The nature and regulation of the alternative oxidase of plant mitochondria

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Introduction

Plant mitochondria are analogous to their mammalian counterparts in terms of the structure and arrangement of the respiratory chain complexes and sites of energy conservation [1]. It is obvious that such a basic electron transport chain was developed at an early stage of evolution and has remained highly conserved throughout the development and divergence of the animal and plant kingdoms. In addition to this basic electron transport chain, plant mitochondria have, however, routes of substrate oxidation and a terminal oxidase not normally encountered in mammalian systems. The distinct characteristic features of the plant mitochondrial respiratory chain are the presence of an NAD(P)H dehydrogenase located on the outer surface of the inner mitochondrial membrane and a cyanide- and antimycin-resistant alternative oxidase [1, 2]. The unique NAD(P)H dehydrogenase is insensitive to rotenone and is coupled to two sites of phosphorylation, a route that bypasses Complex I possibly owing to either presence of an additional dehydrogenase located on the outer surface of the inner mitochondrial membrane or alternatively may represent a second ubiquinone reduction site for Complex I (which is rotenone-insensitive).

Nature of the alternative oxidase

One of the most characteristic features of plant mitochondria is the possession of a finite level of substrate oxidation that is insensitive to inhibition by cyanide, azide, carbon monoxide, antimycin and myxothiazol [2]. The degree to which mitochondrial respiratory activity is insensitive to cyanide can vary from as little as a few percent in the case of potato tubers to as much as 100% in the case of the mitochondria isolated from the spadices of Arum maculatum and Sauroutherium guttatum [2]. It should be stressed that the level of cyanide-resistant respiration in the anroid species is not trivial, rivalling those found in insect flight muscle with rates exceeding 1–2 μmol O₂ min⁻¹ mg⁻¹ of protein.

Cyanide-resistant respiration is not restricted to the plant kingdom, however, since a similar oxidase is also found in fungi, a large number of yeasts and various protista including several members of the brucei group of African trypanosomes [2].

Well-established features of the plant alternative oxidase include its branch point from the main respiratory chain on the substrate side of Complex III at the level of ubiquinone, the reduction of O₂ to H₂O and not H₂O₂ or O²⁻ and inhibition by

primary hydroxamic acids, n-propylgallate and di-
sulphiram [1, 2]. In its membrane-bound form, tri-
carboxylic acid cycle intermediates and external
NADH can act as respiratory substrates whereas
once solubilized artificial quinols (e.g. duroquinol or
UQH2) can effectively act as electron donors.
When succinate or external NADH are used as
respiratory substrates, electron flux from reduced
ubiquinone to O2 via the alternative oxidase is non-
phosphorylating whereas with NAD-linked sub-
strates ATP can be synthesized at Complex I [2].
Information on the topological orientation of the
alternative oxidase suggests it is located toward the
matrix side of the inner mitochondrial membrane
[3]. Although several laboratories have attempted to
isolate and purify the alternative oxidase from
thermogenic tissues using a variety of detergents
[2], none have succeeded to date in obtaining a
totally purified preparation, and in the majority of
cases SDS/PAGE of the resulting preparations
shows major bands in the 30–39 kDa region and
numerous minor polypeptides. Spectrally, the
preparations show no cytochrome absorption or the
presence of flavins or any novel iron-sulphur
centres. Metal analysis reveals the presence of 3–6
nmol copper/mg of protein [4] and in some cases
iron [5], but no zinc or manganese. Both the copper
and iron were found to be e.p.r. silent. As indicated
previously, if H2O and not H2O2 or O2+ is the
primary product of oxygen reduction, this requires
that a coupled transition metal complex be present
in the catalytic site of the oxidase. It therefore
becomes important to determine conclusively what
mets are specifically associated with the oxidase
and this, based upon current preparations, remains
essentially unknown.

Perhaps the greatest recent advance in our
understanding of the nature of the alternative oxidase
has been obtained from the work of McIntosh
and colleagues [6, 7]. They used a partially purified
preparation of the Sauromatum oxidase to raise
polyclonal and monoclonal antibodies and found
that these antibodies reacted with three bands on
Western immunoblots at molecular masses of 35,
36 and 37 kDa. The appearance of these bands was
found to correlate with the presence and activity of
the oxidase in various tissues [6]. The antibodies
could immunoprecipitate 50% of duroquinol ox-
dase activity [7] and more conclusively cross-
reacted with two polypeptides, at 36.5 and 37 KDa,
in Neurospora crassa mitochondria [8] in which the
alternative pathway had been induced to appear by
the addition of chloramphenicol to inhibit mito-
chondrial protein synthesis. No cross-reactivity was
observed with mitochondria from uninduced
Neurospora which lack alternative oxidase activity.
Such observations clearly establish that the three
cross-reactive polypeptides in Sauromatum of
molecular masses 35, 36 and 37 kDa are associated
with the alternative oxidase.

