Two Functional Thioredoxins Containing Redox-sensitive Vicinal Dithiols from the Chlamydomonas Outer Dynein Arm*

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Ramila S. Patel-King, Sharon E. Benashski, Alistair Harrison, and Stephen M. King‡

From the Department of Biochemistry, University of Connecticut Health Center, Farmington, Connecticut 06032-3305

We describe here the molecular cloning and analysis of the M, 14,000 and 16,000 outer arm dynein light chains (DLCs) from Chlamydomonas flagella. Within the outer arm, the M, 14,000 DLC apparently is associated with the intermediate chains at the base of the soluble dynein particle; the M, 16,000 DLC interacts directly with the α dynein heavy chain. Sequence analysis indicates that both molecules are novel members of the thioredoxin superfamily and share ~30% sequence identity with thioredoxin from Penicillium. Both DLCs have a perfect copy of the thioredoxin active site (WCGPCK); the M, 16,000 DLC also contains the canonical P-loop motif (AX_S(5)GKS). There is a single gene for both DLCs within Chlamydomonas and only single messages that were up-regulated more than 10-fold upon deflagellation were observed on Northern blots. Both recombinant DLCs were specifically eluted from a phenylarsine oxide matrix with β-mercaptoethanol indicating that they contain vicinal dithiols competent to undergo reversible oxidation/reduction. Furthermore, we demonstrate that outer (but not inner) arm dynein may be purified on the basis of its affinity for phenylarsine oxide suggesting that the predicted redox-sensitive vicinal dithiols exist within the native complex.

Flagellar dyneins are molecular motors responsible for generating the force required for interdoublet microtubule sliding which ultimately is converted to flagellar bending (see Warner et al. (1989) for reviews). The related cytoplasmic dyneins are involved in a wide variety of intracellular motile events including axonal transport, endosome and Golgi movement, positioning of the mitotic spindle and nuclear migration (Corthesy-Theulaz et al., 1992; Li et al., 1993; Paschal and Vallee, 1987; Schroer et al., 1989; Xiang et al., 1994). Both the flagellar and cytoplasmic isoforms are highly complex assemblies containing multiple motor subunits associated with a variety of accessory proteins (see Holzbaur et al. (1994) and Witman et al. (1994)). Members of this latter group of polypeptides are thought to target the motor to the appropriate cargo (King et al., 1991, 1995; Paschal et al., 1992) and to modify motor activity to achieve regulated movement (Barkalow et al., 1994; King and Patel-King, 1995b; Stephens and Prior, 1992). However, although considerable progress has recently been made (Gill et al., 1994; Hughes et al., 1995; King and Patel-King, 1995a, 1995b; LeDizet and Piperno, 1995; Mitchell and Kang, 1991, 1993; Ogawa et al., 1995; Paschal et al., 1992; Wilkerson et al., 1995), the molecular identity and function of many of these accessory proteins remains unexplored.

One of the best characterized dyneins is the outer arm from flagella of Chlamydomonas. This complex consists of three dynein heavy chains (the α, β, and γ DHCs; ~520 kDa each) which contain the ATPase and microtubule motor domains, two intermediate chains of 76.5 and 63 kDa (IC78 and IC69, respectively) and 8 different light chains (DLCs) of M, 8,000-22,000, several of which are present in multiple copies (reviewed in Witman et al. (1994)). Structurally, the outer arm appears as three globular head domains interconnected by stems to a common base (Goodenough and Heuser, 1984). Each globular head and stem is formed from a single DHC. Each DHC is also tightly associated with at least one DLC, and we have recently determined that one of the γ DHC-associated DLCs is a novel Ca2+ binding member of the calmodulin superfamily (King and Patel-King, 1995b). At least for the β DHC, the tightly bound DLC interacts with the N-terminal 160 kDa of the DHC (Sakakibara et al., 1993) and therefore is predicted to be located in the stem region rather than the globular head domain. The remaining DLCs apparently interact with the two ICs in a distinct subcomplex located at the base of the soluble dynein particle (Mitchell and Rosenbaum, 1986; Witman et al., 1991). Both ICs are known to be essential for assembly of the outer arm (Mitchell and Kang, 1991, 1993; Wilkerson et al., 1995) and one (IC78) is involved in attachment of this dynein to its cargo (King et al., 1995). The functional significance of the various DLCs associated with the ICs is much less clear. They may be involved in interactions with other axonemal components located on or near the doublet microtubules or, as dynein arms overlap in situ, they may interact directly with the globular head domains of the adjacent dynein particle.

