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Complex Formation with the Activator RACo Affects the Corrinoid Structure of CoFeSP

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Supporting Information

ABSTRACT: Activation of the corrinoid [Fe-S] protein (CoFeSP), involved in reductive CO_2 conversion, requires the reduction of the Co(II) center by the [Fe-S] protein RACo, which according to the reduction potentials of the two proteins would correspond to an uphill electron transfer. In our resonance Raman spectroscopic work, we demonstrate that, as a conformational gate for the corrinoid reduction, complex formation of Co(II)FeSP and RACo specifically alters the structure of the corrinoid cofactor by modifying the interactions of the Co(II) center with the axial ligand. On the basis of various deletion mutants, the potential interaction domains on the partner proteins can be predicted.

ATP-dependent electron transferases are enzymes capable of transferring an electron from a redox-active site with moderate redox potential to a site with low oxidation potential at the expense of ATP.[1] Three classes of ATP-dependent electron transferases are currently known and include the nitrogenase reductase,[2] the activators of 2-hydroxyacyl-CoA dehydratases,[1] and the recently discovered reductive activators of corrinoid enzymes (RACE proteins).[3−5] The corrinoid iron−sulfur protein (CoFeSP) connects the two branches of the reductive acetyl-CoA pathway, allowing the overall reduction and condensation of two molecules of CO_2 to activate acetic acid.[6] RACo, the reductive activator of CoFeSP, belongs to the family of RACE enzymes and hydrolyzes ATP to transfer one electron from a [2Fe-2S] cluster on RACo to the corrinoid-bound Co(II) ion on CoFeSP.[5] The reduction of inactive Co(II)FeSP to active Co(I)FeSP is necessary when Co(I)FeSP becomes accidentally oxidized, which happens in vitro after ∼100 methylation cycles.[7] Formation of a complex between RACo and CoFeSP depends on the oxidation state of CoFeSP, and stable complexes have so far been observed for only Co(II)FeSP.[5]

This work is dedicated to analyzing how complex formation prepares the Co(II)FeSP−RACo complex for the energy demanding electron transfer. The transport of an electron against a redox potential gradient could be facilitated by modulating the potential of the electron-donating cofactor to increase its reducing power or by making the electron-accepting cofactor a better oxidant. In either case, altering the reduction potential of a cofactor requires changes in its direct environment, e.g., the first and second coordination spheres in the case of metal-containing cofactors. Here, we used resonance Raman (RR) spectroscopy to detect specific structural changes in the cofactor environment of RACo ([2Fe-2S] cluster) and Co(II)FeSP (corrinoid and [4Fe-4S] cluster) induced by complex formation.

The RR spectrum of CoFeSP, obtained with 413 nm excitation, is dominated by three bands in the region between 1400 and 1700 cm^{-1}, which can readily be attributed to the corrinoid cofactor.[8−11] For the oxidized Co(II)FeSP state, these bands, which originate from C═C stretching modes of the corrin macrocycle, are observed at 1604, 1543, and 1498 cm^{-1} (Figure 1B). The frequencies of these modes are sensitive to the oxidation state of the Co ion and undergo a small downshift of 4−5 cm^{-1} upon reduction to Co(I) by dithionite (Figure 1A).[8] Also, formation of a complex of Co(II)FeSP with its activator RACo is reflected by changes in the RR spectrum (Figure 1C) that are exclusively ascribed to the corrinoid because the [2Fe-2S] cluster of RACo and the [4Fe-4S] cluster of Co(II)FeSP do not give rise to RR bands in this frequency range (Figure 1D and Figure S1B of the Supporting Information). Compared to the free Co(II)FeS protein, the RACo-binding induced changes include an upshift for the most intense bands to 1607 and 1545 cm^{-1} and a 2 cm^{-1} downshift of the 1498 cm^{-1} band. In the case of the deletion mutant of CoFeSP that lacks the [4Fe-4S] cluster, the complex binding constant is lowered such that the RR spectrum displays a superposition of the RR bands of the RACo-bound and the free CoFeSP protein (Figure S1E of the Supporting Information), implying that the lack of the [4Fe-4S] cluster does not affect the active site conformation of CoFeSP and the structural consequences of RACo binding. The same results are obtained for a complex of Co(II)FeSP with a deletion mutant of RACo in which the [2Fe-2S] cluster has been removed (Figure S1D of the Supporting Information). These findings indicate that the peptide segment of RACo accommodating the [2Fe-2S] cluster is not involved in those interactions with Co(II)FeSP that affect the corrin structure.

