Histidine 129 in the 75-kDa Subunit of Mitochondrial Complex I from *Yarrowia lipolytica* Is Not a Ligand for [Fe₄S₄] Cluster N5 but Is Required for Catalytic Activity*

Revised for publication, October 8, 2004, and in revised form, November 24, 2004
Published, JBC Papers in Press, November 30, 2004, DOI 10.1074/jbc.M411488200

Antje Waletko, Klaus Zwickert, Albina Abdrahamanova, Volker Zickermann, Ulrich Brandt, and Stefan Kerscher‡

From the Universität Frankfurt, Fachbereich Medizin, Institut für Biochemie I, F-60590 Frankfurt am Main, Germany

Respiratory chain complex I contains 8–9 iron-sulfur clusters. In several cases, the assignment of these clusters to subunits and binding motifs is still ambiguous. To test the proposed ligation of the tetranuclear iron-sulfur cluster N5 of respiratory chain complex I, we replaced the conserved histidine 129 in the 75-kDa subunit from *Yarrowia lipolytica* with alanine. In the mutant strain, reduced amounts of fully assembled but destabilized complex I could be detected. Deamino-NADH:ubiquinone oxidoreductase activity was abolished completely by the mutation. However, EPR spectroscopic analysis of mutant complex I exhibited an unchanged cluster N5 signal, excluding histidine 129 as a cluster N5 ligand.

The N-terminal part of the 75-kDa subunit contains three highly conserved iron-sulfur cluster motifs and is homologous to the N-terminal portion of iron-only hydrogenases (15). In the x-ray structure of the iron-only hydrogenase from *CpI* of *Clostridium pasteurianum*, these motifs ligate one [Fe₂S₂] and two [Fe₄S₄] iron-sulfur clusters (16). Recently, direct evidence for the presence of three iron-sulfur clusters in the heterologously expressed 75-kDa subunit from *Paracoccus denitrificans* has been presented (17). In the same study, the fast relaxing [Fe₄S₄] cluster N5 has been assigned to the second binding motif of the 75-kDa subunit, an unusual HXXCCXXXXXXCC motif that contains three cysteines and a histidine. Secondary structure predictions using the PROF algorithm (26) suggest that this sequence element, both in hydrogenases and complex I from various organisms, forms a loop bounded by α-helices (Fig. 1). To test the proposed assignment of this sequence as a cluster N5 ligation motif in fully assembled complex I, we mutated the conserved histidine 129 of the *Y. lipolytica* 75-kDa subunit to alanine.

MATERIALS AND METHODS

The *Y. lipolytica* deletion strain nuamΔ (ura3–302, leu2–270, lys11–23, nuam::URA3, NUGM-Htg2, NDH2s, MatB), in which a 1.7-kb ClaI/KpnI fragment corresponding to codons 34–598 of the NUAM gene encoding the 728-amino acid precursor of the 75-kDa subunit of complex I is replaced with the *Y. lipolytica URA3* gene (1.6 kb) oriented in opposite direction to the original NUAM open reading frame, was constructed by the double homologous recombination strategy published previously (18). A wild type and a PCR-generated H129A mutant version of the NUAM gene were then subcloned as 5.2-kb SalI fragments (after an internal SalI site in the NUAM gene) into the replicative plasmid pUB4 (19) and transformed into strain *nuamΔ*, resulting in the strains *nuamΔ*, pNUAM-H129A (mutant). The presence of the point mutation in large scale cultures of strain *nuamΔ*, pNUAM-H129A was verified by the isolation of total DNA from 5-ml aliquots and the direct sequencing of PCR products.

Complex I catalytic activities in *Y. lipolytica* mitochondrial mem-

---

*This paper is available on line at http://www.jbc.org*
Cluster N5 in the 75-kDa Complex I of Y. lipolytica

