Spectroscopic Studies of the ncd Motor Domain·ADP Complex: CD Spectrum of ADP Induced by Binding to the Motor Domain of ncd†

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ABSTRACT: Previously, we reported that the nucleotide-free ncd motor domain exhibited a near-UV CD spectrum different from that of the ordinary ncd motor domain·ADP complex [Shimizu and Morii, (1996) *J. Biochem. 120*, 1176−1181]. In the present study, we exchanged the bound nucleotide ADP with N^6-methylADP (MeADP) which has a UV absorption spectrum different from that of ADP. The resultant ncd motor domain·MeADP complex gave a near-UV CD spectrum different from that of the ordinary ncd motor domain with bound ADP. This result indicates that the bound nucleotide contributes to the near-UV CD spectra to a considerable extent although ADP or MeADP free in solution gives a spectrum with negligible peaks and troughs. In addition, the absorption intensity of ADP or MeADP at the peak wavelengths decreased to a considerable extent upon binding to the nucleotide-free ncd motor domain. It is suggested that interaction between adenine moiety and chromophore(s) of the protein contributed to the spectral changes of ADP. A candidate chromophore is Tyr^{442} which is stacked with the adenine moiety at a distance of 0.43 nm. On the other hand, we detected an intensity decrease of tryptophanyl fluorescence upon binding of a nucleotide to the nucleotide-free ncd motor domain, while at the same time tyrosyl fluorescence increased. The fluorescence changes, as well as the UV absorption change described above, gave similar rates upon addition of a nucleotide to the nucleotide-free ncd motor domain. Therefore, they are likely to originate from the same conformational change of the protein.

The product of a *Drosophila* gene *nonclaret disjunctional*, ncd, is a kinesin-related protein (*1, 2*). The ncd expressed in bacteria and purified has been shown to have both microtubule-stimulated ATPase activity and motor activity to move microtubules in vitro. The most remarkable characteristic of ncd motility is that the directionality, microtubule minus end-directed, is opposite of that of kinesin. In addition, the ncd motor domain, homologous to that of kinesin, is on the C-terminal of the polypeptide, in sharp contrast to the N-terminal motor domain of kinesin.

One of the unique features of the motor domain of kinesin and its family members, including ncd, is the tight binding of ADP (*3*). While ADP-free kinesin motor domain, which exhibits the initial burst of phosphate production upon ATP addition, has been prepared (*4, 5*), removal of the tightly bound ADP from the ncd motor domain by EDTA treatment resulted in inactivation and/or denaturation (*6*−*8*). Adding a high concentration of NaCl such as 0.5 M to the solution, however, prevented the ADP-free ncd motor domain from irreversible denaturation, at least for a while.

Previously (*6*), we measured and compared the CD spectra of this ADP-free ncd motor domain to the ordinary ncd motor domain with bound ADP. The far-UV CD spectra were almost identical, suggesting that the backbone structure was largely unaffected, if at all, by ADP removal. On the other hand, the near-UV CD spectra in the range 245−290 nm were considerably different from each other. We interpreted this result to be evidence that the removal of the tightly bound ADP affected the local conformation of the motor domain. However, there remained a possibility that the CD spectral difference was due to ADP; free ADP in solution gave negligible CD peaks and troughs in this region (e.g., ref *9*) but its binding to the special region of the protein might have brought about a spectral change due to some interaction of the adenine moiety of the ADP molecule with a chromophore of the protein.

In the present study, we reinvestigated the near-UV CD spectra of the ncd motor domain in more detail by exchanging the bound nucleotide ADP with NDP. Exchange was only partial with most NDPs, indicating a much higher affinity for ADP than for NDPs or NTPs. However, MeATP[1] expelled more than 90% of the bound ADP. The resultant ncd motor domain·MeADP complex gave a near-UV CD spectrum different from that of the original ncd motor domain·ADP complex, indicating that at least some part of the CD spectral characteristics was due to the bound nucleotide. We also observed changes in the UV spectra of

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[1] Abbreviations: DTT, dithiothreitol; EGTA, ethylene glycol bis-(aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; GST, glutathione S-transferase; MeADP, N^6-methyladenosine 5′-diphosphate; MeATP, N^6-methyladenosine 5′-triphosphate; Mops, 3-morpholinopropanesulfonic acid; NDP, nucleoside 5′-diphosphate; NTP, nucleoside 5′-triphosphate.