In order to understand how flagellar dynein functions, it is obviously necessary to determine the structure and role of all the component polypeptides. Therefore, we have initiated a molecular analysis of the DLCs within the Chlamydomonas outer arm. Here we demonstrate that the M, 14,000 and 16,000 DLCs, which are associated with ICs and with the α DHC, respectively, are novel members of the thioredoxin superfamily containing perfect copies of the thioredoxin active site. Affinity chromatography on phenylarsine oxide indicates that both molecules are functional as thioredoxins and we demonstrate that outer arm (but not inner arm) dynein can be purified on the basis of redox-sensitive vicinal dithiols. These are the first redox-sensitive proteins to be found associated with a microtubule-based molecular motor.

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‡ To whom correspondence should be addressed. Tel.: 860-679-3347; Fax: 860-679-3348; E-mail: king@panda.uchc.edu.
Dynein Purification and Peptide Sequencing—Flagellar axonemes were prepared from Chlamydomonas reinhardti strain 1132 by standard methods (Witman, 1986). Outer arm dynein was extracted by treatment of the axonemes with 0.6 M NaCl and purified by sucrose density gradient centrifugation. Dynein containing fractions were concentrated in a Centricon 30 (Amicon, Danvers, MA) that had been preincubated with 1% Tween 20 in Tris-buffered saline. The concentrated samples were electrophoresed in a 5–15% acrylamide gradient gel and blotted to polyvinylidene difluoride membrane (Immobilon P, Millipore, Billerica, MA) in 10 mM NaHCO3, 3 mM Na2CO3, 0.01% SDS, 20% methanol. The blot was stained with Amido Black and the regions containing the M14,000 and 16,000 DLCs were excised. These strips were incubated with trypsin and peptides eluting from the membrane were purified by reverse phase chromatography on an Aquapore RP-300 (C18) column. Peptides were sequenced using an Applied Biosystems model 492A sequencer.

Molecular Cloning and Analysis—To obtain a region of the M14,000 DLC for use as a probe, two gene specific primers incorporating the Chlamydomonas codon bias and either an EcoRI or XhoI site at the 5’ end were designed. A forward primer (5’-GGCCGATCCGAGCTCAGGAGA-3’) based on the sequence QVVEVFQ and a reverse primer (5’-GGCTCTCCGAGCTGAGGAGAC-3’) based on the peptide sequence DDLEENGAYCTSGAGGAGAAC-3’) were used in a polymerase chain reaction. The 100-μl sample contained 1 μg of each primer, 1 μl of first strand cDNA made from RNA derived from cells actively regenerating their flagella, 20 μl TrisCl, pH 8.8, 10 μM KCl, 6 mM (NH4)2SO4, 1.5 μg MgCl2, and 0.1% Triton X-100. This was heated to 95°C for 5 min, quenched on ice, and 2 μl of the PCR product (5 μl) and 1 μl of an Avanti total RNA (Calbiochem, La Jolla, CA) added. The sample was overlaid with mineral oil, placed in a thermocycler, and subject to 40 rounds of the following regime: 96°C for 30 s, 35°C for 2 min, 75°C for 2 min. This was followed by a final 10 min at 75°C. The reaction was analyzed in an agarose gel and a small fraction reamplified using the above procedure. Ultimately, a single prominent band was obtained and this was subcloned into the EcoRI and XhoI sites of plBluescript SK+ (Stratagene).

