 3.7 at 20 mM. DKH365 elutes at the same position as monomeric DKH340 in gel filtration experiments. This indicates that DKH365 is also monomeric under these conditions.

DKH365 would correspond to a rat kinesin construct of 359 residues (Figs. 1b and 2) including the complete motor domain, linker, and five complete turns of the a7 helix, part of the dimerization domain. The x-ray scattering patterns from DKH365 recorded in the range 0.1 nm$^{-1} < s < 2.0$ nm$^{-1}$ for protein concentrations from 1 to 16 mg/ml in Fig. 4 yield the apparent molar masses and radii of gyration presented in Ta-
composite curve can be reasonably well fitted (Fig. 5) by that
yield a composite scattering curve of dimeric DKH365. The
responds most closely to the scattering from pure dimers was
with protein concentrations (Fig. 1a). The curves and dimer (asymmetric model). The radius of gyration of the symmetric
dependent monomer-dimer equilibrium of DKH365 in solution and in the model
generated from the truncated rat kinesin structure. The systemic deviations between these curves at very small angles can be attributed to the fact that the solutions of DKH365 are not entirely monodisperse.

Drosophila Kinesin DKH381—The kinesin fragment DKH381 is predominantly dimeric in solution as proven by sucrose density centrifugation and gel filtration (28). It has been shown that the protein tends to aggregate at low salt concentration but is mostly monodisperse at NaCl concentration of 100 mM or higher. This Drosophila kinesin construct corresponds to a rat kinesin dimer (residues 2–369) plus six additional residues at the C-terminal end of the polypeptide chain. Because the difference is very small, the six missing residues were not included in the model.

The molar mass computed from the composite scattering curve from DKH381 in Fig. 6 is 75 ± 8 kDa suggesting that the protein is a dimer in solution (the value estimated from the primary sequence of the dimer is 80 kDa). The maximum dimension of the particle and its radius of gyration are 13 ± 1 nm and 3.82 ± 0.05 nm, respectively, in agreement with the atomic model in Fig. 1a. The scattering curve computed from this model yields an excellent fit (Fig. 6) to the experimental data with $\chi = 0.74$ at $V = 103$ nm$^3$ and $\delta_r = 24$ e/nm$^3$. Clearly DKH381 forms stable dimers in solution, and the atomic model in Fig. 1a is fully compatible with the solution scattering data.

To check whether or not a DKH381 construct with twofold symmetry would also be compatible with the scattering data, the model in Fig. 1a was symmetrized and compared with the experimental data. The resulting value of $\chi = 0.90$, which, although still acceptable, is worse than that provided by the asymmetric model. The radius of gyration of the symmetric model ($R_g = 4.0$ nm) significantly exceeds the experimental value In fact, major systematic deviations ($\chi = 1.15$) are observed between the experimental data and the scattering curve of the asymmetric model in the initial part of the curves ($s < 0.22$ nm$^{-1}$). In contrast the asymmetric model yields a good fit in this region with $\chi = 0.83$. The initial portion of the curve contains most information about the quaternary structure (i.e. the organization of the dimer), whereas its outer part is dominated by the contribution from the internal structure of the monomers. Inspection of the deviation at $s < 0.22$ nm$^{-1}$ also indicates that the theoretical curve from the asymmetric dimer corresponds to a more extended structure than the experimental one. Given that the model was symmetrized so as to obtain the most compact mutual positions of the monomers and that all other possible symmetric configurations would yield even larger radii of gyration, it is very improbable that DKH381 would have a 2-fold symmetry axis in solution. Also, if the experimental curve were slightly influenced by aggregation, the difference with the calculated and the symmetric model would be even larger.

Drosophila Kinesin DKH392—The extensively characterized kinesin construct DKH392 (25–29) has been shown to be a dimer in solution containing one tightly bound ADP per head domain. The comparable rat kinesin construct would have 385 residues, thus containing 16 additional C-terminal residues.

The molar mass of DKH392 computed from the primary sequence of the dimer is 83 kDa, and the estimate (80 ± 10 kDa) obtained from the composite scattering curve from the construct in Fig. 7 is also compatible with a dimeric structure. The maximum dimension of the particle and its radius of gyration ($14 \pm 1$ nm and $4.17 \pm 0.07$ nm, respectively) are larger.

