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Adenosine Triphosphate Synthesis and the Natural Electron Acceptor for Synthesis of Serine from Glycine in Leaves

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Mitochondria from the leaves of higher plants (Kisaki *et al.*, 1971; Bird *et al.*, 1971) and from rat and avian livers (Sanadi & Bennett, 1960; Kawasaki *et al.*, 1966) catalyse a reaction between two molecules of glycine to form one molecule each of serine, CO_2 and NH_3. Kisaki *et al.* (1971), using mitochondria from leaves of *Spinacia oleracea*, found that the reaction was faster in N_2 than in air and that NAD^+ was necessary as the electron acceptor. By contrast, the reaction catalysed by mitochondria from leaves of *Nicotiana tabacum* required aerobic conditions, was accompanied by the synthesis of ATP and ADP, and used one atom of O_2 for each molecule of serine formed (Bird *et al.*, 1972). The affinity of the reacting system for O_2 was high (Fig. 1). The reaction was inhibited by inhibitors of the electron-transport system in mitochondria and was little affected by added NAD^+. The immediate acceptor of electrons arising from conversion of glycine into serine is therefore linked to part of the electron-transport system of mitochondria.

The rate of reaction was measured by using [1-^{14}C]glycine as the substrate. The $^{14}CO_2$ evolved was absorbed in 2M-NaOH and measured by liquid-scintillation counting. Reaction vessels were shaken throughout the reaction period. The ADP phosphorylated was measured by a modification (Nobel, 1967) of the method of Nielsen & Lehninger (1955). Leaves were obtained from tobacco plants (*Nicotiana tabacum* var. White Burley) kept in darkness for 24h. Leaf lamina was ground with 4vol. of medium (pH7.7) containing 0.4M-sucrose, 20mM-sodium citrate, 0.2M-tris–HCl, 10mM-KH_2PO_4, 5mM-EDTA and 1% (w/v) of bovine serum albumin. Coarse debris was removed by squeezing through two layers of muslin and filtering through cottonwool. The filtrate was centrifuged (100*g* for 10min) and the pellet discarded. The supernatant liquid was centrifuged (38000*g* for 10min) and the pellet obtained was resuspended in an appropriate volume of medium (pH7.7) containing 0.4M-sucrose, 20mM-sodium citrate, 4mM-$MgCl_2$, 10mM-KH_2PO_4 and 1% (w/v) of bovine serum albumin.

Ferricyanide could act as the final electron acceptor instead of O_2 (Table 1). Serine synthesis was strongly inhibited by antimycin A whether O_2 (Bird *et al.*, 1972) or ferricyanide (Table 1, Expt. A) was the final electron acceptor. Amytal (2mM) inhibited serine synthesis by less than 20%. With liver mitochondria oxidation of substrates linked through NAD^+ to the electron-transport chain is completely inhibited by 1.8mM-Amytal (Jailing *et al.*, 1955); measurements made with an oxygen electrode (Rank Bros.), with pyruvate as substrate, confirmed that this is also true for tobacco leaf mitochondria. Therefore oxidation associated with the synthesis of serine from glycine does not proceed through the internal pool of NAD^+ (Lehninger, 1951) in mitochondria, and coupling site I of the electron-transport system is not involved. Ferricyanide accepts electrons between coupling sites II and III (Lee *et al.*, 1967) and should halve phosphorylation where oxidations involve only phosphorylation sites II and III. Table 1, Expt. B, shows that in the presence of ferricyanide

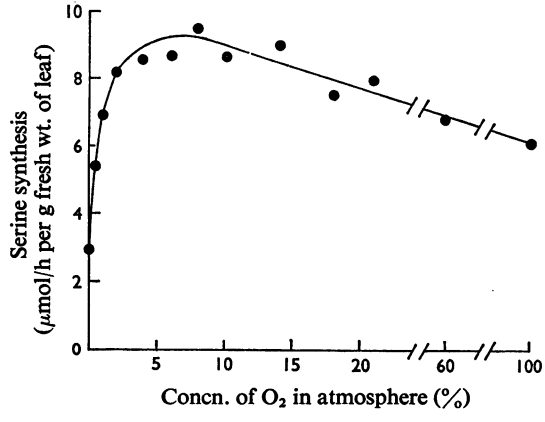

Fig. 1. *Rate of serine synthesis at various oxygen concentrations*

Reaction mixtures (pH7.7; 0.66ml) contained particles from 0.1g of leaf, 1μmol of NAD^+, 0.1μmol of pyridoxal phosphate, 2μmol of ADP, 10μmol (0.5μCi) of [1-^{14}C]glycine, 200μmol of sucrose, 10μmol of sodium citrate, 5μmol of KH_2PO_4, 2μmol of $MgCl_2$ and 5mg of bovine serum albumin. The reaction vessels were evacuated and filled with measured volumes of O_2 and N_2 to give atmospheres with the O_2 content shown. During the reaction the vessels were kept on a mechanical shaker at 25°C.

Table 1. *Effects of Amytal, ferricyanide and antimycin A on serine synthesis from glycine and accompanying phosphorylation of ADP*

Reaction mixtures were as described in Fig. 1 except for the addition, where indicated, of antimycin A (6.67nmol), $K_3Fe(CN)_6$ (2μmol) and Amytal (1.33μmol) or the omission of [1-^{14}C]glycine.

Atmosphere in the reaction vessel	Additions to reaction mixtures	Rate of synthesis (μmol/h per g fresh wt. of leaf) Serine	ATP	ATP increase caused by the presence of glycine
Expt. A				
Air	—	4.32	—	—
Air	$K_3Fe(CN)_6$	5.28	—	—
Air	Amytal	3.66	—	—
N_2	—	0.50	—	—
N_2	$K_3Fe(CN)_6$	5.80	—	—
N_2	Amytal	0.60	—	—
N_2	Amytal + $K_3Fe(CN)_6$	4.70	—	—
N_2	Antimycin A	0.18	—	—
N_2	Antimycin A + $K_3Fe(CN)_6$	0.46	—	—
Expt. B				
Air	Glycine absent	—	6.65	—
Air	—	4.56	12.21	5.56
N_2	Glycine absent	—	3.26	—
N_2	—	0.92	2.81	–0.45
N_2	$K_3Fe(CN)_6$, glycine absent	—	5.51	—
N_2	$K_3Fe(CN)_6$	4.18	8.50	2.99

in N_2 the increase in phosphorylation caused by the presence of glycine was only 54% of that where O_2 was the final electron acceptor.

The results confirm our view that NAD^+ reduction is not important during serine synthesis from glycine in leaves, but that the electron-transport system of mitochondria coupled to phosphorylation sites II and III is responsible for the oxidation involved and that two molecules of ATP are incidentally synthesized for each molecule of serine formed.

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