ORGANIC LETTERS

2006
Vol. 8, No. 7
1275−1278

Two New Chiral EDTA-Based Metal Chelates for Weak Alignment of Proteins in Solution

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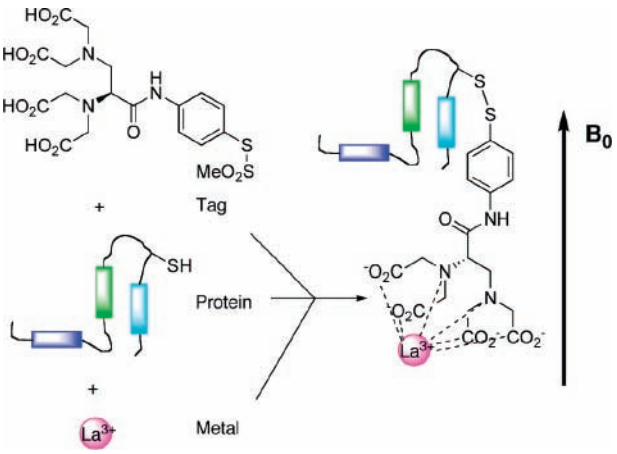
Received December 16, 2005

ABSTRACT



A short synthesis of EDTA-based metal chelates that can be attached to the cysteine residue of a protein via a disulfide bond is described. The complexes were used after coordination of lanthanides to align trigger factor and apo-calmodulin in solution to yield residual dipolar couplings and pseudocontact shifts. Alignment tensors for the new tags are linearly independent compared to those of previously published tags.

Residual dipolar couplings (RDCs) improve the speed and accuracy of structure determination for biomolecules[1] by solution NMR. In addition, dynamics within globular proteins[2] or between protein domains[3] can be measured using RDCs.

Successful measurement of RDCs requires the partial orientation of samples in a strong magnetic field using either external alignment media or paramagnetic alignment of diamagnetic proteins.

We and other groups have therefore developed paramagnetic tags that can be attached to diamagnetic proteins either as fusion proteins[4] or via a covalently linked tag[5] (Figure 1) attached to cysteine. Therefore, we describe in this communication the synthesis of another set of paramagnetic tags whose alignment tensors differ from previously described tags. Similar to the external alignment media, structures can be defined more precisely, if more than one alignment tensor

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10.1021/ol053049o CCC: $33.50
Published on Web 03/09/2006

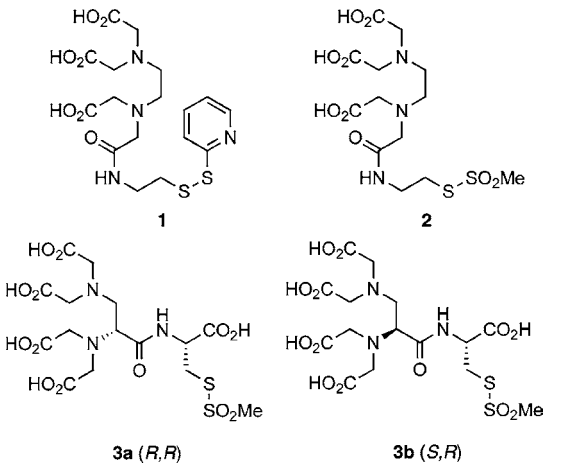
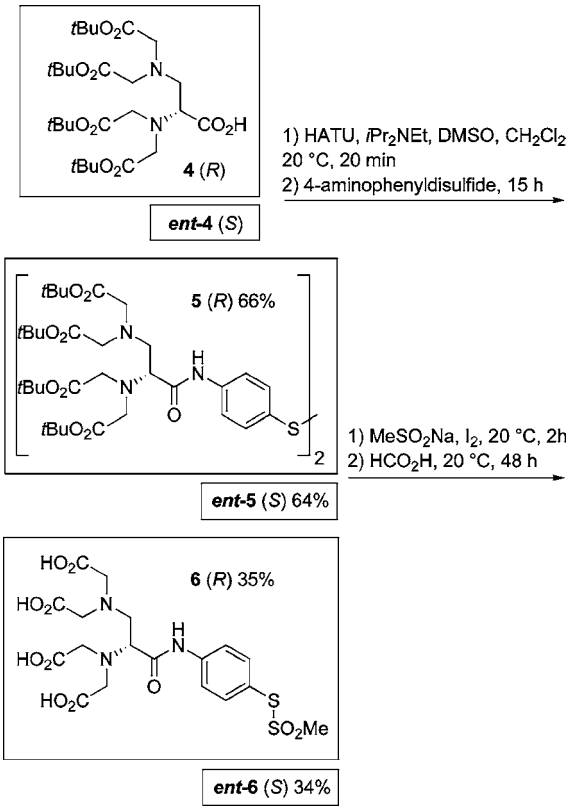


