Cross-linking of $\beta$ heavy chain subunit of dynein from sea urchin sperm flagella by dimethyl suberimidate

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ABSTRACT

The dynein $\beta$ heavy chain and the intermediate chain 1 (IC1) from sea urchin sperm flagella cosediment at 11S through sucrose density gradient centrifugation. When the isolated $\beta$/IC1 complex was treated with a bifunctional cross-linker, dimethyl suberimidate (DMS), and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, four high molecular mass products appeared on the gel. Immunoblotting of the products with affinity-purified polyclonal antibodies against $\beta$ heavy chain and IC1 revealed that these were cross-linked products of $\beta$ heavy chain and IC1. Treatment of $\beta$/IC1 complex with DMS activated the ATP hydrolysis and decreased its sensitivity to inhibition by vanadate. These results indicate that dynein $\beta$ heavy chain and IC1 associate with each other to form a complex and suggest that the intramolecular interactions within $\beta$/IC1 complex are involved in the process of ATP hydrolysis.

Dynein is a force-generating 'motor ATPase' which is involved in eukaryotic ciliary and flagellar movements and other microtubule-based cell motility (1, 12). Outer-arm dynein isolated from sea urchin sperm flagella is composed of two heavy chains ($\alpha$ and $\beta$), three intermediate chains (IC1, IC2 and IC3) and several light chains. Dialysis of the outer-arm dynein against a low ionic strength solution causes dissociation of the complex into the aggregates of $\alpha$-heavy chain, $\beta$/IC1 complex and IC2/IC3 complex (18). It has been shown by in vitro motility assay that the $\beta$/IC1 complex itself can induce the sliding of singlet microtubules (15).

Recent studies on the molecular structure of dynein heavy chains have supplied a lot of information on the primary and secondary structures of dynein heavy chains (3, 13). However, the intramolecular interactions within dynein heavy chains or interactions of a dynein heavy chain with intermediate chains have not yet been elucidated.

Bifunctional imido esters have been widely used in studying the structures of multisubunit proteins (14). In order to obtain some information on the interactions within dynein complex, I used a bifunctional cross-linker, dimethyl suberimidate (DMS). When the $\beta$/IC1 complex isolated from sea urchin sperm flagella was treated with DMS, four cross-linked products (I-IV) with molecular masses higher than that of $\beta$ heavy chain appeared on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gel (Fig. 1, a and b). In order to reveal the molecular composition of these cross-linked products, DMS-treated dynein was separated by SDS-PAGE, transferred on a polyvinylidene difluoride membrane and immunostained with affinity purified polyclonal antibody against $\beta$ heavy chain, IC1, IC2 or IC3 (Fig. 1b). The immunoblot showed that all of the cross-linked products reacted with anti-$\beta$ and anti-IC1 antibodies but not with anti-IC2 and anti-IC3 antibodies (Fig. 1b), indicating that they were the results of the cross-linking between $\beta$ heavy chain and IC1.

On the other hand, an indistinct band between $\beta$ heavy chain and IC1 was observed on SDS gel (asterisks in Fig. 1, a and b). This band reacted with

Abbreviations: DMS, dimethyl suberimidate; IC, intermediate chain; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; Vi, inorganic vanadate.
Fig. 1 SDS-PAGE and immunoblots showing the cross-linking of β/IC1 complex with DMS. Outer-arm dynein and β/IC1 complex were prepared from sperm flagella of the sea urchin, *Anthocidaris crassispina*, by the method described previously (6, 9). Before cross-linking, the β/IC1 complex (0.25 mg/ml) was exhaustively dialyzed against 50 mM triethanolamine buffer (pH 8.5) containing 0.2 mM EDTA. Then 10 vol of β/IC1 solution was mixed with 1 vol of several concentrations of DMS (Wako Pure Chemical Industries, Osaka, Japan) that were dissolved in the same buffer as described above. After incubation of the mixture at 20°C for 3 h, the reaction was terminated by the addition of 1 vol of 1 M lysine. Cross-linked products were analyzed by SDS-PAGE by the method of Laemmli (11) with 3.5% acrylamide gel containing 4 M urea used in the separating gel (a). The β/IC1 complex treated with 1 mM DMS at 20°C for 1 h were separated by SDS-PAGE and transferred onto a PVDF membrane (Millipore, Bedford, U.S.A.). Then, the membrane was stained with Coomassie brilliant blue R-250 (lane 1) or immunostained with affinity purified rabbit polyclonal antibody against β heavy chain (lane 2), IC1 (lane 3), IC2 (lane 4) or IC3 (lane 5) (b). Affinity purification of antibodies and immunoblotting were performed by the method described previously (5). I, II, III and IV, cross-linked products between dynein β heavy chain and IC1; asterisk, cross-linked products between IC2 and IC3.