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the nucleotides. Upon addition of ATP to the nucleotide-free ncd motor domain, the fluorescence of tryptophanyl and tyrosyl residues changed at similar rates, suggesting that changes in the protein conformation take place upon nucleotide binding.

MATERIALS AND METHODS

Commercially obtained ATP, GTP, and ITP were purified, while MeATP was prepared and purified as described (*10*). α-^{32}P-ATP (PB 171, Amersham) was used without further purification.

Preparation of the ncd motor domain was the same as described (*11*). The molecular mass and the mean residue weight of the ncd motor domain were taken to be 41 300 and 113.2, respectively. The removal of ADP from the motor domain in the presence of EDTA and a high salt was done in the same manner as described (*6*). The protein concentration was determined by the method of Lowry et al. (*12*), using bovine serum albumin as a standard.

The exchange of nucleotide was performed according to the following method. One-half of a milliliter of solution of the ncd motor domain containing 20 mM Mops-NaOH (pH 7), 0.5 M NaCl, 2 mM $MgCl_2$, 0.5 mM EGTA, and 0.5 mM DTT was added with 2 mM NTP and dialyzed against 3 mL of the same solution with 2 mM NTP. The dialyzing solution was changed once. Then, the dialysate was gel filtrated through a prepacked gel exclusion column (10DG, BioRad) in the presence of 0.1 mM NTP with the same buffer solution.

The assessment of the exchange of the nucleotide was done in two ways. (1) The ncd motor domain was labeled with radioactive ADP by incubating it with α-^{32}P-ATP (see ref *11*). The resultant radioactively labeled ncd motor domain was treated with NTP as above. After treatment, the remaining radioactivity was measured by Cerenkov counting to estimate the exchange. (2) We checked the UV absorption spectrum of the substance released from the ncd motor domain upon acid addition. ncd motor domain (not radiolabeled) treated as above was added with final 0.3 M perchloric acid to precipitate the protein. We then checked the UV absorption spectrum of the supernatant. This method is based upon characteristic UV spectra of various nucleotides.

The CD spectra were measured using a Jasco J-600 or J-630 CD spectropolarimeter with a temperature-controlled cuvette holder at 20 °C as described previously (*6, 13*). Fluorescence and UV absorption spectra were obtained using a Shimadzu RF-5000 spectrofluorometer and a Jasco U-Best V-560 or a Beckman DU-70 spectrophotometer, respectively.

RESULTS AND DISCUSSION

Near-UV CD Spectra of the ncd Motor Domain with NDP. To see whether the near UV CD spectral difference between the ncd motor domain with bound ADP and the ADP-free ncd motor domain was due at least in part to ADP, we attempted to replace the bound ADP with other NDP. Addition of NTP or NDP to the nucleotide-free ncd motor domain might have been a method to make the ncd motor domain·NDP complex. However, addition of ATP or ADP to the nucleotide-free ncd motor domain only partially (up to 80%) restored the original near-UV CD spectrum (*6*).