Recently Rhoads & McIntosh [9] have used the
monoclonal antibodies to isolate a cDNA clone
encoding the alternative oxidase protein from
Sauromatum. This clone encodes a deduced poly-
peptide sequence of 349 amino acids having an esti-
mated molecular mass of 38.9 kDa. The first 60–65
amino acids appear to encode a putative mitochondr
al transit peptide, suggesting that the mature pro-
tein has a calculated molecular mass of 32 kDa.
Unfortunately the sequence does not shed any light
on the nature of any cofactors associated with the
oxidase since it does not reveal any motifs common
to any known metal binding sites. The protein is
very hydrophilic, with charged residues distributed
over much of its mass. It contains significant num-
bers of tyrosine and histidine residues and several
ionic motifs which may play a role in metal cofactor
binding. Three regions of the deduced protein are
predicted to be strongly a-helical and within two of
these regions are spans of relatively high hydropho-
bicity. These sequences are likely to produce mem-
brane-spanning helices, as deduced from a hydropathy
analysis using the algorithm of Kyte and Doolittle [2].

More recently, Sakajo et al. [10] have isolated a
cDNA clone encoding a protein involved in cyanide-resistant respiration in the yeast
Hansemula anomala. Analysis of the deduced amino
acid sequence of this clone reveals that it also poss,
sees two membrane-spanning a-helices with 63% se-
quency similarity to the hydrophobic regions of
the plant protein. Again there is little information as
to the nature of any cofactors required for catalytic
activity of this enzyme, although it should be noted
that induction of the oxidase in Hansemula anomala
requires ferrous iron [11]. Obviously Hansemula anomala clearly provides an interesting system for
further characterization of the alternative oxidase
and for comparison with the plant oxidase.

Regulation of the alternative oxidase
The mechanism by which electron flux is parti-
tioned between the main respiratory pathway and
the alternative oxidase has, until recently, remained
unclear. While the ubiquinone pool has long been
recognized as the branch point of the alternative
pathway from the main respiratory chain, consider-
able uncertainty surrounded the mechanism by
which electron flow on to the alternative pathway is
actually regulated. Some answers to this question are, however, now emerging as a result of the development of a voltammetric technique to continuously monitor the steady-state redox poise of the ubiquinone pool in isolated plant mitochondria. Moore and colleagues [12, 13] have demonstrated that in cyanide-sensitive mitochondria, electron flow through the main respiratory chain is linearly dependent upon the redox state of the ubiquinone pool [12]. However, in cyanide-resistant mitochondria under ADP-limiting conditions, the relationship between the respiratory rate and the redox state of ubiquinone was distinctly non-linear and the quinone pool and the rate of electron flow through the alternative pathway was not apparent until the reduction level of the quinone pool reached 35–40%, and then increased disproportionately on further reduction [13]. Although these results, to a first approximation, conformed to a previous model postulated by Bahr and Bonner [14], it was found that the resulting data would not fit the Bahr and Bonner model. This suggested that this model could not completely account for the observed relationship between the redox poise of the quinone pool and the rate of electron flow through the alternative pathway. More recently, Siedow and Moore [2, 15] have proposed a kinetic model, based upon quinone-pool assumptions, which appears to provide a closer fit to the experimentally observed results than that obtained with previous models. The kinetic model of Siedow and Moore is based upon a mechanism in which reduction of the alternative oxidase by ubiquinol proceeds via two sequential two-electron transfer reductions. It allows them to derive a rate equation by altering the values of the equilibrium constants associated with the reversible electron transfer steps. The model appears to adequately account for the situation where the alternative pathway is present but not engaged; and explain why in some tissues it can be engaged under state-3 conditions (i.e. in the presence of excess ADP) whereas in others it can not be engaged until respiration is severely limited.

In summary, the kinetic model quantitatively accounts for the observed regulation of electron flow through the alternative pathway better than previous models. The model also suggests that this regulation follows quinone-pool behaviour, but in a manner that is consistent with the original predictions of Bahr and Bonner.


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