RESULTS

As a qualitative test for complex I assembly, mitochondrial membranes from strain nuamΔ, pNUAM-H129A were analyzed by BN-PAGE. As illustrated in Fig. 2, a fully assembled enzyme appeared to be present in the mutant strain, but the intensities of all subunit bands were strongly reduced compared with those in the parental strain. In contrast, electron transfer from NADH to the artificial acceptor HAR (23) was only slightly reduced in mitochondrial membranes from strain nuamΔ, pNUAM-H129A and still amounted to 74% of the parental strain value (Table I). This result could be explained by the presence of subcomplexes in the membranes from this mutant; because FMN bound to the 51-kDa subunit is sufficient to catalyze the non-physiological reaction, subcomplexes containing this subunit can show NADH:HAR activity. These subcomplexes may have escaped detection by BN-PAGE because of their instability. At any rate, it was clear that the H129A mutation had markedly reduced complex I stability. It should be noted that significant residual NADH:Har activity was also found in the nuamΔ strain carrying the empty plasmid pUB4 (Table I), although no assembled complex I was detectable by BN-PAGE in this strain (not shown). We also mutated several cysteines in all three iron-sulfur binding motifs of the 75-kDa subunit to alanine, but in all cases we failed to detect complex I assembly or NADH:Har activity above the level observed in strain nuamΔ, pUB4 (data not shown).

Specific deamino-NADH- and decylubiquinone activity of membranes from strain nuamΔ, pNUAM-H129A was below detection level. This finding demonstrated that even the fully assembled fraction of complex I (Fig. 2) in the mitochondrial membranes lacked ubiquinone reductase activity. The mutant strain exhibited very low growth yields in complete media (14–6 times less than that commonly obtained for the parental strain), suggesting that a considerable portion of assembled complex I had dissociated during the purification procedure. This finding supports the notion that the mutation H129A had destabilized complex I.

EPR spectroscopy was performed with complex I from strain nuamΔ, pNUAM-H129A and from the parental strain. At a temperature of 40 K and a microwave power of 1 milliwatt, a single binuclear cluster called N1 could be detected (Fig. 3A). At a temperature of 12 K and a microwave power of 1 milliwatt, signals arising from clusters N1, N2, N3, and N4 could be seen (Fig. 3B). Cluster N5 signals were detected at 5 K and a microwave power of 100 milliwatts (Fig. 3C). Confirming previously published results (13), the g values of cluster N5 from wild-type Y. lipolytica complex I were g∥ = 2.002, 1.93, and ~1.89. The g∥ and g∥∥ signals of cluster N5 were difficult to assign, because they overlapped with the g∥∥ and g∥∥∥ signals of clusters N1 and even more with N4. Interference by the N4 signal also was much more severe, as this cluster was not completely power saturated under the EPR conditions used. However, the g∥ signal of cluster N5 was clearly separated from any other EPR signal in isolated complex I from Y. lipolytica. The spectra shown in Fig. 3C clearly show that the cluster N5 g∥ signals of both preparations were virtually identical in terms of peak intensity, peak width, and field position (Fig. 3C). This result indicated that cluster N5 was still present in complex I from strain nuamΔ, pNUAM-H129A and that its geometry was unaffected. Virtually identical results were obtained in two independent preparations of the mutant enzyme.

DISCUSSION

We have replaced a strictly conserved histidine residue (His-129) in the 75-kDa subunit of Y. lipolytica complex I with alanine to test whether this residue is a ligand of iron-sulfur cluster N5, as has been proposed recently (17). As the EPR
signature of cluster N5 was completely unchanged in complex I purified from the mutant strain, we could exclude this possibility. Our results are in contradiction with a recent study with the H129A mutation. Although we also observed destabilization of Y. lipolytica complex I by the H129A mutation, it obviously did not affect the EPR spectra of the purified enzyme.

A more fundamental problem with complex I from P. denitrificans is that the g signal values of cluster N5, detected at 5 K, are very close to those of cluster N4, detected at 12 K (17). Distinction between these clusters relies on the assumption that the cluster N4 signals of P. denitrificans complex I are completely power saturated at 5 K and a microwave power of 100 milliwatt, which in our hands is clearly not the case for cluster N4 of Bos taurus and Y. lipolytica complex I. Therefore, although at least the g signals of clusters N4 and N5 are well separated in the latter two model organisms, the assignment of EPR signals to cluster N5 of P. denitrificans complex I is more difficult. It should be noted that the cluster N4 EPR signals were also found to be somewhat affected by the H106C mutation in P. denitrificans complex I (17). Thus, there is another possibility in regard to how the findings in P. denitrificans and Y. lipolytica could be reconciled. If the iron-sulfur cluster assignment within the 75-kDa subunit was revised, the mutation of the conserved histidine in Y. lipolytica may have resulted in the removal of a cluster N1b ligand, leaving the EPR signature of cluster N5 unchanged. The histidine to cysteine exchange in P. denitrificans as such may then have been EPR-silent, but it may have caused a structural alteration that affected the EPR patterns of both clusters N4 and N5.