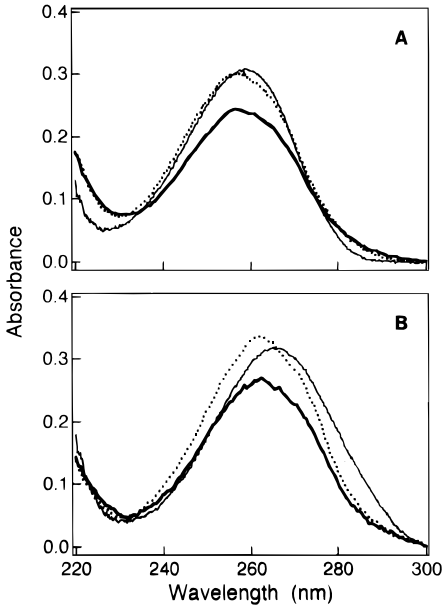

FIGURE 1: Absorption spectra of ATP and MeATP. Absorption spectra of ATP (A) and MeATP (B) were measured at a concentration of 0.02 mM in the presence of 50 mM Mops-NaOH and 2 mM $MgCl_2$ (thin, solid lines) or in the presence of 0.3 M perchloric acid (dotted lines). Forty micromolar ordinary ncd motor domain, or 40 μM ncd motor domain, which had been treated with MeATP for exchange of the bound nucleotide as described in Materials and Methods, was mixed with an equal volume of 0.6 M perchloric acid, and the resultant supernatant after clarification by centrifugation was measured in the same manner (thick, solid lines), shown in A and B, respectively. Note that the spectrum of the acid supernatant of the protein is similar in pattern to that of authentic material in acid in either case. Smaller absorption of acid supernatant of the protein than that of authentic material, although they were intended to be the same concentration, may indicate that some protein was not native, having no bound nucleotides. It should be noted that the absorption spectrum of ATP (or MeATP) and that of ADP (or MeADP) are indistinguishable.

Instead, therefore, we incubated the ncd motor domain with a large excess of NTP, which was followed by gel filtration in the presence of NTP as described in detail in Materials and Methods.

The extent of the replacement was one of our major concerns. As described in Materials and Methods, we incorporated radioactive ADP into the ncd motor domain by incubating it with α-^{32}P-ATP. ATP, or more generally NTP, when incorporated is converted into ADP or NDP at the ncd active site (*3, 11*). The loss of radioactive ADP from the ncd motor domain after treatment with MeATP was more than 90%, while treatment with GTP or ITP only resulted in about 50% loss. This indicates that the affinity of ADP is so high that it does not allow easy exchange with GTP or ITP (which are converted into GDP or IDP at the active site as stated above) although GTP and ITP supported the in vitro microtubule motility by GST/MC1, an ncd construct (*14*).

As MeATP seemed to be promising and in order to rule out the possibility that ncd motor domain had just lost its ADP without incorporating MeATP, we added perchloric acid to denature and precipitate the protein, then measured the UV spectrum of the acid supernatant. The spectrum was almost superimposable to that of free (authentic) MeATP in pattern (Figure 1). Thus, we concluded that, after treating the ncd motor domain, as described herein, most of the bound ADP was replaced with MeATP. It should be noted that,

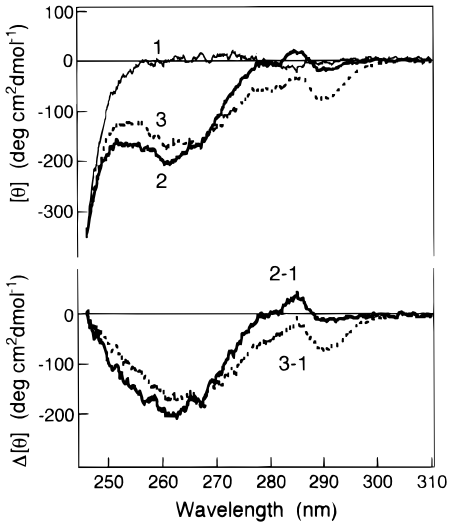

FIGURE 2: Near-UV CD spectra of ncd motor domain. The upper half of the figure indicates the near-UV CD spectra of (1) the nucleotide-free ncd motor domain, (2) the ordinary ncd motor domain with bound ADP, and (3) the ncd motor domain containing mostly MeADP after the nucleotide exchange treatment described in Materials and Methods. The solution contained 0.02 mM protein, 20 mM Mops-NaOH (pH 7.0), 0.5 M NaCl, 4 mM $MgCl_2$, and 2 mM EDTA. The measurements were done at 20 °C by circulating water through a cuvette holder. The cuvette light path was 10 mm. The molar ellipticity, θ, was calculated on the basis of the amino acid residue concentration; the mean residue weight was taken to be 113.2. Note that the spectra (2) and (3) are considerably different. The lower half of the figure indicates the difference spectrum between (3) and (1) shown as (3−1) and that between (2) and (1) shown as (2−1). The negative peak of (2−1), the ADP-bound form versus the nucleotide-free form, is at 260−262 nm while that of (3−1), the MeADP-bound form versus the nucleotide-free form, is at 266−268 nm. A peak and a trough at 285−290 nm are thought to be due to tryptophanyl residue(s) (see text).

after binding to the enzyme active site, MeATP was presumably converted into MeADP; the bound nucleotide should be MeADP, although we have not directly confirmed it. It may be noteworthy, however, that even adenylylimidodiphosphate (AMPPNP), a nonhydrolyzable ATP analogue for many enzymes, is cleaved by ncd (*15*), and that the resultant ADP analogue, adenosine 5′-phosphoroamidate (AMPPN), remains at the active site (E. Sablin, personal communication).