Figure 1. EDTA-based metal chelates.

can be implemented for the protein under investigation. In addition, for the study of domain motions, it is essential to align one domain by the paramagnetic tag and to study the induced alignment on the others.[3]

The enantioselective synthesis of two novel tags is described in Scheme 1. The optically active (*R*)-2,3-bis[di-

Scheme 1. Preparation of Tags **6**



(*tert*-butyloxycarbonylmethyl)amino]propionic acid **4**[5d] was coupled to 4-aminophenyl disulfide, using HATU as the condensation reagent to give the compound **5** in 66% yield. The iodine oxidative sulfenylation[6] of the sodium methanesulfinate with disulfide **5a** afforded the protected thiosulfonate, which was deprotected without purification with formic acid followed by HPLC purification to give the target tag **6**. In a similar manner, the enantiomeric acid ***ent*-4** gave the thiosulfonate ***ent*-6**.

The new tags were attached to the proteins apo-calmodulin (apo-CaM)[7] and trigger factor.[8] An ^{15}N,^{1}H HSQC spectrum of trigger factor is shown in Figure 2 for tag **6** loaded with

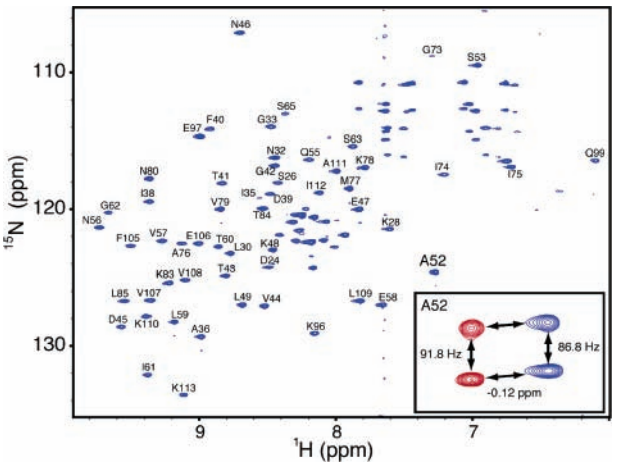


Figure 2. ^{15}N,^{1}H HSQC of the ^{15}N-labeled S100C mutant of trigger factor tagged with **6**. The full loading of the tag with Dy^{3+} is obvious from the absence of any peaks from the isotropic spectrum of trigger factor. The inset shows the isotropic (red) and anisotropic (blue) ω_1-coupled resonances of A52.

Dy^{3+}. The overview spectrum indicates the high quality of the sample. No isotropic peaks were detected. NH resonances even in close proximity to the tag site (C100) can be observed, as for example Q99. This is because the metal is farther away from the protein backbone for **6** and ***ent*-6** (16 Å) than for the tags **3a** and **3b** (13 Å). Inlays show expansions of the isotropic (red) and anisotropic (blue) ω_1-

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coupled peaks of residue A52. The alignment tensors for the previously published **3a** and **3b** as well as the new tags **6** and ***ent*-6** are compared by determining their intertensor angles, which are shown in Table 1 for both proteins trigger

