FEBS Letters 587 (2013) 2143–2149

FEBS Letters
journal homepage: www.FEBSLetters.org

Protein–protein interactions between intermediate chains and the docking complex of *Chlamydomonas* flagellar outer arm dynein

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ARTICLE INFO

Article history:
Received 23 May 2013
Accepted 23 May 2013
Available online 6 June 2013

Edited by Dietmar J. Manstein

Keywords:
Dynein
Cilia
Flagella
Microtubule
Chlamydomonas

ABSTRACT

Outer arm dynein (OAD) is bound to specific loci on outer-doublet-microtubules by interactions at two sites: via intermediate chain 1 (IC1) and the outer dynein arm docking complex (ODA-DC). Studies using *Chlamydomonas* mutants have suggested that the individual sites have rather weak affinities for microtubules, and therefore strong OAD attachment to microtubules is achieved by their cooperation. To test this idea, we examined interactions between IC1, IC2 (another intermediate chain) and ODA-DC using recombinant proteins. Recombinant IC1 and IC2 were found to form a 1:1 complex, and this complex associated with ODA-DC in vitro. Binding of IC1 to mutant axonemes revealed that there are specific binding sites for IC1. From these data, we propose a novel model of OAD-outer doublet association.

Structured summary of protein interactions:
IC2 physically interacts with **DC2** and **DC1** by anti bait coimmunoprecipitation (View interaction)
DC2 physically interacts with **IC2** and **IC1** by anti bait coimmunoprecipitation (View interaction)
IC2 and **IC1** physically interact by cross-linking study (View interaction)
IC2 and **DC1** physically interact by cross-linking study (View interaction)
DC2 and **DC1** physically interact by cross-linking study (View Interaction: 1, 2)
DC1 and **IC1** physically interact by cross-linking study (View interaction)
IC2 binds to **IC1** by anti bait coimmunoprecipitation (View interaction)
IC2, **DC1** and **DC2** physically interact by cross-linking study (View interaction)
DC2, **IC1** and **DC1** physically interact by cross-linking study (View interaction)

1. Introduction

Axonemal dyneins in cilia and flagella are attached to the doublet microtubules and generate force against the adjacent doublet microtubule. Outer arm dynein (OAD), which generates ~70% of total propulsive force in the axoneme [1], binds to specific sites on the A-tubule with a regular spacing of 24 nm. How binding to specific axonemal sites is achieved remains a fascinating unanswered question. Also, it is important for understanding the mechanisms underlying human diseases called primary ciliary dyskinesia (PCD), since they are mostly caused by defects in OAD assembly [2].

Chlamydomonas OAD consists of three heavy chains (HCs: α, β, and γ), two intermediate chains (ICs: IC1 and IC2), and 11 light chains (LCs). It is a complex macromolecular system with three globular "heads" composed of the C-terminal regions of the HCs, and a "tail" comprising the HC N-terminal regions, and the ICs and LCs. At the base of the tail, an additional structure called the outer-dynein-arm docking complex (ODA-DC) is present and mediates the binding of OAD to the doublet microtubule. The ODA-DC is composed of three subunits, DC1, DC2 and DC3. It is preassembled in the cytoplasm and transported into flagella independently of OAD [3,4].

IC1 and the ODA-DC are considered important for OAD-doublet association. IC1 was shown by chemical crosslinking to directly bind to α-tubulin [5]. The ODA-DC also must be important for OAD-doublet microtubule binding, since mutants lacking the

Abbreviations: BMH, bismaleimidohexane; IC, intermediate chain; LC, light chain; OAD, outer arm dynein; ODA-DC, outer dynein arm docking complex

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http://dx.doi.org/10.1016/j.febslet.2013.05.058

ODA-DC lack OAD in the axoneme, even though a complete OAD complex is assembled in the cytoplasm [4,6]. Binding of the ODA-DC and OAD involves interaction between a LC (LC7b) and DC2 [7]. Studies using a mutant lacking DC3 indicate that DC1 and DC2 are responsible for the binding of OAD to the ODA-DC [8].