Figure 2 shows the near-UV CD spectrum of ncd motor domain·MeADP complex, together with that of the nucleotide-free ncd motor domain, and that of the original ncd motor domain with bound ADP. The CD spectra were quite different between the ADP-bound form and the MeADP-bound form. The difference spectrum, shown in the lower part of the figure, was similar to the absorption spectrum of ADP or MeADP. This result strongly suggests that the near-UV CD spectrum was, to a large extent, brought about by the nucleotide, although nucleotides free in solution showed negligible peaks and troughs in this wavelength zone. The nucleotide, normally quiet in terms of CD spectra, became active upon binding to the enzymic site of the ncd motor domain. Possible reasons for this change will be discussed below.

We also measured the CD spectra of the ncd motor domain which had been treated for the exchange of the nucleotide with GTP or ITP. Although the exchange was only around 50%, the near-UV CD spectra were much different from that

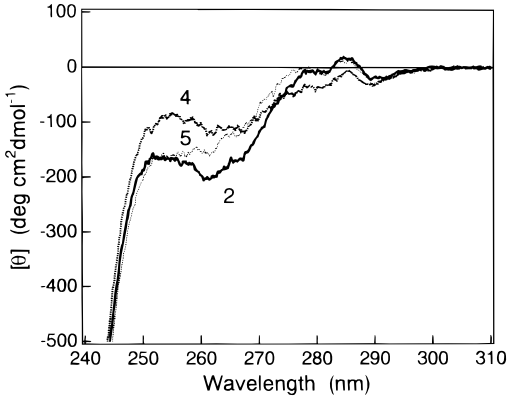

FIGURE 3: Near-UV CD spectra of the ncd motor domain having different nucleotides. Shown herein are the near-UV CD spectra of (2) the ordinary ncd motor domain with ADP, (4) the ncd motor domain treated with GTP, and (5) the ncd motor domain treated with ITP. Solution ingredients and other conditions as well as the definition of θ were the same as described in the legend to Figure 2. Even after extensive treatment with GTP or ITP, the ncd motor domain still contained about 50% of radiolabeled ADP which had been incorporated as described in text, indicating that the exchange of ADP to GDP or IDP was only up to about 50%. Nonetheless, the near-UV CD spectra were quite different, strongly suggesting the contribution of the nucleotide to the near-UV CD spectra.

of the ncd motor domain·ADP complex (Figure 3). This may augment the above argument that a considerable part of the near-UV CD spectra could be ascribed to the bound nucleotide.

Changes in UV Absorption Spectra of Nucleotides. We were curious whether the near UV CD spectral changes would be accompanied by absorption spectral changes. It was not possible to directly compare the spectra of free nucleotides with those of bound nucleotides with precision if we exchanged the nucleotides as described above. For example, we could not know the exact percentage of the ncd motor domain having nucleotide. Therefore, we first removed the bound ADP from the ncd motor domain by EDTA treatment in the presence of high salt and added ATP or MeATP, and compared the spectra with those of free nucleotides using a tandem cuvette. Since only up to 80% of the nucleotide-free ncd motor domain remained potentially active, thus able to bind nucleotide, we added a substoichiometric amount of nucleotide, 12.5 μM ATP or MeATP, to 25 μM ncd motor domain (post-mixing concentrations).

As seen in Figure 4, 30 s after the addition of ATP or MeATP, the difference of the UV spectra was obvious; the absorption decreased upon mixing of a nucleotide with the protein. After 5 min, the decrease was more profound but a further decrease was small. The negative peak was observed at 258 and 263 nm for ATP and MeATP, respectively. The magnitude of the decrease was considerably large; at 12.5 μM, the absorbance of free ATP at 259 nm is 0.19 and that of free MeATP at 266 nm is 0.20, so, an almost 30% decrease at peak positions occurred. The spectra shown in Figure 4 are quite different from each other, depending on the nucleotides, indicating that the difference was mostly due to the nucleotides themselves.