For the M16,000 DLC, we employed a 3’ RACE procedure (Frohman et al., 1988) utilizing a gene specific primer (5’-GGCGGATCCGAGCTGAGGAGAAAC-3’) based on the peptide sequence DDLEEN and incorporating an EcoRI site, and a dig(dT)20 adaptor primer (5’-GGCCGACGGTGACTACTTTTGGATC-3’). The reaction was performed as described above except that the anneal temperature was increased to 45°C. The product was cloned into plBluescript SK+.

For both DLCs, the cloned regions then were used to probe a Chlamydomonas cDNA library made in λZapI from RNA derived from cells that were undergoing flagellar regeneration (Wilkinson et al., 1995). cDNA containing phagemids were rescued using helper phage. The longest clones were sequenced (from double-stranded DNA) on both strands using Sequenase v2.0 (U. S. Biochemical Corp.) and a 7-deaza-dGTP sequencing kit. Several regions of high GC content also were sequenced (in both directions) using single stranded DNA templates. Northern and Southern blots were prepared and processed by standard methods (Sambrook et al., 1989) using the conditions described by King and Patel-King (1995a).

Computational Methods—Sequence assembly was performed using the GCG software package (Devereux et al., 1984). Pairwise and multiple alignments were made using GAP and PILEUP, respectively. The PROSITE data base was searched using MOTIFS. Secondary structure predictions were made using PHD (Rost and Sander, 1993) and molecular models built with SWISS-MODEL (Peitsch and Jongeneel, 1993). Homology searches were performed using the BLAST service at NCBI. Predictions were made using PHD (Rost and Sander, 1993) and the PROSITE database was searched using MOTIFS. Secondary structure predictions were made using Raptor (Cohen and Schiller, 1994) and Mol-Mol (Kabsch and Sander, 1983).

RESULTS

Peptide Sequencing—Following high salt extraction from the axoneme, the Chlamydomonas outer dynein arm is obtained as two particles sedimenting at ~18S and 12S in sucrose density gradients (Piperino and Luck, 1979; Pfister et al., 1982). The 18 S particle consists of the α and β DHCs, both ICs, and the M20,000, 19,000, 16,000, 14,000, 11,000, and 8,000 DLCs. The electrophoretically purified M14,000 and 16,000 DLCs were digested with trypsin in situ and peptides eluting from the membrane were purified by reverse phase chromatography (Fig. 1, a and b). Two peptides were sequenced from each protein and yielded a total of 26 (out of 28) and 26 (out of 27) unambiguous residue assignments for the M14,000 and 16,000 DLCs, respectively.

Molecular Cloning of the M14,000 DLC—In order to clone the M14,000 DLC, we designed primers based on parts (namely OVVFQ and FYSVSSE) of the two peptide sequences shown in Fig. 1a. These were used in a polymerase chain reaction with first strand cDNA made from RNA derived from cells regenerating their flagella as the template. A product of ~120 bp was obtained. Clones containing the O VVFQ primer sequences were used to screen a λZapI generated from RNA of the M14,000 and 16,000 DLCs. The sequence SWC continuing from the forward primer (OVVFQ) Peptide sequencing gave the continuing sequence SXX; however, note that the phenylthiohydantoin derivatives of both Trp and Cys residues are not identified under the peptide sequencing conditions used here. Furthermore, this product encoded a Lys (i.e. a tryptic cleavage site) immediately N-terminal to the FYSVSSE sequence used as the reverse primer. We concluded that this product likely encoded part of the M14,000 DLC. The 120-bp polymerase chain reaction product was therefore used to screen a Chlamydomonas cDNA library made in the vector λZapI that was enriched for flagellar sequences (Wilkinson et al., 1995). Multiple positive clones were obtained and one of the longest sequenced on both strands from double stranded DNA. The entire DNA sequence for the M14,000 DLC is shown in Fig. 2. This clone contains a single long open reading frame that predicts a protein of 129 residues with a mass of 14,179 Da and a pl of 8.34. This protein contains both peptide sequences that were obtained from the electropheretically purified molecule (26/26 residues correct) as well as the predicted Lys residues N-terminal to both tryptic fragments. As only 14 residues were used for the design of the original primers, this confirms that the clone does indeed en-
code the Mr 14,000 DLC. In addition, there are two in-frame stop codons upstream of the putative translation initiation site and the coding region terminates with a single stop codon followed by 523 bp of 3'-untranslated region before the poly(A) tail.