Despite the postulated importance of IC1 and the ODA-DC for OAD attachment to the doublet microtubule, the available data indicate that both IC1 and ODA-DC have rather weak affinity for axonemal doublet microtubules. First, OAD cannot bind to the doublets in mutant axonemes that lack the ODA-DC; this suggests that the IC1-doublet microtubule interaction is not very strong. Second, the ODA-DC binding to the doublet appears to be incomplete without OAD, because the amount of the ODA-DC attached to outer-doublets is reduced in the axoneme of mutants that cannot assemble OAD (such as *oda2*(ΔHCγ), *oda4*(ΔHCβ) and *oda6*(ΔIC2)) [6]. Thus, the ODA-DC cannot bind to the doublet strongly enough without OAD, while OAD cannot bind to the doublet without the ODA-DC. The inter-dependence of OAD and the ODA-DC in their microtubule binding suggests that there must be some unknown protein–protein interaction(s) between OAD, the ODA-DC and the doublet that strengthen OAD docking and assembly.

To further explore the mechanism of OAD–doublet microtubule association, in this study we performed protein–protein interaction analyses between IC1, IC2, DC1, DC2, and microtubules. We established an expression system for these proteins using insect culture cells and used the recombinant proteins for biochemical analyses. Our results suggest that, although individually IC1 and the ODA-DC attach OAD only weakly to the doublet microtubules, they associate with each other through multiple interactions, and that together this association strengthens OAD attachment to the doublets.

2. Materials and methods

For details see Supplementary information.

2.1. Strains and culture of Chlamydomonas reinhardtii cells

The following mutants of *Chlamydomonas reinhardtii* were used: *oda1* (ΔDC2) [9,10], *oda3* (ΔDC1) [9,11], *oda6* (ΔIC2) [9,12], *oda9* (ΔIC1) [9,13], *ida4* (Δp28) [14], and *ida5* (Δactin) [15]. Double mutants of *oda1ida5*, *oda3ida4*, *oda6ida4*, and *oda9ida4* were produced by the standard procedure [16]. All cells were grown in Tris–acetate-phosphate (TAP) medium with aeration at 25 °C, on a 12 h/12 h light/dark cycle [17].

2.2. Preparation of Chlamydomonas axonemes

Flagellar axonemes were isolated from *Chlamydomonas reinhardtii oda1* strain by the method previously described [18]. Axonemes were resuspended in HMDEK (30 mM Hepes, pH 7.4, 5 mM $MgSO_4$, 1 mM dithiothreitol, 1 mM EGTA, and 50 mM potassium acetate).

2.3. Preparation of recombinant IC1, IC2, DC1 and DC2

IC1, IC2, DC1 and DC2 were expressed in *Sf21* cells by baculovirus system. IC1 was tagged with 6 × His at the N-terminus (for IP) or the C-terminus (for electroporation experiments), and not tagged for experiments that assayed co-purification with IC2. Other proteins were 6 × His-tagged at the C-terminus except for DC1 or DC2 used in co-purification experiments. Recombinant proteins were purified by Ni–NTA agarose (QIAGEN, Hilden, Germany) as described by the manufacturer, with slight modifications (0.6 M NaCl was added to all the solutions).

2.4. Protein electroporation

Electroporation was used to introduce recombinant proteins into live *Chlamydomonas* cells as described in [19]. Briefly, autolysin-treated cells were mixed with a recombinant protein (0.5–1.0 mg/ml), and an electric pulse was applied with an ECM600 electroporation system (BTX, Holliston, MA, USA). Cell images were observed under a dark-field microscope and recorded using a video camera.

2.5. Preparation of porcine brain tubulin and polymerization of cytoplasmic microtubules

Tubulin was purified from porcine brain by cycles of assembly and disassembly in vitro in a high-molarity PIPES buffer [20]. Microtubule pellets were resuspended in HMDEK containing paclitaxel.

2.6. Immunoprecipitation

Protein A-agarose beads (Roche) were washed with blocking buffer (TBS, pH 7.2, 3% BSA (w/v), 1%Triton-X100 (v/v)), incubated with the anti-DC2 antibody [3] or anti-IC2 antibody (sigma), and then incubated with purified recombinant proteins. The resultant beads were resuspended with SDS sample buffer.

2.7. Chemical crosslinking of immunoprecipitated products

Recombinant proteins were mixed and treated with the chemical crosslinker bismaleimidohexane (BMH) (Pierce Chemical, Rockford, IL, USA) for 1 h at room temperature [5]. Reactions were terminated by the addition of SDS–PAGE sample buffer containing 2-mercaptoethanol.