Monitoring the change of absorption at 260 nm after the addition of ATP, we found the change had a rate of 0.07 s^{-1}. This value is consistent with that reported previously for 2′(3′)-*O*-(*N*-methylanthraniloyl)adenosine 5′-triphosphate

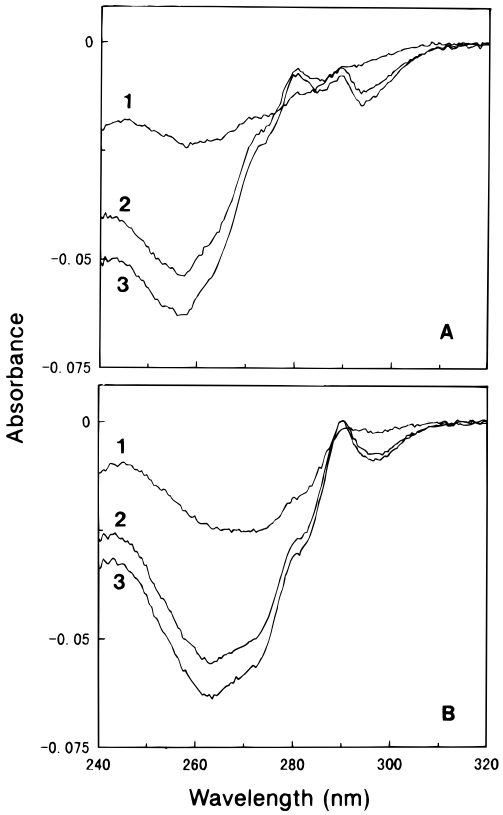

FIGURE 4: Difference absorption spectra of ATP or MeATP in the presence and absence of the nucleotide-free ncd motor domain. A tandem cuvette with two compartments whose light paths were 4.5 mm each and a cuvette with a single compartment with 9 mm light path were used. Fifty micromolar nucleotide-free ncd motor domain in 20 mM Mops-NaOH (pH 7), 4 mM $MgCl_2$, 2 mM EDTA, and 0.5 M NaCl and another solution with 25 μM ATP (A) or MeATP (B) but without the protein were put into the compartments of the tandem cuvette separately. A one-to-one mixture of the above solutions was put into the other cuvette, and the difference spectra were measured. The measurements were done at room temperature (ca. 22 °C). (1), (2), and (3) in the figures indicate that the spectra were recorded 30, 300, and 600 s after the mixing, respectively. Upon mixing, the absorption decreased in the entire region recorded, and negative peaks were seen around 258 and 263 nm in the case of ATP and MeATP addition, respectively. In addition, peculiar patterns were observed between 275 and 300 nm, probably due to tryptophanyl residue(s).