The RR bands of Co(II)FeSP in the frequency region between 250 and 400 cm^{-1} (Figure 2A) are unambiguously attributed to the modes of the [4Fe-4S] cluster because these bands are not detected in the RR spectrum of the deletion

Received: June 14, 2012
Revised: August 22, 2012
Published: August 27, 2012

dx.doi.org/10.1021/bi300795n | *Biochemistry* 2012, 51, 7040−7042

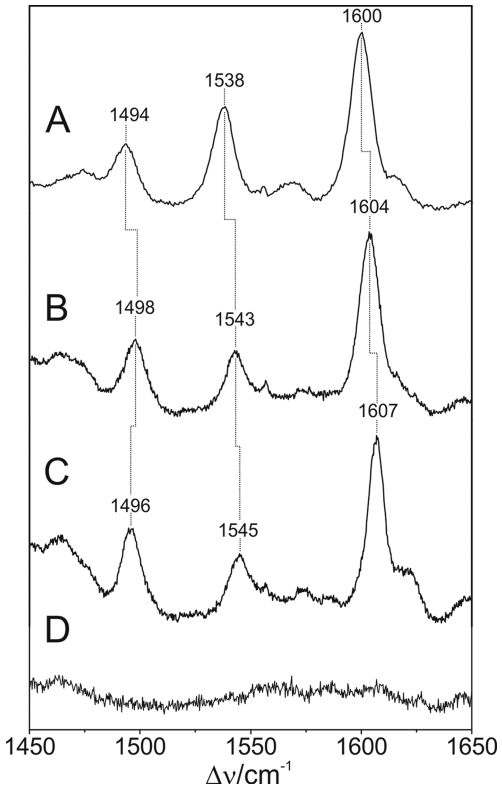

Figure 1. RR spectra of (A) Co(I)FeSP, (B) Co(II)FeSP, (C) the complex of Co(II)FeSP and RACo, and (D) RACo. The spectra, obtained with 413 nm excitation, display the frequency range of the C=C stretching modes of the corrin macrocycle.

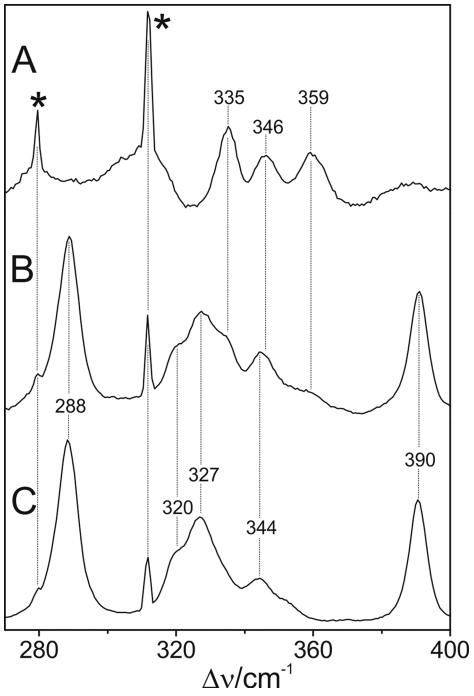

Figure 2. RR spectra of (A) Co(II)FeSP, (B) the complex of Co(II)FeSP and RACo, and (C) RACo. The spectra, obtained with 413 nm excitation, display the frequency range of the fundamentals of the [Fe-S] centers. Peaks labeled with asterisks are due to non-lasing emission lines of the Kr^+ ion laser.

mutant lacking the [4Fe-4S] cluster (Figure S3I of the Supporting Information). Thus, there are no spectral contributions from the corrinoid cofactor in this region. The band pattern significantly differs from that of the [2Fe-2S] cluster of RACo (Figure 2C). Furthermore, at 413 nm excitation, the resonance enhancement for the [2Fe-2S] cluster is distinctly stronger such that these bands dominate the RR spectrum of the Co(II)FeSP−RACo complex whereas those of the [4Fe-4S] cluster can be detected only as shoulders (Figure 2B). To analyze the involvement of the [Fe-S] centers in complex formation, we have subtracted the RR spectrum either of the free Co(II)FeSP or of the free RACo from that of the complex to obtain the spectra of the bound [2Fe-2S] or [4Fe-4S] cluster, respectively. These spectra reveal no differences compared to the RR spectra of corresponding free proteins such that we can safely rule out (mutual) structural perturbations of the [Fe-S] clusters upon complex formation (Figure S4 of the Supporting Information).