The Mr 14,000 DLC clone was used to probe genomic DNA obtained from Chlamydomonas strain S1D2 (Fig. 3a). Single major bands were observed in samples digested with BamHI and Smal suggesting that there is only one gene for this DLC within Chlamydomonas. On Northern blots, a single message of ~1.34 kb was observed in RNA obtained from nondeflagellated cells (Fig. 3b, NDF). As expected for an integral axonemal component, the amount of message for this DLC was induced more than 10-fold upon deflagellation (Fig. 3b, 30' post DF).

Molecular Cloning of the Mr 16,000 DLC—Initially, a 3' RACE procedure using a gene specific primer based on the sequence DDLEEN and an oligo(dT) adaptor was employed and yielded a product of ~600 bp. Sequence analysis revealed that the correct continuing sequence (PMYLA) was predicted following the forward primer. Therefore, we used this product to screen the λZapII cDNA library and isolated multiple positive clones. The entire sequence for the 1019-bp Mr 16,000 DLC clone is shown in Fig. 4. The clone contains a single open reading frame encoding a protein of 156 residues with a mass of 17,365 Da and a pI of 7.76. There is a single in-frame stop codon in the 5'-untranslated region. The reading frame ends with a single stop codon followed by a 451-bp 3'-untranslated region before the poly(A) section. The predicted protein contains both peptide sequences (see Fig. 1b); 26/26 residues correct. This confirms that the clone indeed encodes the Mr 16,000 DLC. Furthermore, the predicted Lys residue is found N-terminal to the SVLPTFR sequence. However, the second peptide is preceded by a Leu rather than a Arg or Lys residue. Thus, it is not clear whether this peptide results from tryptic digestion at a noncanonical site or is due to contamination by a protease of different specificity.
Southern and Northern blot analysis of the Mr 16,000 DLC is shown in Fig. 5. Single bands are observed in genomic DNA digested with BamHI and PstI suggesting that there is a single gene for this protein within Chlamydomonas (Fig. 5a). Following digestion with BamHI and PstI, only single bands were observed indicating that there is one gene for this DLC in Chlamydomonas. Standards are indicated at the left (kilobases). b, Southern blot of total RNA (20 μg) obtained from non-deflagellated cells (NDF) and from cells that had been deflagellated and allowed to undergo flagellar regeneration for 30 min (30 min post DF). Standards are shown at the left (kb). A single message of ~1.33 kb is evident in the induced sample.

FIG. 5. a, Southern blot of 10 μg of genomic DNA derived from Chlamydomonas strain S1D2 and digested with BamHI, PstI, PvuII, and SmaI. The blot was probed with the full-length cDNA for the Mr 14,000 DLC. Following digestion with BamHI and PstI, only single bands were observed indicating that there is one gene for this DLC in Chlamydomonas. Standards are indicated at the left (kilobases). b, Southern blot of total RNA (20 μg) obtained from non-deflagellated cells (NDF) and from cells that had been deflagellated and allowed to undergo flagellar regeneration for 30 min (30 min post DF). Standards are shown at the left (kb). A single message of ~1.33 kb is evident in the induced sample.
Thioredoxin-like Dynein Light Chains

Dockonas DLCs and thioredoxins from chicken, rhesus macaque, Emericella, and Penicillium generated by the GCG program PILEUP is shown in Fig. 6b. The probability ($p_n$) scores calculated for the various matches by BLAST are shown in Fig. 6c. Both Chlamydomonas flagellar proteins contain perfect copies of the thioredoxin active site, namely WCGPCK, and are thus novel members of that family of sulfhydryl oxidoreductases. The Mr 16,000 DLC also contains a perfect copy of the P-loop consensus sequence ((A/G)X4GK(T/S)) for a nucleotide binding site (76AKEHRGKS83).