However, because the study by Yano et al. (17) clearly demonstrates the presence of three iron-sulfur clusters in the heterologously expressed 75-kDa subunit of P. denitrificans, this proposal raises another problem. If all three motifs in the 75-kDa hold an iron-sulfur cluster, why did the H129A mutation not have any effect on the EPR spectra of Y. lipolytica complex I? Possible answers are that cluster N1b is either not present or undetectable by standard EPR spectroscopy in complex I from Y. lipolytica. Although complex I from bovine heart mitochondria and various other sources is known to contain two EPR detectable binuclear iron-sulfur clusters called N1a and N1b (9), only one binuclear iron-sulfur cluster (called N1b) could be detected in complex I from Y. lipolytica to date. The stoichiometry of clusters N1/N2/N3/N4 was determined as 1:1:1:1 (13). As the N1 signal of Y. lipolytica complex I was not detectable in a subcomplex including the 75-kDa but lacking the 24-kDa subunit, which in bovine heart complex I contains binuclear cluster N1a, it seems tempting to speculate that also in the Y. lipolytica enzyme the 75-kDa subunit may carry a second binuclear cluster that has, to date, escaped detection by

---

**Table I**

| Strain                     | NADH:HAR activity µmol min⁻¹ mg⁻¹ | Percent of activity | dNADH:DBQ activity µmol min⁻¹ mg⁻¹ | Percent of activity |
|----------------------------|-----------------------------------|---------------------|-----------------------------------|---------------------|
| NuamΔ,pNUAM-WT             | 0.84                              | 100                 | 0.290                             | 100                 |
| NuamΔ,pNUAM-H129A          | 0.62                              | 74                  | <0.01                             | <3                  |
| NuamΔ,pUB4                 | 0.28                              | 33                  | <0.01                             | <3                  |

**Fig. 3.** EPR spectra of purified complex I from mutant H129A (6.3 mg/ml) and parental strain (3.7 mg/ml). A microwave frequency of 9.47 GHz and a modulation amplitude of 0.64 millitesla were used, as well as a spectrum at 40 K and a microwave power of 1 milliwatt (A), a spectrum at 12 K and a microwave power of 1 milliwatt (B), and a spectrum at 5 K and a microwave power of 100 milliwatt (C). Characteristic signal positions of individual clusters are indicated. The region between g = 1.9 and g = 1.85 reflects contributions from all iron-sulfur clusters detectable under these conditions.

---

2 V. Zickermann, K. Zwicker, M. Bostina, M. Radermacher, and U. Brandt, manuscript in preparation.
EPR. If the HXXXCXXXXXXX motif ligated this EPR silent cluster, it would have been removed by the H129A mutation, offering a straightforward explanation of why ubiquinone reductase activity was lost.

As a consequence, one has to conclude that in complex I the use of the iron-sulfur binding motifs has changed from iron-only hydrogenases. Either the histidine in question that is conserved in all known complex I sequences has acquired a different function in complex I, or the motif ligating a binuclear cluster in hydrogenase binds a tetranuclear cluster (N5) in complex I and vice versa.

Acknowledgments—We thank Gudrun Beyer for excellent technical assistance and Nikki Bolcevic for help in the isolation of strain nuam/H9004. We thank Maja Aleksandra Tocilescu for preparing the BN-PAGE gels shown in Fig. 2 and Hermann Schägger for help with interpretation of the gels.