2.8. Co-precipitation assay of recombinant proteins with axonemes

The purified proteins were mixed with axonemes and incubated for 20 min at 4 °C. The samples were centrifuged at 20,000×*g* for 12 min at 4 °C. Pellets were washed with the same buffer, and then resuspended in SDS–PAGE sample buffer forimmunoblotting. Signals were detected by chemiluminescence. The amount of ICs was calculated from the luminescence intensity and a calibration curve determined with known amounts of purified ICs.

3. Results

3.1. Expression and purification of recombinant IC1, IC2, DC1 and DC2

We used a baculovirus system to obtain protein samples. This system yielded much greater amounts of recombinant IC1 and IC2 than the in vitro translation system used in a previous study [21] and allowed us to perform quantitative biochemical studies. Recombinant IC1 and IC2 were successfully expressed in insect culture cells, with ~50% of the produced proteins being soluble (Fig. 1A). Recombinant IC1 and IC2 tagged with 6 × His were partially purified with Ni–NTA agarose beads (Fig. 1B). When IC1 without a His-tag and His-tagged IC2 were co-expressed, they could be co-purified with Ni–NTA (Fig. 1B), suggesting that these proteins are associated with each other in the cultured cell. This idea was further supported by the observation that anti-IC2 antibody immuno-precipitated both IC2 and IC1 from the mixture of these proteins (Fig. 3A). Densitometry of Coomassie blue-stained gels of co-purified IC1–IC2His indicated that the stoichiometry of IC1 and IC2 is 1:1; this assumes that they have equal affinity for the dye. As one copy of each of these proteins is present in the

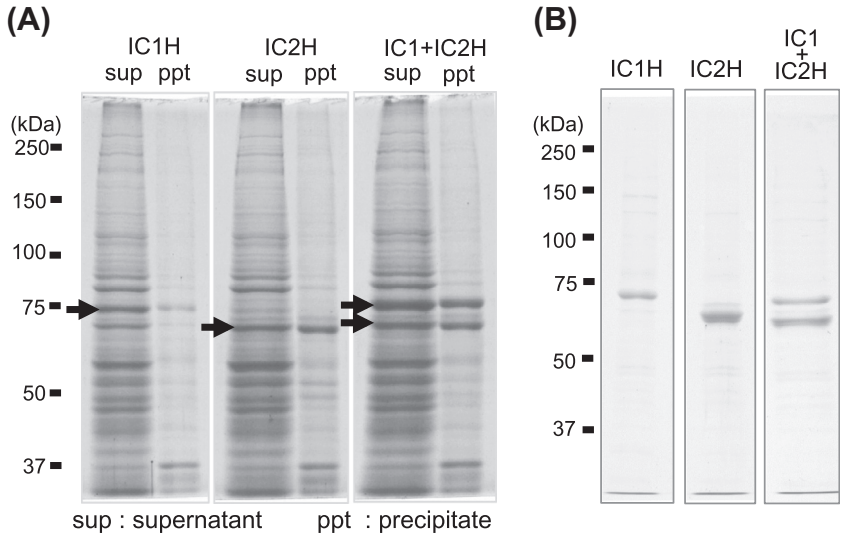

Fig. 1. Expression and purification of IC1 and IC2. All gels were stained with Coomassie Brilliant Blue. (A) Supernatants and precipitates from the lysate of *Sf21* cells expressing IC1–6 × His (Mol. Wt 76,305), IC2–6xHis (Mol Wt. 63,534) or co-expressing IC1 (not tagged) and IC2–6 × His. All proteins were solubilized by 50–60% (arrows). (B) IC1-His and IC2-His were partially purified by Ni–NTA chromatography. Co-expressed IC1 and IC2–6 × His were co-purified by Ni–NTA chromatography as well (IC1 + IC2H).

