Journal of Bioenergetics and Biomembranes, Vol. 30, No. 1, 1998

Foreword

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This Special Issue focuses on structure, function, regulation, and evolution of the respiratory heme-copper oxidases. These enzymes are molecular micromachines that transduce the free energy released in the "combustion chamber" of a binuclear oxygen reduction site—by redox-linked proton translocation—into an electrochemical proton gradient across the mitochondrial or bacterial membrane (Babcock and Wikström, 1992). The interest in this enzyme superfamily stems largely from this unique function as an energy transducer. But it is also due to the key role in physiology: over 90% of the biological O_2 consumption on Earth is catalyzed by these enzymes. To paraphrase the late M. R. Lemberg (1969), "the general significance of cytochrome oxidase greatly exceeds that of hemoglobin, its much studied and much more completely known chemical relative." The significance of this statement has only increased with the subsequent realization of how widespread the heme-copper oxidases are in Nature, and how well both structure and function have been conserved during evolution. For this reason chemists have been intrigued to explore the catalytic mechanism of O_2 activation (cf. Babcock and Wikström, 1992; Ferguson-Miller and Babcock, 1996; Kitagawa and Ogura 1997), which apparently does not lead to measurable release of toxic by-products. In close relation to this, physicists have for years studied the structures and properties of the magnetically coupled metals of the dioxygen reduction site. Regulation of the heme-copper oxidases is still not fully understood, but some control properties seem related to the function of nuclear-encoded subunits (Kadenbach *et al.*, p. 25). The evolution of this class of enzymes presents another intriguing problem. Which came first, O_2 in the atmosphere or the prototype of the heme-copper oxidases? Although perhaps surprising, Castresana and Saraste (1995) have argued a good case for the latter (see Hendriks *et al.*, p. 15).

Resolution of the first two structures of heme-copper oxidases at the atomic level by X-ray crystallography (Iwata *et al.*, 1995; Tsukihara *et al.*, 1995, 1996; Ostermeier *et al.*, 1997; see Yoshikawa *et al.*, p. 7) is clearly the most notable single achievement in this field since the previous Special Issue on this topic, edited by Shelagh Ferguson-Miller (1993). The impact is noticeable in virtually all articles. It has certainly contributed to the large number of color pictures, which may also be a sign of confidence, however, that I hope is justifiable. On the other hand, and without depreciating the importance of the crystal structures, it is clear that they have not solved the mechanisms of function. While the crystal structures do indeed represent the end of one arduous road in oxidase research (Beinert, 1995), they also signify the beginning of another. We now need a combination of structural and dynamic studies to achieve functional understanding, and this Special Issue presents several examples toward this end.

As the previous Special Issue was purely a North American endeavor, this issue comprises authors from "the rest of the world," with one notable exception: the article by Regan *et al.* (p. 35) serves as an example of the many fruitful intercontinental collaborations in oxidase research, but it is also warranted by its own merits as a pioneering attempt at identifying electron transfer paths in the heme-copper oxidases. Brunori *et al.* (p. 41) provide another insightful contribution to this topic. There has also been notable progress in our knowledge on proton transfer pathways, which are key players in both oxygen reduction and proton-pumping (Brzezinski and Ädelroth, p. 99; Konstantinov, p. 121; Pfitzner *et al.*, p. 89; Wikström *et al.*, p. 139). This progress is largely due to a combination of site-directed mutagenesis and functional studies guided by the structure. Other significant advances are related to the chemistry of O_2 reduction and the structure and reactivity of the binuclear center metals (Kitagawa and Ogura, p. 71; Torres *et al.*, p. 63; Watmough *et al.*, p. 55). Kannt *et al.* (p. 81) have contributed with a novel study

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0145-479X/98/0200-0003$15.00/0

Table I. Key Residues[a]

Bovine heart mito cyt aa_3	*P. denitrificans* cyt aa_3	*Rh. sphaeroides* cyt aa_3	*Escherichia coli* cyt bo_3	Property
H61	H94	H102	H106	Heme *a* ligand
H378	H413	H421	H421	Heme *a* ligand
H376	H411	H419	H419	Heme a_3 ligand
H240	H276	H284	H284	Cu_B ligand
H290	H325	H333	H333	Cu_B ligand
H291	H326	H334	H334	Cu_B ligand
W236	W272	W280	W280	Close to **H291**
Y244	Y280	Y288	Y288	Covalent bond to **H240**
V243	V279	V287	V287	O_2-channel
T309	T344	T352	T352	H-bonded to **H-290**
K319	K354	K362	K362	H^+-input (K-pathway)
T316	T351	T359	T359	H^+-input (K-pathway)
D91	D124	D132	D135	H^+-input (D-pathway)
N80	N113	N121	N124	H^+-input (D-pathway)
N98	N131	N139	N142	H^+-input (D-pathway)
S157	S193	S201	T201	H^+-input (D-pathway)
E242	E278	E286	E286	H^+-input (D-pathway)
M71	I104	I112	M116	Near **E242**
D364	D399	D407	D407	Near H^+-output path?
R438	R473	R481	R481	H-bond to heme a_3 Δ propionate
R439	R474	R482	R482	H-bond to heme *a* Δ propionate
H368	H403	H411	(H411)	Mg ligand
D369	D404	D412	(N412)	Mg ligand
E198 (SUII)	E218 (SUII)	E254 (SUII)	—	Mg ligand
H161 (SUII)	H181 (SUII)	H217(SUII)	—	Cu_A ligand
H204 (SUII)	H224 (SUII)	H260(SUII)	—	Cu_A ligand
C196 (SUII)	C216 (SUII)	C252(SUII)	—	Cu_A ligand
C220 (SUII)	C220 (SUII)	C256 (SUII)	—	Cu_A ligand
M207 (SUII)	M227 (SUII)	M263 (SUII)	—	Cu_A ligand
E198 (SUII)	E218 (SUII)	E254 (SUII)	—	Cu_A ligand

[a] The table lists a selection of amino acid residues, many of which are frequently referred to in the articles, and their analogs in the most frequently studied oxidases from four species. The oxidase from bovine-heart mitochondria is taken as the basis set (in boldface).

of electrostatic interactions in the enzyme and their possible functional implications. The work pioneered by Kadenbach and his collaborators on nuclear-encoded subunits of the mitochondrial oxidases is followed up here by a description of how these enzymes may be regulated by adenine nucleotides. Orii (p. 47) presents an analysis of the absorption spectra of the enzyme's two heme groups, which leads to conclusions that certainly will stir up discussion. Hendriks *et al.* (p. 15) analyze the evolutionary origin of the heme-copper oxidases, and especially their relationship to enzymes involved in denitrification. Finally, possible mechanisms of the proton translocation machinery in these enzymes are discussed by Kannt *et al.* (p. 81), Papa *et al.* (p. 109), Rich *et al.* (p. 131) and Wikström *et al.* (p. 139).

I am grateful to the authors who quite spontaneously made this Special Issue a forum for open and imaginative new developments and ideas, and I hope that the following pages will serve, as did the previous Special Issue, as a significant stimulus for further exploration in the next few years to come. I would also like to thank Anna Aagaard (Göteborg) and Anne Puustinen (Helsinki) for their help with the "Table of Key Residues," and Martin Karpefors (Göteborg), Peter Brzezinski (Göteborg), and Jeffrey Regan (Pasadena) for providing the cover illustrations. Finally, I am grateful to the Editor-in-Chief, Peter Pedersen

(Baltimore), for his suggestion to compile this issue, and for his patience with the special editor's performance.

Helsinki, November 10, 1997

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