affinity-purified anti-IC2 and IC3 antibodies (Fig. 1b), indicating that this resulted from the cross-linking between IC2 and IC3 which contaminated the β/IC1 fraction as a complex through sucrose density gradient centrifugation.

Cross-linking of the β/IC1 complex with DMS activated ATPase activity (Fig. 2). When the concentration of DMS was increased to 0.5 mM, the ATPase activity of β/IC1 complex was activated up to 2-fold, then it gradually decreased with increasing concentration of DMS. The β/IC1 complex was also treated with increasing concentration of methyl acetimidate which is a monovalent reagent to amidinate amino-groups without cross-linking and can be used as a control. The result showed that ATPase activity gradually increased, however, at the most up to 1.2-fold only of the initial activity even at 2.0 mM. This suggests that the activation of ATP hydrolysis by DMS is not due to the chemical modification of amino-groups, but to the cross-linking within dynein complex. Other two reagents for amino-groups modification, O-methylisourea
and N-hydroxysuccinimide, had almost no effect on ATPase activity even at 2 mM.

Vanadate is a well-known potent inhibitor of dynein (2) and is considered to replace Pi in the dynein-ADP-Pi intermediate by forming a more stable dynein-ADP-Pi complex (17). By treating the β/IC1 complex with DMS, the sensitivity of the complex to vanadate decreased (Fig. 3a). The concentration of vanadate to inhibit ATPase activity to 50% was 50 μM in the case of DMS-untreated β/IC1 complex, whereas the value increased to 500 μM in the case of DMS-treated complex.

Furthermore, vanadate-mediated photocleavage is one of the characteristics of dynein heavy chain (4) and could be related to the enzymatic properties of dynein (4, 8). The preparation of β/IC1 complex was treated with 1 mM DMS and subsequently subjected to photocleavage in the presence of ATP and vanadate. When the band intensities on SDS-PAGE gel of a major cross-linked product, band II, and unreacted β heavy chain were measured by a densitometer, significant suppression of cleavage of band II was observed in contrast to the unreacted β heavy chain (Fig. 3b). This result, along with the result shown in Fig. 3a, implies that cross-linking of β/IC1 complex with DMS might result in decreasing the lifetime of dynein-ADP-Pi intermediate, which causes the formation of a less stable dynein-ADP-Pi complex.

The results described above suggest that interactions of β chain with IC1 or intramolecular interactions within β heavy chain are involved in the process of ATP hydrolysis. There have been studies on the interaction of dynein with microtubules by using a zero-length cross-linker, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (10, 16). The present study showed that DMS could be also a potent cross-linker to study dynein ATPases, especially for the interactions within dynein complex.

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Fig. 3 Effects of DMS treatment on the vanadate-mediated properties of β/IC1 complex. (a) Vanadate inhibition of ATP hydrolysis by DMS-treated (●) and -untreated (○) β/IC1 complex. Isolated β/IC1 complex was incubated at 20°C for 3 h in the presence and absence of 1 mM DMS. After the reactions were terminated by lysine, ATPase activities were measured in the presence of several concentrations of vanadate. (b) Quantitative analysis of the decrease of β heavy chain (○) and a cross-linked product of β/IC1 complex (●) by Vi-mediated photocleavage. The β/IC1 complex was treated with 1 mM DMS at 20°C for 3 h. Then, photocleavage was performed by the method of Gibbons et al. (4) with some modifications (5). Proteins were separated by SDS-PAGE and the band intensities of band II and β heavy chain were measured by a densitometer.