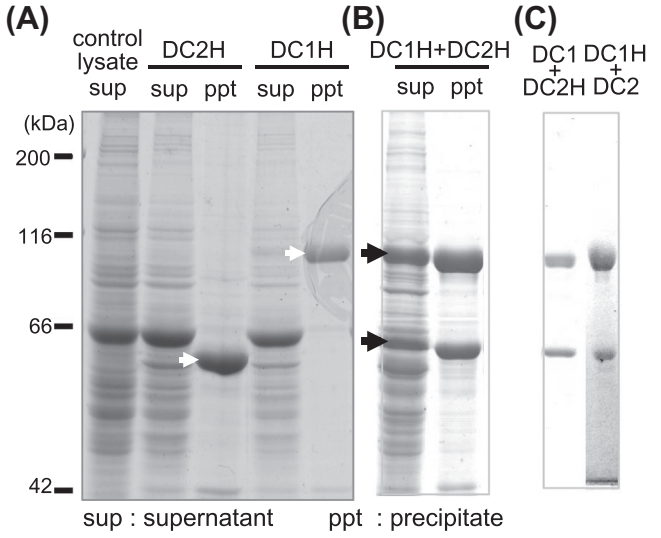

Fig. 2. Expression and purification of DC1 and DC2. All gels were stained with Coomassie Brilliant Blue. (A) Supernatants and precipitates from the lysate of *Sf21* cells expressing DC1–6 × His (Mol. Wt. 83,381 (migrates at M_r ~105,000)) or DC2–6 × His (Mol. Wt. 62,234). Neither protein was soluble when expressed singly (white arrows). (B) Co-expression of DC1–6 × His and DC2–6 × His. A large fraction of both proteins was soluble (black arrows). (C) DC1 and DC2 were co-purified by Ni–NTA chromatography even when only one component was His-tagged.

native outer dynein arm [22], we conclude that they form a heterodimer; however, this must be verified in future studies. We hereafter refer to the recombinant protein complex of IC1 and IC2 as "IC1–2".

DC1 and DC2 were almost totally insoluble when expressed singly (Fig. 2A). However, when co-expressed, as much as 30–40% of the expressed proteins became soluble (Fig. 2B). DC1 without a His-tag co-purified with His-tagged DC2 on Ni–NTA agarose beads, and vice versa (Fig. 2C). The stoichiometry of DC1 and DC2, estimated as above, was 1:1. As IC1, IC2, DC1 and DC2 have been shown to be present in equimolar amounts in the outer dynein arm [10,22], DC1 and DC2 may form a heterodimer as well; however, this must also be verified in future studies. The DC1–DC2His complex remained soluble after dialysis against a physiological buffer. These data suggest that DC1 and DC2 are soluble only when they form a complex. We hereafter refer to the DC1–DC2His complex as "DC1–2".

The recombinant proteins thus obtained were assayed for their functional activity using an electroporation-mediated protein delivery method [19,23]. Recombinant IC1His, IC2His, or DC1–2 was introduced into mutant cells that lacked the respective proteins and an inner-arm dynein, *oda9ida4*, *oda6ida4*, *oda1ida5*, and *oda3ida4,* and were thus immotile. Electroporation induces deflagellation, which upregulates transcription of flagellar components [24]. These non-motile mutants become motile if outer arm dynein assembles from introduced recombinant proteins together with other subunits in the cytoplasmic pool or with newly synthesized subunits. After introduction of the recombinant proteins and incubation for a few hours, 0.3–1.2% of cells displayed flagellar motility, albeit slower than the *ida4* or *ida5* mutant (Supplementary Table 1; Supplementary movies). The low level of motility recovery was most likely due to inefficient delivery of proteins of high molecular weight such as IC1 (76,305 Da) and IC2 (63,534 Da), and the low concentrations (0.3–1.0 mg/ml) of recombinant proteins that we had to use. Because there was a difference in the motility recovery rate between separate introduction of IC1 or IC2 and introduction of the IC1–2 complex, we suggest that part of the reasons was rapid degradation of the introduced proteins, which could take place when these proteins cannot readily form OAD/ODA-DC complexes; ICs and the subunits of ODA-DC in the cytoplasm are thought to undergo degradation when not forming complexes [3,4]. Despite these experimental limitations, it is clear that some motility was restored after delivery of recombinant ICs and DCs, verifying that they are functional as the subunits of OAD and the ODA-DC in vivo.

3.2. Interactions between IC1–2 and DC1–2 complexes

The tail domain of OAD has been shown to be in close apposition to the ODA-DC on the doublet microtubules [25]. However, thus far, the only protein–protein interaction between OAD and the ODA-DC that has been biochemically identified is between a dynein light chain (LC7b) and the ODA-DC subunit DC2; whether or not ICs are involved is unclear. We therefore tested whether there is any interaction between IC1–2 and DC1–2.