According to the crystal structure of CoFeSP, the corrinoid cofactor is located in a cleft between subunit CfsB and the C-terminal part of subunit CfsA such that the periphery of the macrocycle is solvent-exposed.[12,13] The distance from the corrinoid cofactor to the [4Fe-4S] cluster is ~60 Å.[13] Thus, it is not surprising that the structural changes of the corrinoid due to complex formation with RACo are not influenced by the [4Fe-4S] cluster. According to our RR data, the interaction domain on RACo must be located on the C-terminal part of the protein because deletion of 100 amino acids at the N-terminus, harboring the [2Fe-2S] cluster, does not affect the structural consequences of complex formation on the active site of CoFeSP (Figure S1D of the Supporting Information).

The corrinoid cofactor is in a base-off configuration in the Co(II) and Co(I) oxidation states, and the Co ion is axially only coordinated by a water molecule.[12,13] Just according to the crystal structure of CoFeSP, there is no candidate for a protein ligand in the vicinity of the Co center that might replace the water ligand upon RACo binding as a possible origin for the observed changes in the RR spectrum. These changes refer to the redox-state sensitive C=C stretching modes between 1490 and 1610 cm^{-1}. In analogy to previous findings for metalloporphyrins,[14] this redox-state sensitivity may result from the translocation of electron density between the metal-centered orbitals and the antibonding molecular orbitals of the macrocycle. With the transition from Co(II) to Co(I), the increased electron density on the metal ion is partially transferred to the antibonding molecular orbitals of the corrin, thereby weakening C=C bonds, which in turn is reflected by the observed frequency downshifts. Conversely, one would expect a transfer in the opposite direction (back-donation) for the oxidation of Co(II) to Co(III). In fact, in an early study of the free B12 cofactor, upshifts of 4 and 10 cm^{-1} were reported for the modes at 1543 and 1604 cm^{-1}, respectively.[8] It is now interesting to note that complex formation causes qualitatively the same frequency shifts, albeit not as large, i.e., from 1543 to 1545 cm^{-1} and from 1604 to 1607 cm^{-1}. A genuine oxidation of the Co center in CoFeSP can safely be ruled out taking into account the fact that the RR spectral changes are also observed in the absence of the [Fe-S] centers as the only possible electron acceptors (Figure S1D,E of the Supporting Information).

Electron paramagnetic resonance (EPR) experiments confirm this conclusion and moreover indicate a conformational change in the axial ligand upon RACo binding (Figure 3).

dx.doi.org/10.1021/bi300795n | *Biochemistry* 2012, 51, 7040−7042

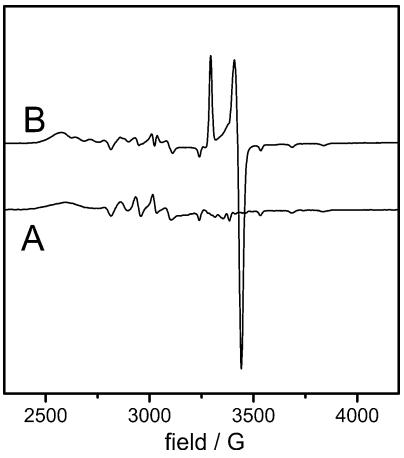

Figure 3. EPR spectra of (A) Co(II)FeSP and (B) the complex of Co(II)FeSP and RACo. The broad low-amplitude signals ranging from 2500 to 3800 G are due to the correnoid cofactor. The sharper and better resolved signals on the low-field side of the complex (B) indicate well-defined ligand−Co interactions in contrast to the broad and less resolved signal in the free Co(II)FeSP (A). The dominant signals in the spectrum of the complex (B) originate from the [2Fe-2S] cluster of RACo.

Specifically, the signal narrowing on the low field side for the CoFeSP−RACo complex points to a tightening of the ligand−Co interactions. It is therefore tempting to assume that alterations of the Co−O(aquo) bond affect the electron density in the corrin macrocycle, which in turn is reflected by the shifts in the C=C stretching frequencies.