Table 1. Axial (Da-HN) and Rhombic (Rhomb) Components of the Alignment Tensors and Angles between Them Achieved with the Four Paramagnetic Tags for Trigger Factor[a]

tag1/tag2	deg angle	tag	Hz Da-HN	rhomb	Å distance
3a/3b	69	**3a**	4.1	0.56	13
3a/6	147	**3b**	4.4	0.38	13
3a/*ent*-6	157	**6**	4.3	0.35	16
3b/6	120	***ent*-6**	4.1	0.47	16
3b/*ent*-6	119	**6**	3.2[b]	0.48[b]	17[b]
6a/*ent*-6	8	***ent*-6**	3.4[b]	0.32[b]	18[b]
6a/*ent*-6	24[b]				

[a] Distances from the tagged sulfur atom to the metal positions are given. [b] These data belong to apo-CaM.

factor and apo-CaM. The distances of the metal position from the sulfur atom of the tagged cysteine are also listed. Structure calculations have been performed in the case of trigger factor with the information from all four alignment tensors and are compared to the structure calculation performed with only one alignment tensor (Figure 3). The

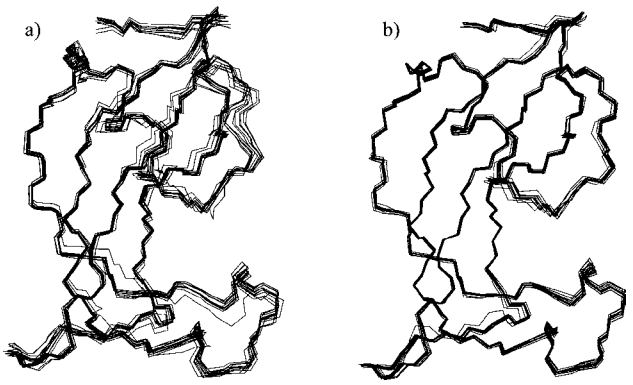

Figure 3. (a) The 10 lowest energy structures of trigger factor calculated using the RDCs induced by the tag **6** loaded with Dy^{3+} as compared to (b) the 10 lowest energy structures of trigger factor in which all four tags were used and loaded with Dy^{3+}. The increase of the precision of the structures is clearly visible and reflected in the rmsd to the mean of 0.353 Å for (a) and 0.202 Å for (b).

structure from four alignment tensors is more precise (rmsd to mean: 0.202 Å) than that calculated from only one alignment tensor (rmsd to mean: 0.353 Å for **6**) as can be inferred from the rmsd of the well-folded regions for the backbone atoms.

The second protein that was tagged with the two compounds **6** and ***ent*-6** is apo-CaM. A titration with the lanthanide was done up to a concentration of 60% of the apo-CaM (Figure 4). No shifts due to metalated CaM are

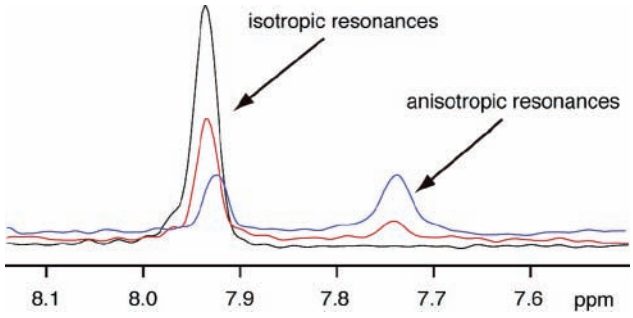

Figure 4. Titration of ^{15}N-labeled S17C mutant of apo-CaM tagged with **6** and loaded with Tb^{3+}. As is obvious from the titration, there is no general broadening of the lines because the tag binds the lanthanide quantitatively. The Ca^{2+} binding sites of apo-CaM especially do not show any indication of binding.

