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NMR insights into dynamics regulated target binding of DLC8 dimer

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Abstract

Conformational dynamics play a crucial role in biological function. Dynein light chain protein (DLC8) acts as a cargo adaptor, and exists as a dimer under physiological conditions and dissociates into monomer below pH 4. In the present NMR study, we identified some dynamic residues in the dimer using chemical shift perturbation approach by applying small pH change. As evidenced by gel filtration and CD studies, this small pH change does not alter the globular structural features of the protein. In fact, these changes result in small local stability perturbations as monitored using temperature dependence of amide proton chemical shifts, and influence the dynamics of the dimer substantially. Further, interaction studies of the protein with a peptide containing the recognition motif of cargo indicated that the efficacy of peptide binding decreases when the pH is reduced from 7 to 6. These observations taken together support the conception that dynamics can regulate cargo binding/trafficking by the DLC8 dimer.

Keywords: Dynein light chain protein; Cargo trafficking; Gel filtration; Nuclear magnetic resonance; Temperature coefficients; pH sensitivity; Circular dichroism

Cytoplasmic dyneins are microtubule-based molecular motors which provide forces for several motility processes in eukaryotic cells [1,2]. These are multisubunit complexes of molecular masses >1 MDa. Among all the subunits of dynein motor complex the light chain (DLC8) is the smallest and is highly conserved among different species. Molecular genetic studies in several organisms including *Drosophila*, *Chlamydomonas*, and *Aspergillus* show that DLC8 has high functional significance [3,4]. DLC8 consists of 89 residues and interacts with a large number of other proteins with diverse biological functions [2,5–8]. For example, in healthy cells, Bim, a pro-apoptotic protein is sequestered away from the sites where pro-survival proteins (Bcl-2) reside by complexation with DLC8. On receiving apoptotic stimuli, the Bim–DLC8 complex releases Bim in the cytoplasm at the site where it can interact with the Bcl-2 proteins [9]. Thus, DLC8 acts as a cargo adaptor in the dynein complex to transport various organelles. A five-residue 'K/RXTQT' sequence has been recognized as the recognition motif in target peptides [10].

DLC8 is a stable dimer in the pH range 7–5, and a stable monomer below pH 4.0 [11,12]. But the protein is functional only as a dimer since the monomer is not capable of binding to the target proteins [13]. The dimer is a symmetric molecule (Fig. 1) with each monomer consisting of two α-helices (α1, residues 15–31; α2, residues 35–50) and five β-strands (β1, residues 6–11; β2, residues 54–59; β3, residues 62–67; β4, residues 72–78, and β5, residues 81–87) [14]. The protruding β3 strand of one monomer pairs in an antiparallel fashion with the β2′ strand of the other monomer. A number of contacts at the interface between the two monomers stabilize the dimeric structure. These include side chain H-bonds: Gln 61–Arg 60′, Tyr 65–Lys 44′, Thr 67–Lys 43′, and hydrophobic interactions: Ile 57/57′, Phe 62/62′, Ser 64/64′, Val 66/66′, and His 55/55′. The dimer binds the target molecules in an antiparallel β-strand fashion through its β3-strand (Fig. 1). It also makes several other contacts with protein residues at the dimer

Abbreviations: DLC8, dynein light chain protein; NMR, nuclear magnetic resonance; HSQC, hetero-nuclear single quantum coherence; CD, circular dichroism; DTT, Dithiothreitol.

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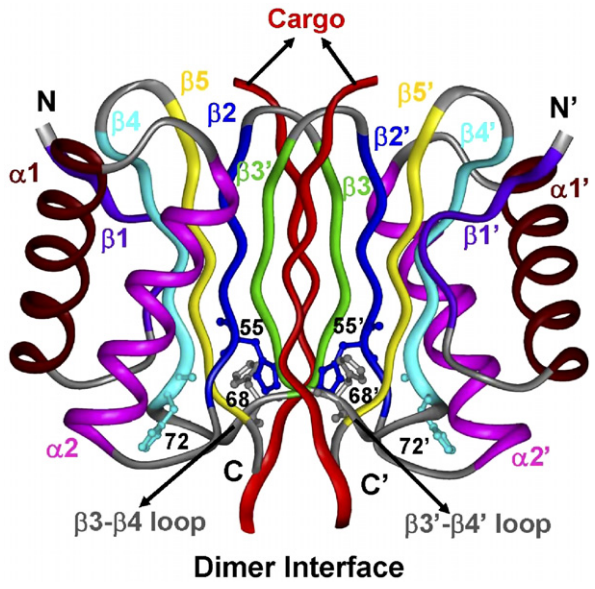

Fig. 1. Structure of the DLC8 dimer (residues 5–89) complexed to a target peptide (PDB id:1cmi). The primed and the unprimed labels distinguish the two monomers. The different α-helix and β-strand secondary structures are identified by color codes: α1 (brown), α2 (pink), β1 (purple), β2 (blue), β3 (green), β4 (cyan), β5 (yellow), and target peptide (red). The side chains of His 55 (blue), His 68 (grey), and His 72 (cyan) are also marked on the structure. DLC8 dimer image was produced using Insight II. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

interface [14]. However, the mechanism of trafficking by the dimer, which requires binding and release of cargo in different regions of the cell, is not very clear. In this context, we present here the regulatory role of protein dynamics in target binding to understand the structure–function relationship of the dimeric DLC8.