The secondary structure for the Mr 14,000 and 16,000 DLCs and for Penicillium thioredoxin were analyzed using PHD (Rost and Sander, 1993); the information base for this prediction included the known structure for thioredoxin from Escherichia coli (Ekland et al., 1984; Jeng et al., 1994) and therefore the accuracy is likely >70% (Rost and Sander, 1993). The regions of the three molecules predicted to be in extended or helical conformations are shown in Fig. 7. Although the Mr 14,000 DLC is only approximately 30% identical to thioredoxin from Penicillium, the predicted secondary structure is very similar. The only apparently significant differences are two small helical regions predicted in the Mr 14,000 DLC for residues 29–32 and 63–70 that are not present in the fungal thioredoxin. The structure prediction for the N-terminal portion of the Mr 16,000 DLC also is quite similar to thioredoxin. However, in this case the C-terminal region is significantly different and is predicted to have an extended section with high helical content.

Molecular models of both DLCs based on the known structure of thioredoxin were built using SWISS-MODEL (Fig. 8). This analysis emphasizes the structural similarities between these dynein proteins and thioredoxin and predicts that the active-site thiols within both DLCs are vicinal (Fig. 8, a and b). Furthermore, this analysis predicts that the Mr 16,000 (but not...
The Mr 14,000 DLC may contain a second vicinal dithiol motif (64TVCAEKC70) that is not identified simply from sequence analysis.

The Mr 14,000 and 16,000 DLCs Bind Phenylarsine Oxide—Identification of these DLCs as novel members of the thioredoxin superfamily raised the question of whether these molecules are functionally active sulfhydryl oxidoreductases. To obtain sufficient protein for functional assays, we expressed both DLCs as C-terminal fusions with maltose-binding protein (MBP-DLC14 and MBP-DLC16). We also obtained, for use as a control, a recombinant protein in which the lacZ gene was fused to MBP (MBP-lacZ; this fusion protein has a mass of 50,843 Da).

One distinguishing feature of proteins containing vicinal dithiols is that they bind reversibly, but with high affinity, to metals such as arsenic, cadmium, and mercury (Hannestad et al., 1982). To test whether the two DLCs indeed contain vicinal dithiols, we attempted to purify the fusion proteins by affinity chromatography on PAO. The basic reaction scheme is shown in Fig. 9a. In the absence of a reductant, a covalent dithioarsine ring structure is formed. Due to steric constraints, the interaction of monothiols with PAO is weak and unstable (see Kalef and Gitler (1994) for further discussion of dithioarsine chemistry). The addition of high concentrations of either a mono- or dithiol reductant causes release of the vicinal dithiol-containing protein from the affinity matrix.

Fusion proteins were reduced with 1 mM dithiothreitol and the reductant (which irreversibly inactivates the arsenical moiety) removed by extensive dialysis. In some experiments, the proteins were purified in the presence of 1 mM β-mercaptoethanol. Subsequently, the samples were incubated with the PAO slurry for 60 min and the matrix then extensively washed. Electrophoretic analysis of the initial preparations and of the unbound fractions are shown in Fig. 9b (leftmost and central lanes). Proteins bound via vicinal dithiols then were eluted by incubation of the PAO resin with 0.5 M β-mercaptoethanol. The composition of those eluates is shown in Fig. 9b (rightmost lanes). Only a very small fraction of the MBP-lacZ fusion protein bound to, and was subsequently eluted from, the PAO matrix. As MBP-lacZ does not contain vicinal dithiols, this

**FIG. 7.** Secondary structures for the Mr 14,000 and 16,000 DLCs and for thioredoxin from *Penicillium* (P34723) were predicted using PHD (Rost and Sander, 1993). E, extended sheet; H, helix. The prediction base contained an experimentally derived thioredoxin structure and, therefore, the expected accuracy is >70%.