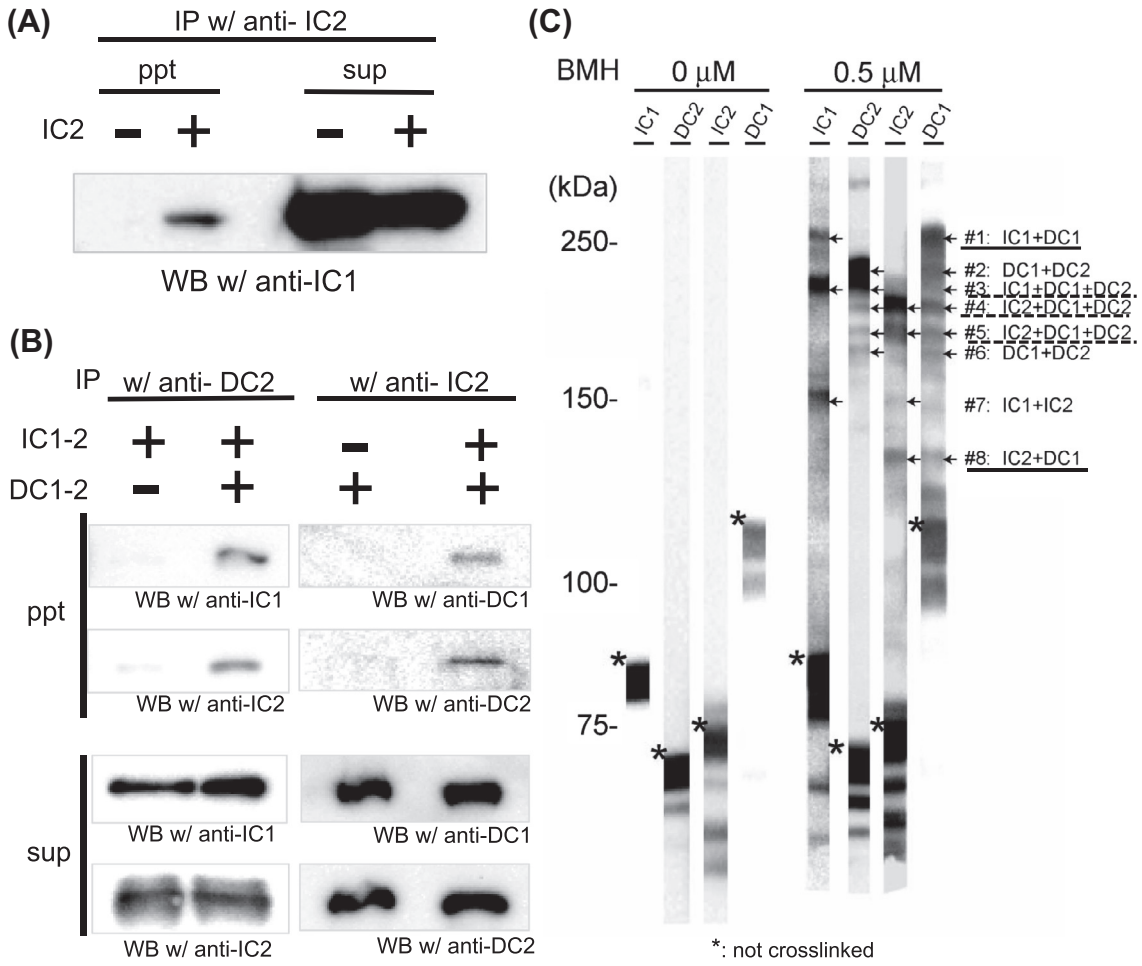

Fig. 3. Association between IC1, IC2, DC1 and DC2. (A) IC1 associates with IC2. His-IC1 sample alone (IC2−) or mixed with IC2 (IC2+) was immunoprecipitated with anti-IC2 antibody and the precipitate and supernatant fractions were assayed by western blotting for the presence of IC1. (B) IC1–2 associates with DC1–2. IC1–2 alone (DC1–2−) or mixed with DC1–2 (DC1–2 +) was immunoprecipitated (IP) with anti-DC2 antibody. Also, DC1–2 alone (IC1–2-) or mixed with IC1–2 (IC1–2+) was immunoprecipitated with anti-IC2 antibody. Western blotting (WB) with antibodies against IC1, IC2, DC1 or DC2 indicates both IC1–2 and DC1–2 were precipitated from the mixture with either antibody, demonstrating that IC1–2 and DC1–2 form a complex. (C) Chemical crosslinking of mixed "IC1–2 + DC1–2" complex with BMH. Immunoblotting against mixed proteins with or without chemical crosslinking using four antibodies is shown. The four blot strips in each sample were probed independently and placed immediately adjacent to each other so that the alignment of the crosslinked product bands could be assessed directly. The bands detected with multiple antibodies were marked with arrows and numbered. The bands #1 and #8 revealed new interactions (underlines). The bands #3, #4, and #5 show the combination of a known interaction (DC1–DC2) and new interactions (#1 or #8). The asterisks represent the proteins that are not crosslinked. For details see Results 3.2.