Materials and methods

Protein expression and purification

DLC8 was expressed and purified as described elsewhere [12].

Gel filtration

Gel filtration was performed at pH 7, 6 and 3 using a Hi Load 16/60 Superdex 75 column (Amersham) with buffer (20 mM phosphate, 200 mM NaCl at pH 7, 6 and 20 mM acetate, 200 mM NaCl at pH 3) at a flow rate of 0.3 ml/min with absorbance monitored at 280 nm using Bio-Rad Bio-Logic LP system. Nearly 1 ml of 100 μM protein was loaded on to the column.

Circular dichroism

Secondary and tertiary structures at pH 7 and 6 for DLC8 dimer and at pH 3 for DLC8 monomer were measured by recording far-UV CD spectra (205–250 nm) and near-UV spectra (260–310 nm) on a JASCO model J-810 spectropolarimeter using 1 nm bandwidth. For far-UV and near-UV CD experiments, a protein concentration of 40 and 150 μM in phosphate buffer at pH 7 and 6 (20 mM phosphate, 200 mM NaCl, and 2 mM DTT), and in acetate buffer at pH 3.0 (20 mM acetate, 200 mM NaCl, and 2 mM DTT) were used, respectively. The far-UV and near-UV CD experiments were recorded using a fused quartz cell with path lengths of 1 mm and 1 cm, respectively.

NMR spectroscopy

NMR sample. For NMR studies the protein purified as described above was concentrated to 1.0 mM. Phosphate buffer (20 mM phosphate, 200 mM NaCl, and 2 mM DTT) was used for the experiments recorded at the pH values 7 and 6, respectively. The final volume in all the samples was ~550 μl (90% H_2O + 10% D_2O).

NMR data acquisition and processing. All the NMR experiments were recorded using a triple channel Varian Unity-plus 600 MHz NMR spectrometer equipped with pulse-shaping and pulse field gradient capabilities. ^1H–^{15}N HSQC was recorded with 128 complex points along ^{15}N (t_1) dimension. The ^1H and ^{15}N carrier frequencies were set at 4.71 and 119 ppm, respectively. Amide proton chemical shifts as a function of temperature in the range 20–40°C were measured by recording seven HSQC spectra with 160t_1 increments. The dimer was stable in the entire temperature range at both the pH values. Peptide binding NMR experiments were carried out using a commercially synthesized (GL Biochem Ltd.) 11 residue peptide fragment (VYTKQTQTTST) of dynein intermediate chain (IC74).

All the data were processed using FELIX on a Silicon Graphic, Inc. work station. Prior to Fourier transformation and zero-filling, data were apodized with a sine-squared weighting function shifted by 60° in both dimensions. After zero-filling and Fourier transformation the final matrix had 4096 and 1024 points, respectively, along F_2 and F_1 dimensions. Amide proton temperature coefficients were obtained from the chemical shifts of the individual residues. The chemical shift data were then analyzed by linear regression to estimate the slope and hence the temperature coefficients.

Results and discussion

Conformational fluctuations in DLC8 dimer

Amide and ^{15}N chemical shifts are sensitive probes for the environment of a given residue in a protein structure. When external perturbations are applied, chemical shift changes take place either due to a permanent change in the structure around one or a group of residues, or when the structure is dynamic there can be changes in the weighted averages of the local conformational fluctuations. We first observed from gel filtration experiments that the pH change from 7 to 6 does not alter the dimeric state of the protein (Fig. 2A). Next, we monitored the secondary and tertiary structure of the DLC8 dimer at pH 7 and 6 using far-UV and near-UV circular dichroism spectra. These spectra are shown in Fig. 2B and C, respectively. For reference CD spectra of the DLC8 monomer (pH 3) are also included in the figure. It is clear from Fig. 2 that there are no noticeable differences at pH 7 and 6 either in the secondary structure or in the tertiary structure of the DLC8 dimer. This is supported by the fact that the ^1H–^{15}N HSQC spectra at the two pH values are very similar so much so that transfer of assignment is readily possible (Fig. S1, supplementary material). However, there are small differences in the actual chemical shift values for some residues, which must be interpreted to reflect on small shifts in the weighted averages of local conformational fluctuations in the protein structure. Therefore, considering the changes, $\Delta H^N(\Delta N) = \delta_7 - \delta_6$, where δ_7 and δ_6 represent