Redox-dependent complex formation requires the recognition of oxidation-state specific attributes of the cofactor. As suggested previously[12] and confirmed by the recent crystal structure of the complex between CoFeSP and the methyltransferase from *Morella thermoacetica*,[15] the corrinoid-bound C-terminal domain of CoFeSP is mobile and allows exposition of the β-side of the corrin macrocycle. It may be that in the CoFeSP−RACo complex a similar conformational change causes a displacement of the corrinoid cofactor and changes the interactions of the Co ion with the axial ligand, leading to the RR-detectable perturbation of the (electronic) structure of the corrin macrocycle. As a consequence, the redox potential of the corrinoid cofactor may be altered to facilitate the reduction by RACo. Note that a recent theoretical study has demonstrated that a reorientation of the water ligand may influence the electron density distribution in the Co(I) state of methyltransferases and thus tune the Co(II)/Co(I) redox potential.[16]

■ ASSOCIATED CONTENT

Supporting Information

Materials and methods for experimental protocols and Figures S1−S4 (including further RR spectra). This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

All authors jointly planned and designed the project. W.M., F.L., and S.E.H. conducted the experiments. W.M., H.D., and P.H. wrote the manuscript.

Funding

This work was supported by the Cluster of Excellence "Unifying Concepts in Catalysis-UniCat", funded by the DFG.

Notes

The authors declare no competing financial interest.

■ REFERENCES

(1) Buckel, W., Hetzel, M., and Kim, J. (2004) *Curr. Opin. Chem. Biol. 8*, 462−467.
(2) Rees, D. C., Tezcan, F. A., Haynes, C. A., Walton, M. Y., Andrade, S., Einsle, O., and Howard, J. B. (2005) *Philos. Trans. R. Soc. 363*, 971−984.
(3) Schilhabel, A., Studenik, S., Vödisch, M., Kreher, S., Schlott, B., Pierik, A. J., and Diekert, G. (2009) *J. Bacteriol. 191*, 588−599.
(4) Ferguson, T., Soares, J. A., Lienard, T., Gottschalk, G., and Krzycki, J. A. (2009) *J. Biol. Chem. 284*, 2285−2295.
(5) Hennig, S. E., Jeoung, J. H., Goetzl, S., and Dobbek, H. (2012) *Proc. Natl. Acad. Sci. U.S.A. 109*, 5235−5240.
(6) Ragsdale, S. W., and Pierce, E. (2008) *Biochim. Biophys. Acta 1784*, 1873−1898.
(7) Menon, S., and Ragsdale, S. W. (1999) *J. Biol. Chem. 274*, 11513−11518.
(8) Mayer, E., Gardiner, D. J., and Hester, R. E. (1973) *J. Chem. Soc., Faraday Trans. 2 69*, 1350−1358.
(9) Salama, S., and Spiro, T. G. (1977) *J. Raman Spectrosc. 6*, 57−60.
(10) Dong, S., Padmakumar, R., Banerjee, R., and Spiro, T. G. (1999) *J. Am. Chem. Soc. 121*, 7063−7070.
(11) Stich, T. A., Seravalli, J., Venkateshrao, S., Spiro, T. G., Ragsdale, S. W., and Brunold, T. C. (2006) *J. Am. Chem. Soc. 128*, 5010−5020.
(12) Svetlitchnaia, T., Svetlitchnyi, V., Meyer, O., and Dobbek, H. (2006) *Proc. Natl. Acad. Sci. U.S.A. 103*, 14331−14336.
(13) Goetzl, S., Jeoung, J. H., Hennig, S. E., and Dobbek, H. (2011) *J. Mol. Biol. 411*, 96−109.
(14) Parthasarathi, N., Hansen, C., Yamaguchi, S., and Spiro, T. G. (1987) *J. Am. Chem. Soc. 109*, 3865−3871.
(15) Kung, Y., Ando, N., Doukov, T. I., Blasiak, L. C., Bender, G., Seravalli, J., Ragsdale, S. W., and Drennan, C. L. (2012) *Nature 484*, 265−269.
(16) Kumar, M., and Kozlowski, P. M. (2011) *Angew. Chem., Int. Ed. 50*, 8702−8705.