When mixed, IC1–2 and DC1–2 were found to be co-immunoprecipitated upon treatment with anti-DC2 antibody (Fig. 3B). Conversely, DC1–2 co-immunoprecipitated with IC1–2 when treated with anti-IC2 antibody (Fig. 3B). These data suggest that ICs and the ODA-DC directly interact with each other. To assess which subunits are responsible for their association, IC1–2 and DC1–2 complexes were mixed and chemically crosslinked with several crosslinkers with different reaction groups and spacer lengths. The results using BMH, which reacts with –SH groups and has a spacer length of 13 Å, showed interactions between two complexes as identified by western blotting using four antibodies. IC1–DC1 and IC2–DC1 are crosslinked with each other and therefore they are within 13 Å of each other (Fig. 3C). The crosslinked product #1 in Fig. 3C (containing IC1 and DC1) is apparently ~250,000 Da. Because the molecular weight of IC1 is ~78,000 and that of DC1 is ~83,000 (although it appears to be ~105,000 in SDS–PAGE), the #1 product may be composed of either 1 IC1 and 2 DC1 or 2 IC1 and 1 DC1 molecules. The crosslinked product #8 is apparently ~180,000 Da, which is close to the sum of the molecular weights of IC2 and DC1. These data suggest that the IC1–2 complex interacts with the DC1–2 complex via IC1–DC1 and IC2–DC1 interactions. The bands #3, #4, and #5 contain three subunits. These bands may involve a novel interaction (either IC1–DC1 or IC2–DC1) in addition to a known interaction between DC1 and DC2. Both #2 and #6 bands contain only DC1 and DC2. The two bands may reflect different stoichiometries or different crosslinking sites between the two proteins.

3.3. Possible docking site for IC1 on the doublet microtubule

Previous studies using co-sedimentation assays and chemical crosslinking showed that IC1, but not IC2, is a microtubule-binding protein [21]. However, only qualitative assessment has been made for IC2 binding. Since we now have sufficient amounts of IC proteins, we reassessed the microtubule-binding activities of both proteins. First, we used porcine brain microtubules and found that IC1 bound cytoplasmic microtubules, whereas IC2 did not (Supplementary Fig. 1). These results are consistent with the previous report [21]. However, although qualitatively the same results were always obtained, the amount of IC1 molecules pelleting with microtubules varied greatly from one experiment to another, making quantitative binding assessments not possible. Despite repeated experiments

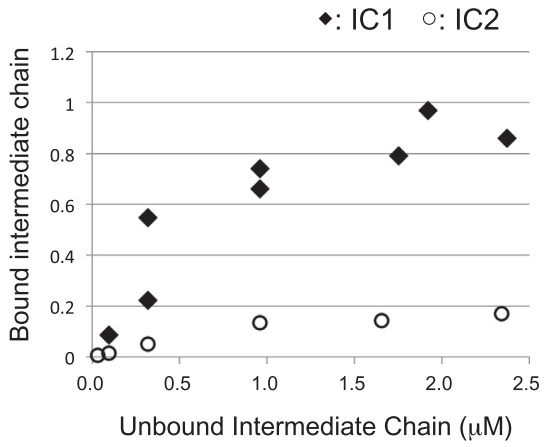

Fig. 4. Binding of IC1 and IC2 to *Chlamydomonas oda1* axonemes. The amount of IC bound was determined by immunoblotting; values were expressed relative to the amount of native IC in wild-type axonemes. IC1 binding saturated at almost the same level as in wild-type axonemes. IC2 did not show marked binding in this concentration range. These results suggest that IC1 preferentially binds to specific sites on the doublet microtubules.

using various conditions, we were unable to determine the cause of this variation; one possibility is that the use of ultracentrifugation, which is necessary to recover IC1-bound microtubules, might have interfered with the assay.