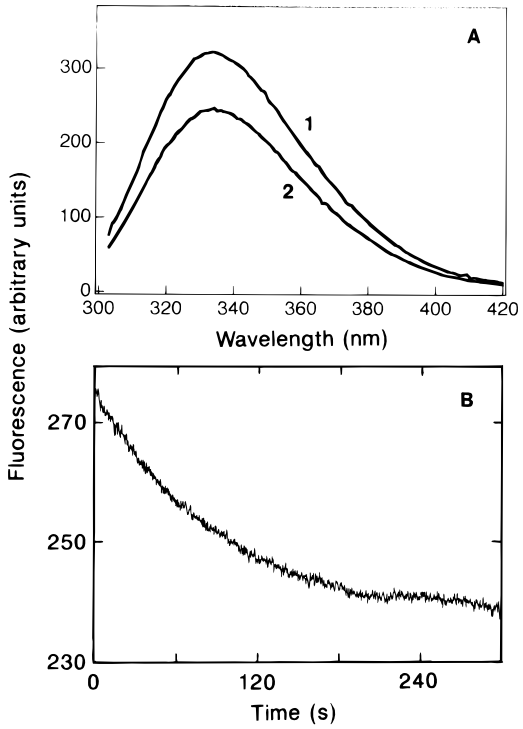

FIGURE 5: Fluorescence of the ncd motor domain upon excitation at 295 nm. (A) Fluorescence spectra. The nucleotide-free ncd motor domain (0.029 mM) in a solution containing 20 mM Mops-NaOH (pH 7.0), 4 mM $MgCl_2$, 2 mM EDTA, and 0.5 M NaCl (1) before and (2) 10 min after addition of final 0.05 mM ATP was excited at 295 nm using a temperature-controlled cuvette holder at 20 °C, and the emission spectra were recorded. The wavelength of the emission peak did not change upon ATP addition, but the emission intensity decreased by about 25%. (B) Time course of fluorescence. The experimental conditions were the same as those in A. After the ATP addition and mixing, the fluorescence intensity at 340 nm was monitored. The rate of fluorescence decay was about 0.1 s^{-1} at 20 °C. It should be noted that the operation was done manually and that the first 15 s are missing in this figure; time 0 in this figure is in fact 15 s after mixing.

(mantATP) fluorescence change upon its binding to the nucleotide-free ncd motor domain (*6*).

Changes in the Protein Itself upon Binding of the Nucleotide. The absorption difference spectra (Figure 4) also show characteristic small peaks at 280 and 290 nm when ATP is added, and a peak at 290 nm and a shoulder at 280 nm in the case of MeATP addition. Since these peaks and the shoulder appeared at the same wavelengths for both nucleotides and since tryptophanyl residues exhibit absorption in this region, we believe that these were due to tryptophanyl residues. This may also be consistent with the observation of a small peak and a trough in the region from 285 to 290 nm when the near-UV CD spectrum of the ncd motor domain·ADP complex was measured (ref *6*; also see Figures 2 and 3).

Therefore, we became interested in tryptophanyl fluorescence if it would change upon ATP binding to the nucleotide-free ncd motor domain. As seen in Figure 5, the fluorescence upon excitation at 295 nm decreased by mixing the nucleotide-free ncd motor domain with ATP. The overall profile of the emission spectra and the peak position did not change. The time course of the change had a rate of about 0.1 s^{-1}. We also found that the quenching of the fluorescence of the nucleotide-free ncd motor domain by acrylamide was nearly identical to that of the ncd motor domain with bound ADP (data not shown), meaning that the tryptophanyl residue(s) responsible for this difference did not change in terms of the extent of exposure to the medium. Only two tryptophanyl residues are contained in the ncd motor domain; one in β1a sheet (Trp^{370}) and the other in L6 loop (Trp^{473}) (*16*). Neither residue is likely to be in direct contact with the bound nucleotide, although Trp^{370} lies fairly close to the active site (Figure 6). Thus, we think that Trp^{370} is likely to be responsible for the changes, which will be discussed in more detail later.

We also investigated the fluorescence of tyrosyl residues. Upon excitation at 275 nm, the fluorescence due to tyrosyl residues appears around 300 nm. As shown in Figure 7, the fluorescence emission spectra changed after the addition of ATP to the nucleotide-free ncd motor domain; the decrease, around 330−340 nm, was due to the tryptophanyl fluorescence as above, but a conspicuous shoulder was seen around 300 nm. Next, the time course of the fluorescence at 300 nm upon excitation at 275 nm after mixing of the