![Fig. 4. X-ray scattering from DKH365 solutions at different protein concentrations (points with error bars) and fits by linear combinations of the scattering from the DKH365 monomer and dimer (solid lines). Curves (1–8) correspond to the protein concentrations in Table I.](http://www.jbc.org/Downloaded from)
than the corresponding values for DKH381 suggesting that the 16 extra amino acids in the α-helical coiled-coil make the construct more extended. The scattering curve computed from the atomic model of DKH381 fits the experimental data with $\chi = 2.06$ for $V = 103$ nm$^3$ and $\delta p_b = 24$ e/nm$^3$ (Fig. 7). Addition of 16 amino acids to each of the monomers of DKH381 to extend the coiled-coil region yields a more complete model. This model provides a somewhat better fit to the experimental data in Fig. 7 ($\chi = 2.00$ at $V = 113$ nm$^3$ and $\delta p_b = 52$ e/nm$^3$). Attempts to add the 16 amino acids in different ways (not extending the coiled-coil interaction region) yielded worse fits than that obtained with the original DKH381 construct.

**Human Kinesin HK379**—This human kinesin construct is a dimer in solution under standard conditions as shown by gel filtration and equilibrium ultracentrifugation (30–31). It has extremely tightly bound ADP in the active site (30). In terms of alignment of the sequence at the C-terminal end, HK379 corresponds to a rat kinesin construct of 381 amino acids (Figs. 1 and 2). In comparison with the rat kinesin crystal structure, HK379 contains 12 additional residues at the C terminus.

The composite x-ray scattering curve from HK379 in Fig. 8 obtained by extrapolation to zero concentration of the experimental curves corresponding to concentrations between 1 and 9 mg/ml yields an estimate of the molar mass of the solute $120 \pm 15$ kDa, which is much larger than the theoretical molar mass of a dimer (85 kDa). The maximum dimension and the radius of gyration ($19 \pm 2$ nm and $5.2 \pm 0.1$ nm, respectively) are also significantly larger than the values expected for a HK379 dimer. This suggests that higher oligomers are present in the solutions of HK379 and that the contribution from these oligomers is not removed by extrapolation to zero concentration. The atomic model of dimeric HK379 was therefore validated against the composite scattering curve omitting the inner part of the data ($s < 0.05$ nm$^{-1}$). In this range a fairly good fit ($\chi = 1.32$) was obtained at $V = 102$ nm$^3$ and $\delta p_b = 24$ e/nm$^3$ (Fig. 8). It can thus be concluded that the solutions of HK379 contain large aggregates at all concentrations but also that the crystallographic model of the dimeric human kinesin is compatible with the outer part of the scattering pattern.

The neutron scattering patterns collected in D$_2$O (not shown) displayed even stronger aggregation effects than the corresponding x-ray data and could not be used for validation of the HK379 model. The difference between the solution in H$_2$O and D$_2$O gives an indication as to the type of interactions, because D$_2$O is well known to enhance hydrophobic interactions (56). The situation was much more favorable for the neutron data collected in D$_2$O when applying gel filtration chromatography as an additional step directly before the measurements. The scattering curve from HK379 in H$_2$O extrapolated to zero concentration of the solute (Fig. 9) yields an estimate of the molar weight of $80 \pm 4$ kDa, and the $D_{max}$ and $R_g$ values of $14 \pm 1$ nm and $4.1 \pm 0.1$ nm, respectively. These values are very close to those expected for a dimer of HK379 and suggest that the H$_2$O

### Table I

**Oligomer content in the DK365 mixtures**

| Concentration (nominal/measured) | $\chi$ | Molar mass | $R_g$ | $V_{max} (\text{molar})$ | $V_{dimer}$ |
|---------------------------------|-------|------------|------|----------------------|-------------|
| mg/ml                           |       | kDa        | nm   |                      |             |
| 1/1                             | 0.61  | 45 ± 5     | 2.70 ± 0.20 | 0.943 ± 0.048 | 0.057 ± 0.023 |
| 3/3                             | 1.04  | 70 ± 7     | 3.46 ± 0.07 | 0.640 ± 0.023 | 0.360 ± 0.011 |
| 5/6.5                           | 1.14  | 71 ± 10    | 3.40 ± 0.04 | 0.616 ± 0.019 | 0.384 ± 0.009 |
| 8/8.8                           | 1.60  | 82 ± 8     | 3.56 ± 0.03 | 0.392 ± 0.014 | 0.608 ± 0.007 |
| 8/10.7                          | 1.41  | 97 ± 10    | 3.76 ± 0.02 | 0.255 ± 0.015 | 0.745 ± 0.007 |
| 10/12.5                         | 2.22  | 118 ± 10   | 4.00 ± 0.02 | 0.000 ± 0.015 | 1.00 ± 0.008 |
| 12/14.9                         | 2.41  | 121 ± 10   | 4.06 ± 0.02 | 0.000 ± 0.015 | 1.00 ± 0.008 |
| 16/16                           | 2.50  | 109 ± 10   | 3.97 ± 0.02 | 0.000 ± 0.013 | 1.00 ± 0.007 |

**Fig. 5.** Composite x-ray scattering curve from DKH365 (points with error bars) and calculated scattering from the generated crystallographic model of the DKH365 dimer (solid line).