Next we examined the binding of IC1 and IC2 to the flagellar axonemes of the *oda1* mutant, which lack both OAD and the ODA-DC. In this experiment, we could recover IC1/IC2-bound axonemes using low-speed centrifugation and assess the amount of bound proteins reproducibly. As observed in the above qualitative experiments, IC1 precipitated with the *oda1* axoneme, while IC2 did not (Fig.4). Thus it is likely that IC2 does not bind stably with either tubulins or other axonemal proteins. (The binding affinities (K*d*s) of IC1 and IC2 to the axoneme, calculated from the binding curves, are 1.08 μM and 1.50 μM, respectively (Supplementary Fig. 2).) Interestingly, the amount of IC1 bound to the axonemes saturated at almost the same level as that of native IC1 in wild-type axonemes: the saturating amount was calculated to be 1.35/24 nm (Fig. 4; Supplementary Fig. 2). The Hill's coefficient was ~1.02, suggesting there is no significant cooperativity in this binding. These results suggest that IC1 preferentially binds to a specific site on the doublet, and not indiscriminately to all available sites on the microtubule walls.

4. Discussion

4.1. Association of Outer Arm Dynein Intermediate Chains in vitro

In this study, we examined biochemical properties of OAD intermediate chains (IC1 and IC2) using recombinant proteins. These ICs are responsible for both assembly and doublet microtubule binding of OAD. In a recently elucidated pathway of OAD assembly in the cytoplasm, ICs and some LCs are first assembled to form an IC–LC complex, and then HCs and the other LCs are assembled on the IC–LC complex with the help of the Ktu/PF13 protein [26]. ICs are thus thought to be the "core" on which OAD assembly occurs; defining protein–protein interactions involving ICs is thus key to understanding the mechanism of OAD assembly.

Our result that IC1 and IC2 form a 1:1 complex in cultured cells is important since it implies that they can associate with each other without LCs. In the case of *Drosophila melanogaster* cytoplasmic dynein, ICs form a homodimer only when LC7 (DYNLRB) and LC8 (DYNLL) or Tctex1 (DYNLT) are bound to the IC [27]. Of these, LC8 is known to be a dimerization enhancer [28]. In *Chlamydomonas* OAD, several LCs have been shown to bind the ICs and form an IC-LC complex [29,30]. Three of them (LC6, LC8, and LC10) belong to the LC8/DYNLL family [30]. Our data raise the possibility that IC1 and IC2 can heterodimerize without the dimerization enhancers. A previous study also proposed the direct interaction between IC1 and IC2 by stepwise dissociation of OAD subunits by detergent or by immunoprecipitation of recombinant proteins expressed using an in vitro translation system [21,31]. However, our SDS–PAGE analysis of cell lysates (Fig. 1A) revealed another aspect of ICs: IC1, IC2, and even IC1–2 are unstable and only ~50% of the recombinant proteins are soluble. In support of this observation, a previous study showed that quadriflagellated temporary dikaryons formed between *oda6*(ΔIC2) and *oda9*(ΔIC1) gametes do not readily recover normal motility, suggesting that these proteins are quickly degraded when not associated [4]. LCs may stabilize the IC1–IC2 interaction in vivo, and the stabilized IC complex may act as the core for OAD assembly [26].

4.2. DC1 and DC2 need to form a complex to be soluble

This study also examined the properties of DC1 and DC2, the two major components of the ODA-DC, using recombinant proteins for the first time [10,11]. They were solubilized only when co-expressed, suggesting their stability is interdependent. Previous studies showed that quadriflagellated temporary dikaryons formed between *oda1*(ΔDC2) and *oda3*(ΔDC1) gametes did not recover normal motility [9], and that DC1 is not present in *oda1* cytoplasm, which lacks DC2, and DC2 is not present in *oda3* cytoplasm, which lacks DC1. [3]. Our results suggest that this is because DC1 and DC2 are unstable and degraded when not associated. Both DC1 and DC2 have coiled-coil domains. Formation of coiled-coils between the two proteins may be responsible for the assembly and stabilization of their complex.