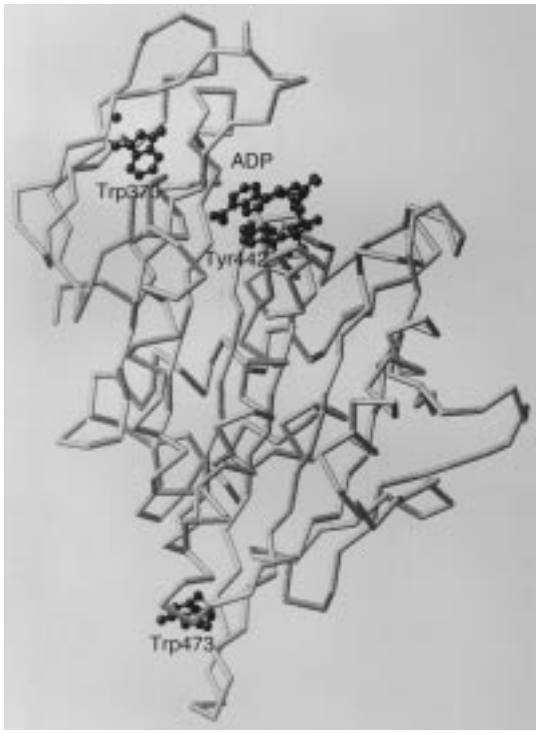

FIGURE 6: Three-dimensional structure of the ncd motor domain. The structure was constructed from the coordinates kindly provided by E. Sablin and R. Fletterick (University of California, San Francisco, CA). The positions of ADP, Tyr^{442}, Trp^{370}, and Trp^{473} are indicated. Note that the adenine of ADP is directly stacked with Tyr^{442}, the distance between the two being 0.43 nm. Trp^{370} is about 1.4 nm away from ADP, and Trp^{473} is much farther away. The estimated interactions relevant to the spectral changes between ADP and those chromophores were the strongest with Tyr^{442} and the weakest with Trp^{473}.

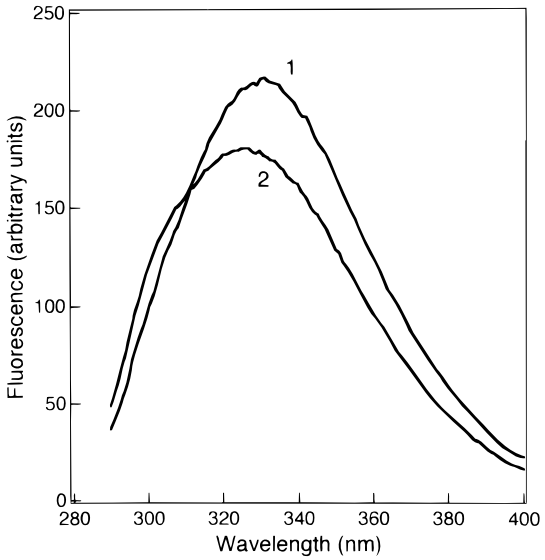

FIGURE 7: Fluorescence spectra of the ncd motor domain upon excitation at 275 nm. Fluorescence spectra were recorded in the same manner as described in Figure 5, except that the excitation was done at 275 nm. At 275 nm, absorption by ATP is not negligible, so that a correction was made for the spectrum after the ATP addition by taking the absorption by ATP to be 27% of the excitation light. The fluorescence decrease around 340 nm seen after the addition of final 0.05 mM ATP is likely to be due to tryptophanyl residue(s). A shoulder around 300 nm appears to be due to tyrosyl residues. It should be noted that, from the time course of fluorescence intensity at 300 nm upon excitation at 275 nm, the fluorescence intensity increase was found to be about 15%.

nucleotide-free ncd motor domain with ATP was measured to reveal a ~15% increase in magnitude. The rate was again very similar, about 0.1 s^{-1}. We therefore conclude that, although the backbone structure remains unaltered upon nucleotide removal, revealed by the far-UV CD spectra (*6*), the binding of the nucleotide induces certain side chain geometry modifications.

As noted above, a small peak at 285 nm and a trough at 290 nm in the difference CD spectrum were consistently observed (Figure 2). In addition, the difference CD spectrum between the nucleotide-free form and the ADP-bound form in the range of far UV indicated a shallow trough around 230 nm, probably a characteristic of tryptophanyl residue(s) (data not shown). Therefore, the tryptophanyl residue(s) was (were) likely to contribute to the difference CD spectrum.

It should be noted again that, according to the structure of the ncd motor domain solved by X-ray crystallography (*16*), the enzymatic active site does not have tryptophanyl residues. However, Trp^{370} lies fairly close to the nucleotide-binding site (Figure 6), which we suspect to be responsible for at least some part of the spectral change. We are now in a process of determining whether this tryptophanyl residue is responsible for those spectral changes by making mutant ncd motor domains.