**Fig. 6.** Composite x-ray scattering curve from DKH381 (1) and calculated scattering from the generated crystallographic model of the DKH381 dimer (2) and the symmetrized dimer (3).
solutions of HK379 analyzed by neutron scattering contain largely dimers. The atomic model of HK379 yields a very good fit ($\chi^2 = 1.15$) at $V = 98$ nm$^3$ and $\delta_{\text{res}} = 0$ (Fig. 9). Lack of contrast between the hydration layer and the bulk water is not surprising for a neutron study in H$_2$O as the neutron scattering length density of water is nearly zero and variations in the density of the bound water hardly influence the scattering pattern (49). The crystallographic model of the dimeric human kinesin is thus corroborated by the neutron scattering data in H$_2$O.

DISCUSSION

We have investigated monomeric and dimeric conventional kinesin constructs using small angle x-ray and neutron scattering (summarized in Table II) and compared members of the conventional kinesin heavy chain subfamily from different species. The most important result of this investigation is that the solution structures of two dimeric constructs, DKH381 and HK379, are very similar to the asymmetric structure of dimeric rat kinesin in the microtubule-independent ADP bound form, the only dimeric kinesin crystal structure solved so far.

This is the third study to examine the solution structure of dimeric kinesin. The first x-ray scattering study (57) compared the solution and crystal structure of dimeric rat kinesin at low resolution at about 5 nm. In their study they found that the radius of gyration ($R_g$) of 4.0 $\pm$ 0.2 nm is somewhat larger but close to the theoretical value of $R_g = 3.6$ nm, which they derived from the atomic coordinates of the rat dimer structure. The construct RK379, which is dimeric in the crystalline state was shown to be also predominantly dimeric in solution. It was suggested that the distance between the heads in the dimer is about 1 nm larger in solution than in the crystalline state. We believe that improved data for the rat kinesin construct covering a broader resolution range should yield a clearer answer.

A second more extended small angle x-ray and neutron scattering study investigated the solution structure of the dimeric human kinesin hKIN420 (58). The authors reported that the radius of gyration ($R_g$) of 4.05 $\pm$ 0.075 nm is significantly smaller than that of the model generated from the crystallographic structure by adding the missing 51 residues to the C terminus ($R_g = 4.5$ nm). They found that the overall experimental scattering pattern poorly fitted to the simulated curve calculated from the model of dimeric rat kinesin. This was attributed to a difference in the orientation of the two head domains of the dimer in solution and in the crystal and a model that is more consistent with the measured data was proposed. This model has a mushroom-like shape rather than the T-like orientation of the catalytic cores found in the crystal structure. The center of mass separations of the catalytic cores in the best fitting model are 0.7–1.0 nm smaller than in the crystal struc-
tecture. We suggest that these observed differences can be explained as follows.

In the present study two much smaller kinesin fragments were used, namely HK379 (residues 2–379) and DKH381 (residues 1–381), which are quite similar to the fragment RK379 (residues 2–379) used for the structure determination. The crystal structure represents 93 and 91% of the entire model (residues 2–379) used for the structure determination. The coordinates that are required, especially at the unknown C-terminal end of the proteins. In contrast, KIN420 is already much longer with an additional stretch of 53 residues at the C terminus of the protein, which makes modeling necessary. Together with the missing 15 residues in loop L11, the coordinates of dimeric kinesin account for only 84% of KIN420. Further difficulties arise because the missing C-terminal region is predicted to also include the first so-called “hinge” region. This region is supposed to give conventional kinesin some flexibility by interrupting the coiled-coil and forming a loop region. The two programs PAIRCOIL (50) and COIL (47) predict that for the human kinesin amino acid sequence this region starts already at about Gly530 and stretches out to about Gly561. It should thus be quite difficult to model this missing region in a manner that takes all these aspects into account. The shorter human kinesin HK379 thus seems more suitable for the comparison between solution and crystal structure.