**FIG. 8.** Molecular models of the Mr 14,000 DLCs were built using SWISS-MODEL. Note that the Mr 16,000 DLC model is truncated as the C-terminal section is sufficiently divergent from thioredoxin that it cannot be modeled by homology. In both space filling models (a and b), the dithiol group of the active site motif WCGPCK is shown in yellow at the lower section of the two molecules. The Mr 16,000 DLC model (b) also predicts a second vicinal dithiol within that molecule. This second dithiol (yellow) is visible in the lower right quadrant of the model. Ribbon diagrams of the Mr 14,000 and 16,000 DLC models are shown in c and d, respectively (red, α helix; yellow, β sheet; blue, turn; white, other). The predicted topologies are very similar to that known for thioredoxin (Ekulf et al., 1984; Jeng et al., 1994).
presumably represents either nonspecific association with the agarose support or, possibly, a weak interaction with PAO via agarose support or, possibly, a weak interaction with PAO via

Fig. 9, a, scheme describing the reaction of a DLC containing a vicinal dithiol with 4-nitrophenylarsine oxide (based on Kalf and Gitler (1994)). A dithioarsine ring is formed between the two sulphydryls and the arsenical; the stability of the ring depends on the precise ring geometry. Dithiol-containing proteins may be eluted from PAO by reduction with mono- or dithiols. b, affinity chromatography of the MBP-DLC14, MBP-DLC16, and MBP-lacZ fusion proteins on phenylarsine oxide. The proteins were reduced with 1 mM dithiothreitol. The reductant was removed by dialysis and the samples incubated with $\beta$-mercaptoethanol-activated PAO resin. After extensive washing, bound protein was eluted with 0.5 M $\beta$-mercaptoethanol. Equivalent volumes of the initial, unbound, and eluted fractions were electrophoresed on an 8% acrylamide gel and stained with Coomassie Blue. c, the reduced DLC fusion proteins were digested with Factor Xa to separate the DLCs from the MBP moiety (left lanes). Following incubation with PAO, bound protein was eluted with 0.5 M $\beta$-mercaptoethanol. Both DLCs bind the PAO matrix whereas MBP does not.

In this report, we describe the molecular analysis of the M$_1$ 14,000 and 16,000 DLCs from the outer arm of Chlamydomonas flagella. These molecules are novel thioredoxins containing perfect copies of the redox-active site that is the major distinguishing feature of this class of sulphydryl oxidoreductases. When expressed as fusion proteins, both DLCs can be purified on the basis of high affinity interaction with arsenic indicating that they contain vicinal dithiol groups (i.e., thiol so arranged within the DLC tertiary structure that they can be oxidized to form a disulfide); these vicinal thiolis are present in the outer arm but not inner arm dynecines from Chlamydomonas flagella. This constitutes the first demonstration of redox-sensitive polypeptides as integral components of a microtubule-based molecular motor.

Thioredoxins act as sulphydryl oxidoreductase (for review, see Holmgren (1985)) and have now been identified in a very wide range of organisms from bacteria to vertebrates. Members of this class of redox-active enzyme are known, or predicted based on in vitro studies, to be involved in a wide variety of regulatory activities. For example, thioredoxin derived from the E. coli host is a component of T7 DNA polymerase that is required for high processivity of the enzyme along the DNA strand (Tabor et al., 1987). Lack of this subunit also has significant consequences for the fidelity of replication (Kunkel et al., 1994). In Chlamydomonas, thioredoxin is intimately involved in the light regulated translation of chloroplast mRNA by manipulating redox potential (Danon and Mayfield, 1994). A Drosophila thioredoxin homologue, encoded at the locus deadhead, is essential for female-specific meiosis, preblastoderm mitosis, and for early embryonic development (Salz et al., 1994). The phenotypes observed suggest that the deadhead protein is not a general protein disulfide reductase but rather is required for post-translational modification of proteins involved in specific developmental processes. Thioredoxin has also been shown to interact with microtubules in vivo (Stemme et al., 1985) and to affect the rate of microtubule assembly in vitro by reducing intratubulin disulfides (Khan and Ludueña, 1991).