REFERENCES
1. Brandt, U., Kerscher, S., Droese, S., Zwicker, K., and Zickermann, V. (2003) FEBS Lett. 545, 9–17
2. Gueguenhé, V., Schilt, A., Weiss, H., Leonard, K., and Friedrich, T. (1998) J. Mol. Biol. 276, 105–112
3. Grigoroff, N. (1998) J. Mol. Biol. 277, 1033–1046
4. Djafarzadeh, R., Kerscher, S., Zwicker, K., Radermacher, M., Lindahl, M., Schägger, H., and Brandt, U. (2000) Biochim. Biophys. Acta 1459, 230–238
5. Kashani-Poor, N., Zwicker, K., Kerscher, S., and Brandt, U. (2001) J. Biol. Chem. 276, 24082–24087
6. Zickermann, V., Restina, M., Hunte, C., Ruiz, T., Radermacher, M., and Brandt, U. (2003) J. Biol. Chem. 278, 29072–29078
7. Friedrich, T. (2001) J. Bioenerg. Biomembr. 33, 169–177
8. Yagi, T., and Matsuno-Yagi, A. (2003) Biochemistry 42, 2266–2274
9. Ohnishi, T. (1998) Biochim. Biophys. Acta 1364, 186–208
10. Rasmussen, T., Scheide, D., Brors, B., Kintischer, L., Weiss, H., and Friedrich, T. (2001) Biochemistry 40, 6124–6131
11. Ohnishi, T., Salerno, J. C., Winter, D. B., Lim, J., Yu, C.-A., Yu, L., and King, T. E. (1975) Biochim. Biophys. Acta 387, 475–490
12. Sled, V. D., Friedrich, T., Leif, H., Weiss, H., Fukumori, Y., Calhoun, M. W., Gennis, R. B., Ohnishi, T., and Meinhardt, S. W. (1993) J. Bioenerg. Biomembr. 25, 347–356
13. Kerscher, S., Kashani-Poor, N., Zwicker, K., Zickermann, V., and Brandt, U. (2001) J. Bioenerg. Biomembr. 33, 187–196
14. Weiss, H., Friedrich, T., Hofhaus, G., and Preis, D. (1991) Eur. J. Biochem. 197, 563–576
15. Smith, M. A., Finel, M., Korolik, V., and Mendz, G. L. (2000) Arch. Microbiol. 174, 1–10
16. Peters, J. W., Lanzilotta, W. N., Lemon, B. J., and Seeffeldt, L. C. (1998) Science 282, 1853–1858
17. Yano, T., Sklar, J., Nakamura-Ogiso, E., Yagi, T., and Ohnishi, T. (2003) J. Biol. Chem. 278, 15514–15522
18. Kerscher, S., Droese, S., Zwicker, K., Zickermann, V., and Brandt, U. (2002) Biochim. Biophys. Acta 1555, 83–91
19. Kerscher, S., Eschemann, A., Okun, P. M., and Brandt, U. (2001) J. Cell Sci. 114, 3915–3921
20. Kerscher, S., Benit, P., Abdrahamanova, A., Zwicker, K., Raisi, I., Karas, M., Rustin, P., and Brandt, U. (2004) Eur. J. Biochem. 271, 3588–3595
21. Schägger, H. (2003) in Membrane Protein Purification and Crystallization: A Practical Guide (Hunte, C., von Jagow, G., and Schägger, H., eds) pp. 105–130, Academic Press, San Diego, CA
22. Kashani-Poor, N., Kerscher, S., Zickermann, V., and Brandt, U. (2001) Biochim. Biophys. Acta 1504, 363–379
23. Gavrivko, E. V., Grivennikova, V. G., Sled, V. D., Ohnishi, T., and Vinogradov, A. D. (1995) Biochim. Biophys. Acta 1230, 23–30
24. Ahlers, P., Zwicker, K., Kerscher, S., and Brandt, U. (2000) J. Biol. Chem. 275, 23577–23582
25. Garofano, A., Zwicker, K., Kerscher, S., Okun, P., and Brandt, U. (2003) J. Biol. Chem. 278, 42435–42440
26. Ouali, M., and King, R. D. (2000) Protein Sci. 9, 1162–1176
Histidine 129 in the 75-kDa Subunit of Mitochondrial Complex I from *Yarrowia lipolytica* Is Not a Ligand for [Fe₄S₄] Cluster N5 but Is Required for Catalytic Activity

Antje Waletko, Klaus Zwicker, Albina Abdrakhmanova, Volker Zickermann, Ulrich Brandt and Stefan Kerscher

*J. Biol. Chem.* 2005, 280:5622-5625. doi: 10.1074/jbc.M411488200 originally published online November 30, 2004

Access the most updated version of this article at doi: 10.1074/jbc.M411488200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 25 references, 7 of which can be accessed free at http://www.jbc.org/content/280/7/5622.full.html#ref-list-1