Theoretical Considerations of the Spectral Changes due to the Interaction of Nucleotide and Chromophores of the Ncd Motor Domain. With creatine kinase, a near-UV CD spectral change, likely due to the bound adenine nucleotide, has been reported (*9*, *17*). The change was ascribed to the dipole−dipole interaction of the 259 nm transition of adenine moiety with linearly polarized π-π^* transitions of a nearby tryptophan residue. The candidate Trp^{223} of the mitochondrial creatine kinase is about 1 nm apart from the adenine, but it could be closer (*18*) and be responsible for the CD spectral change of the adenine moiety. In the case of adenylate kinase, the absorption spectra of bound ATP were different from that in aqueous solutions (*19*). However, no CD spectral changes were reported (*20*). A coupling of two transition moments of the base of a nucleotide and a chromophore of a protein enhances the CD Cotton effects when the two interacting moments are not parallel. When they are parallel, only absorption spectra will be affected (*21*).

In the case of the ncd motor domain, candidate chromophores are Trp^{370} as above and Tyr^{442} (Figure 6). Analogous to the case of creatine kinase, Trp^{370} might seem to be a good candidate, but it is 1.4 nm apart from the adenine of the bound ADP. Moreover, the β1b strand runs between Trp^{370} and the adenine. On the other hand, Tyr^{442} exists in direct contact (0.43 nm) with the adenine moiety. According to the expressions given by Kägi et al. (*9*), the rotational strength corresponding to CD intensity was estimated by the coupled oscillator theory of optical rotation. Generally, the rotational strength is approximately proportional to the dipole strengths and to the geometric factor (G_{ij}), which depends on the vectors of transition moment dipoles and the vector of their distance. The calculations were carried out for a pair of mutually perpendicular transition dipoles in each aromatic plane of chromophore, one of which was laid along the longitudinal direction of chromophore for convenience. By using the atomic coordinates of the ncd motor domain,

the geometric factors with respect to the adenine moiety were 0.10, 1.34, and -0.02 nm^{-2} for Trp^{370}, Tyr^{442}, and Trp^{473}, respectively. Thus, the largest geometric factor was found for the coupling of the adenine and Tyr^{442}.

Next, the rotational strengths for the adenine moiety were calculated to be 2.0, -4.3, and -0.3×10^{-40} cgs units for the interactions with Trp^{370}, Tyr^{442}, and Trp^{473}, respectively. In these calculations, the parameters were adopted from those by Kägi et al. (*9*) and the dipole strength of tyrosine was assumed to be one-quarter of that of tryptophan, which is proportional to the ratio of their absorption coefficients. Possibilities of the dipole interactions other than the ones described above, in addition to some unknown factors, would cause additional ambiguities in the calculations of CD intensity. However, the summation of these calculated rotational strengths, which corresponds to the CD intensity of adenine region, was negative enough to explain the large negative CD band around 262 nm. Going into detail, this band would be attributed mainly to the ADP-Tyr^{442} interaction and would be negligibly affected by the ADP-Trp^{473} interaction (Figure 6). We would also expect that the ADP-Trp^{370} interaction made a small, but not negligible, contribution. Interestingly, the molar ellipticity at 262 nm of the ncd motor domain·ADP complex was -70000 deg cm^2 (dmol of protein)$^{-1}$. This intensity is almost the same in amplitude as that reported for creatine kinase (+68000).

In the amino acid sequence, Tyr^{442} is immediately after the ATP-binding consensus sequence, GXXXXGST. Among kinesins, this position is, without exceptions, occupied with an aromatic amino acid. So far, three-dimensional structures of three motor domains have been solved; ncd, human kinesin, and yeast Kar3 (*22*). The human kinesin has His^{93} and yeast Kar3 has Phe^{482} at this position. In all three cases, these chromophores form stacks with adenine, probably contributing to the tight binding of ATP and ADP to these motor domains (*23*).

The difference in amino acid species at this position might explain why the CD spectral change upon ADP removal was smaller with kinesin motor domain (*6*); the transition moment of His^{93} of the human kinesin should be much weaker than that of Tyr^{442} of ncd. In addition, when we tried to exchange ADP of the ncd motor domain with GDP, the near-UV CD change became much smaller (Figure 3), which might be ascribed to a difference in the spatial arrangement of the transition moment of the base part of GDP from that of ADP. We are also in the process of making mutant ncd and kinesin motor domains at these positions to obtain direct evidence for such interactions.

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