In almost all cases, thioredoxins contain the active site sequence WCGPCCK. Outside of this, the overall amino acid conservation between members of this superfamilly is relatively low (30–40% identity), although there apparently is a much greater level of similarity at the secondary and tertiary structure levels. The dynine-associated thioredoxins we describe here follow this same pattern. Within Chlamydomonas, thioredoxins have been identified from both the chloroplast and cytosol (Decottignies et al., 1991; Stein et al., 1994). Pairwise comparison between these proteins and the flagellar DLCs (not shown) indicate that the Chlamydomonas DLCs are more related to bona fide Chlamydomonas thioredoxins than they are to the chicken isozyme. This suggests an evolutionarily ancient origin for the flagellar proteins. Note that comparison between the chloroplast and cytosolic Chlamydomonas isozymes reveals only 33% sequence identity and emphasizes

To determine whether redox-sensitive dithiols occur within flagellar dynein, we extracted both inner and outer arms from Chlamydomonas axonemes with 0.6 M NaCl and assessed the ability of the various dynein species to specifically bind to PAO. Electrophoretic and immunological analysis of the resulting fractions is shown in Fig. 10, a-c. Approximately 50% of the $\alpha$, $\beta$, and $\gamma$ outer arm DHCs were found to specifically bind to and be eluted from the PAO matrix with $\beta$-mercaptoethanol. In contrast, inner arm DHCs belonging to arms 12 and 13 (see Table I in Piperno (1995)) and a high molecular weight flagellar membrane protein show no affinity for the matrix. Inner arm DHCs 1a and 1b which comprise inner arm I are not resolved from the outer arm DHCs in this gel system. However, inner arm I also contains an IC of $-140$ kDa; no component of this approximate mass in the high salt extract showed significant affinity for PAO (not shown).

### DISCUSSION

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$$\text{DLC}_16 \rightarrow \text{DLC}_14$$
in this regard, it is intriguing that the two DLCs described here) rather than to the DHCs which obviously are related to each other in the two dynein classes (see LeDizet and Piperno, 1985). Treatment of the outer arm dynein with thiol-modifying reagents on dynein ATPase activity have indicated that the outer arm dynein contains of reactive cysteines. As the outer arms but not inner arms may be purified on PAO, this implies that the redox-sensitive interaction is likely due to accessory proteins (such as the DLCs described here) rather than to the DHCs which obviously are related to each other in the two dynein classes and might be expected to behave similarly in such an assay. In this regard, it is intriguing that the γ DHC is readily purified from the remainder of the outer arm upon high salt extraction (Pfister et al., 1982) and does not interact directly with either the M, 14,000 or 16,000 DLCs. As the binding appears to be quite specific, the γ DHC may reassociate with the αβ dimer upon the latter’s binding to PAO. Alternatively, the γ DHC might bind an additional thioredoxin-like subunit. This DHC is known to interact with three DLCs (Pfister et al., 1982): a M, 18,000 protein that we have recently shown to be a novel homologue of calmodulin (King and Patel-King, 1995b), and two copies of a M, 22,000 polypeptide. By analogy with the α subunit (which contains the α DHC and the M, 16,000 DLC), it is possible that the M, 22,000 DLC also is a thioredoxin homologue.

Assuming that the thioredoxin homologues we have identified within the outer arm indeed undergo a redox interaction with other thiols in the flagellum, this necessarily implies the presence of a thioredoxin reductase and a flagellar source of reducing equivalents. Alternatively, these DLCs might function to regulate enzymatic activity while the outer dynein arm is being assembled and stored in the cytoplasm. In this scenario, the DLCs would presumably be able to tap the same stores of reducing equivalents as does the cytosolic thioredoxin.

The M, 16,000 DLC contains a perfect copy of the canonical P-loop motif for nucleotide binding sites (Walker et al., 1982), raising the possibility that this protein binds nucleotide. There are, however, several reasons for caution in this interpretation. Photoaffinity labeling of purified outer arm dynein with photoactive analogues of ATP consistently failed to show significant label associated with any of the DLCs (King et al., 1989; Pfister et al., 1985). Also, we note that this region of the M, 16,000 DLC in fact forms part of a relatively well conserved primary structural element within the thioredoxin superfamily. On balance, it seems more likely that the presence of this motif is serendipitous rather than indicating a specific functionality.

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4 A. Harrison and S. M. King, unpublished observation.
Quantitative densitometry of Coomassie Blue-stained gels gave a stoichiometry of 1.46–1.65 copies of the.

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