observed,[9] indicating that all the metal is bound to the tag and none to the protein. This is due to affinity of the tag to lanthanides in the 10^{-18} M range, while CaM binds them only with 10^{-9} M. High-quality ω_1-coupled ^{15}N,^{1}H HSQC spectra were recorded, and RDCs were extracted for 26 residues for **6** and 30 for ***ent*-6**. The RDCs of helix A were excluded due to their poor fit to the alignment tensor derived from the rest of the residues in the N-terminal domain. One probable explanation is the dynamics of helix A with respect to the rest of the domain that has been detected by fluorescence anisotropy measurements.[10] Further investigations of the dynamics of apo-CaM are under way. The metal positions could be determined as shown in the stereoplot for the tags **6** and ***ent*-6** in Figure 5 from the pseudocontact shifts

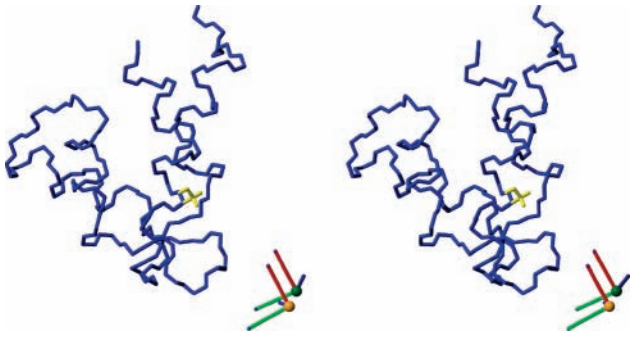

Figure 5. Stereoplot of apo-CaM with the metals and the principal axes of the alignment tensors indicated by axes in green, blue, and red. The green dot indicates the position of the metal for **6**, and the orange for ***ent*-6**. The distance to the sulfur of cysteine (yellow) is approximatley 17 Å.

by fitting them to a previously solved NMR structure.[11]

The correlation plot has a *Q*-factor of 0.206 and a slope of 0.938, which indicate the quality of the data as well as of the derived alignment tensor (Figure 6).

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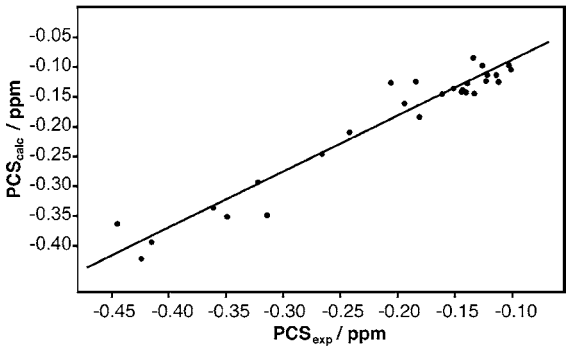

Figure 6. Correlation plot of the pseudocontact shifts for the S17C mutant of apo-CaM tagged with ***ent*-6** loaded with Tb^{3+}. The slope is 0.938, and the *Q*-factor 0.206. The position of the metals was determined by minimizing the *Q*-factor against a previously solved NMR structure.[11]

In conclusion, we developed two new tags, inducing new alignments that are linearly independent from those induced by previously published tags. The tags are introduced at a single cysteine site that can be incorporated at any given protein position. For trigger factor the additional alignment tensors allowed us to improve the precision of the structure. The extremely large affinity of the tag to lanthanides allows investigation of proteins that have tight metal binding sites such as apo-CaM and is therefore a versatile tag for all kinds of proteins.

Acknowledgment. This work was supported by the MPG, the DFG (SFB 492 Z-project), and the Fonds of the Chemical Industry. We thank Kerstin Overkamp and Gerhard Wolf for performing the HPLC purification and ESI-MS analyses. We thank Karin Giller for site-directed mutagenisis. We acknowledge measurement of ESI-HRMS spectra by Dr. Holm Frauendorf, University of Göttingen. Frank Hambloch, University of Göttingen, is acknowledged for conducting the elemental analyses, and Evelyn Pfeil, University of Göttingen, for measurement of the specific rotations. We also want to thank Dr. Christoph Farès, Dr. Karel Kubicek, and Dr. Laurent Verdier for useful discussions.

Supporting Information Available: Experimental procedures and spectroscopic data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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