One reason for choosing the longer kinesin construct KIN420 was the aggregation reported for shorter dimeric constructs (28, 41). In fact, under our experimental conditions, we observed aggregation and the presence of higher oligomers in the solutions of HK379 at all protein concentrations, as well as for the other constructs used at protein concentrations above 10 mg/ml. This effect was even stronger in solutions of D2O. For the x-ray data this problem was circumvented by omitting the inner part of the scattering pattern (s < 0.05 nm⁻¹). This approach is valid, because the contribution of very large aggregates mainly influences the very small angle part of the curves. In this range a fairly good fit was obtained (χ² = 1.32), and it can be safely concluded that this short human kinesin fragment is compatible with the crystallographic rat kinesin model. To further confirm this result HK379 was subjected to a rapid gel filtration chromatography immediately before collecting neutron data at 6 °C in D2O. In this case the calculated radius of gyration of 4.1 ± 0.1 nm was close to that calculated from the model (generated by adding the missing C-terminal residues to the crystal structure), and the comparison between crystal and solution structure yielded a good fit (χ² = 1.15). The Drosophila construct DKH381, which resembles the RK379 crystal structure even better because of a shorter C-terminal coiled-coil region, gave a further confirmation. Its aggregation behavior, which is highly dependent on the salt concentration, is very well characterized (28). Above 100 mM NaCl the S20,w value of 5.0 S is already consistent with the absence of aggregation. Therefore, we collected the scattering pattern at 200 mM NaCl, without modeling the missing residues in loop L11 and at the C terminus. An excellent fit consistent with the crystal structure was obtained (Fig. 6).

The purification methods and sample treatment used in this study were somewhat different from that of Stone et al. (58). We have avoided dialysis to speed up the purification process. Instead of performing an additional ultracentrifugation step just before the measurements, gel filtration chromatography was used to separate the solute protein from aggregates.

Another interesting result of our study is the concentration-dependent monomer-dimer equilibrium of DKH365 that is monomeric in solution at concentrations of up to about 1 mg/ml (25 μM). At about 10 mg/ml (0.25 mM) it is mostly dimeric with a tendency to form higher oligomers (Table I). These results on the concentration-dependent behavior of DKH365 are in agreement with two other studies, performed at lower concentrations, which investigate the oligomeric state at concentrations up to 10 μM and 20 μM, respectively. Jiang et al. (28) showed that DKH365 is monomeric in gel filtration experiments and in solution at concentrations up to 0.05 μM (1.2 μg/ml) used for ATPase assays. Sucrose density centrifugation yielded an S20,w value of 3.5 at 1.0 μM (40 μg/ml) and a slightly increased value of 3.7 at a concentration of 20.0 μM (0.8 mg/ml). At a concentration of 25 μM (1.0 mg/ml) the DKH365 solution already contains about 6% dimeric portion (Table I), which could explain the increase.

In another study, Correia et al. (55) investigated the oligomeric state of three different Drosophila kinesin constructs using sedimentation velocity and sedimentation equilibrium methods. The smallest construct, K341, was found to be monomeric up to a concentration of 10 μM. Construct K366 is only one residue longer than DKH365. Sedimentation velocity experiments on K366 at different concentrations up to 4 μM (160 μg/ml) yielded an S20,w value of 3.25 S. Data from sedimentation equilibrium studies up to a concentration of 10 μM (0.4 mg/ml) could be best described with a 1-2-4-8 model and revealed the presence of a small amount of higher oligomers. An important conclusion that can be drawn from the present study on the concentration-dependent behavior of DKH365 is that when studying dimeric motor proteins, care should be taken to use sufficiently long kinesin constructs.

In the future, x-ray and neutron solution scattering will be useful as a straightforward check to investigate whether in vivo mutants of conventional kinesin have a distorted overall solution shape compared with the wild-type motor dimer. This is especially true for mutants in the core-neck interface, the
region responsible for dimer formation. Additionally, several members of the same subfamilies can be compared with test whether they all display the same overall conformation. We are investigating whether it is possible to detect nucleotide-dependent conformational changes of dimeric conventional kinesin and not in solution in the absence of microtubules. Biochemically well characterized dimeric motors are available from which the bound ADP can be reversibly removed (28, 30) and reloaded with e.g. non-hydrolyzable ATP analogs. Especially in combination with neutron scattering, solution scattering curves of nucleotide-free motors could be compared directly to those with bound nucleotide (either ADP or ATP analogues), which have been formed in situ by adding the nucleotide derivatives. This approach may also be useful in finding crystallization conditions for motors in an ATP-like state.

Acknowledgments—This work would not have been possible without the support of several people. We thank Sharon Endow for the plasmid pDKH337. Plasmid pHKH379 was a gift kindly provided by Ronald Vale. Plasmids pDKH365, pDKH381, and pDKH392 were a kind gift from David Hackney. Jean Pierre Andrieu of the Laboratoire d’Enzymologie Moléculaire at the IBS performed the N-terminal sequencing of human kinesin.

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J. Biol. Chem. 2001, 276:1267-1275.
doi: 10.1074/jbc.M007169200 originally published online October 4, 2000

Access the most updated version of this article at doi: 10.1074/jbc.M007169200

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