4.3. IC complex interacts with the ODA-DC

The tail domain of OAD and the ODA-DC are localized next to each other [25]. Furthermore, OAD and the ODA-DC can be co-purified in a single "23S dynein" particle when extracted from axonemes with high-salt buffers containing Mg^{2+} [10]. However, the only interaction thus far found between OAD and the ODA-DC is between LC7b and DC2 [7]. Our immunoprecipitation experiments demonstrated that IC1–2 directly binds DC1–2, most likely through interactions between IC1–DC1 and IC2–DC1. Since OAD cannot stably bind to the axoneme without the ODA-DC, the binding between OAD and the ODA-DC must be strong. We suggest this strong binding is accomplished by multiple interactions, at least in part, between LC7b–DC2, IC1–DC1 and IC2–DC1.

4.4. Possible docking site for the IC1 on the axoneme

Among the 16 subunits of OAD, IC1 is the only subunit located in the basal portion of the motor that has been shown to have microtubule binding activity. Our results confirmed that IC1 is a microtubule binding protein and IC2 is not. Also, our co-sedimentation assay of IC2 with *oda1* axonemes showed that IC2 does not bind either microtubules or axonemal proteins other than the ODA-DC and integral outer arm components. A recent study that analyzed the structure and the effect of a modified IC2 protein introduced into the axoneme has suggested that IC2 interacts with the dynein regulatory complex (DRC) [32]. Our results suggest that the binding between IC2 and DRC is weak and should be supported by the other OAD components. In contrast, IC1 apparently binds to specific site(s) on the axoneme, and the binding saturates at the same level as in native axonemes. It is somewhat surprising that IC1 binding should saturate at the normal level, since IC1, with

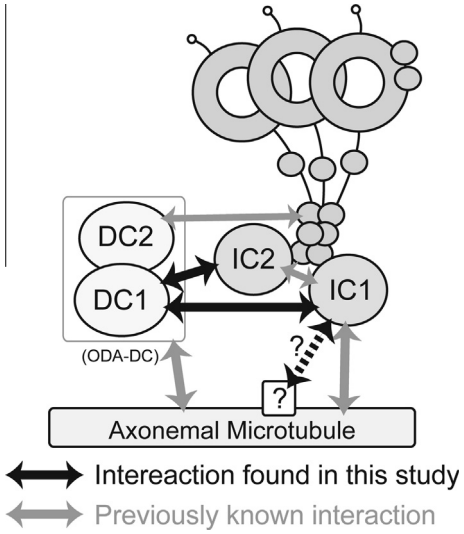

Fig. 5. Model of OAD-outer doublet microtubule binding. Three rings with projections represent heavy chains, and small circles represent light chains. Previously found interactions are shown with gray arrows (IC1-IC2, LC7b-DC2, IC1-tubulin, and the ODA-DC-axoneme). The interaction between IC1 and IC2 was reconfirmed in this study. Molecular interaction newly found in this study is direct interaction between the IC1–2 complex and the ODA-DC presumably via interaction between IC1-DC1 and IC2-DC1 (black arrows). Also, stoichiometric binding of IC1 to the axoneme suggests a third interaction in OAD-axoneme binding (a dotted arrow with "?") next to IC1-tubulin and the ODA-DC-axoneme. IC1 may bind with a specific binding site (a box with "?") on the doublet microtubule.

its microtubule binding activity, could potentially bind anywhere on the bare microtubule surface. One possibility is that the surface structure of the outer-doublet microtubules differs significantly from that of cytoplasmic microtubules due to variations in post-translational modifications.

4.5. A new model

A new model of OAD-outer doublet binding based on our findings concerning protein–protein interactions at the base of OAD is shown in Fig. 5. Our understanding of how OAD subunits are assembled and how OAD is bound to axonemal microtubules has been significantly advanced. The next challenge is to elucidate the mechanism by which the specific binding of IC1, as well as the ODA-DC, is accomplished.

Acknowledgements

We thank Dr. Manabu Yoshida (University of Tokyo) and Dr. Kaoru Yoshida (Toin University of Yokohama) for their help on the microtubule binding assay, and Dr. Masafumi Hirono (University of Tokyo) for helpful discussion. KW is supported by grants from Japan Society for Promotion of Sciences (#22770189, #25291058, #25113507, and #25117506) and a grant from Yamada Science Foundation. SMK is supported by grant GM051293 from the National Institutes of Health.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.febslet.2013.05.058.

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