AN ENERGY DEPENDENT INCORPORATION OF AMINO ACIDS INTO THE PROTEIN OF PLANT MITOCHONDRIA [1, 2]

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It has been demonstrated that mitochondrial enzymes from higher plants are able to catalyze the synthesis of the amide bond of glutamine (5), and the peptide bonds of glutamyl-cysteine and glutathione (6) directly from the constituent amino acids. During the course of these studies with isolated mitochondria it was observed that, in addition to performing the syntheses outlined above, the mitochondria were also incorporating the free amino acids into the mitochondrial protein itself. In view of the possible significance of such phenomena for the general problem of protein metabolism in plants, and in view of the complete lack of information on the incorporation of radioactive amino acids into plant proteins, this observation has been examined in more detail. The present communication concerns the apparent coupling of energy production by the mitochondria to the incorporation of amino acids.

Mitochondria were isolated from bean hypocotyl homogenates by the method of Millerd et al (2), with minor modifications as described previously (6). They were incubated with C^{14}-labeled amino acids for one hour at 25°C in potassium phosphate buffer of pH 7.1 plus the additions listed in table I. The reaction was stopped with trichloracetic acid and the mitochondria reisolated by centrifugation at 10,000 × g for 15 minutes. The sedimented protein was washed several times with 5 % trichloracetic acid and with 1 % potassium hydroxide. The protein was assayed for radioactivity by standard techniques.

Isolated mitochondria incorporated each amino acid incubated with them at slow but measurable rates (table I). The rate of incorporation was markedly increased by the addition of Krebs cycle intermediates and adenylic acid. This increase was strongly inhibited by the respiratory inhibitor, hydrogen cyanide, by anaerobiosis, and by dinitrophenol, which Bonner and Millerd (1) have shown to be a potent inhibitor of oxidative phosphorylation in plants. These results indicate that at least a portion of the incorporation of these amino acids into mitochondrial protein is dependent on the energy produced by respiration.

It has been established that respiratory energy is conserved by the plant in the energy-rich bonds of adenosine triphosphate (1). That adenosine triphosphate can function as the energy donor for amino acid incorporation is indicated by its ability (table I) to replace the Krebs cycle intermediates in increasing incorporation. Incorporation at the expense of added ATP is unaffected by hydrogen cyanide, suggesting that the requirement for oxidative metabolism is, in fact, due to a requirement for an energy supply and that this is bypassed by adenosine triphosphate. The inability of adenylic acid alone to increase incorporation provides further evidence that the energy-rich bonds of adenosine triphosphate are the necessary factor for incorporation.

It appears, therefore, that the adenosine triphosphate produced during plant respiration may actively participate in the turnover of amino acids in mitochondrial protein. The fact that this turnover occurs at the same time that the mitochondria are catalyzing various respiratory and synthetic functions [oxidation of organic acids (2), phosphorylation of adenylate (1) and synthesis of hexose phosphate (4), acetyl-CoA and acetoacetate (3), and simple amides and peptides (5, 6)] gives a further indication of the complex spectrum of metabolic reactions that are associated with mitochondria.

TABLE I

THE EFFECT OF VARIOUS ACTIVATORS AND INHIBITORS ON INCORPORATION OF AMINO ACIDS BY MITOCHONDRIA

ADDITIONS TO SYSTEM *	μM AMINO ACID * INCORPORATED/GM PROTEIN		
	glycine	*glutamic acid*	*aspartic acid*
1. None	0.03	0.08	0.09
2. Adenylic acid + Mg^{+}	0.04	0.10	0.10
3. Same as no. 2 + citrate	0.11	0.23	0.21
4. Same as no. 2 + α-ketoglutarate	0.10	0.21	0.20
5. Same as no. 2 + malate	0.09	0.20	0.20
6. Same as no. 3 + HCN	0.05	0.11	0.11
7. Same as no. 3, incubated under nitrogen	0.07	0.15	0.14
8. Same as no. 3 + dinitrophenol	0.04	0.08	0.10
9. Same as no. 2 + ATP	0.20	0.39	0.33
10. Same as no. 9 + HCN	0.21	0.38	0.35

* Concentrations of all additions to system was 0.001 M. Concentration of the added amino acids was 0.01 M. The general conditions utilized for incubation were as described by Bonner and Millerd (1).

The author wishes to thank Dr. James Bonner for his helpful comments.

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[1] Received September 2, 1953.

[2] Supported in part by the Polychemicals Department, E. I. du Pont de Nemours and Co.

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It is of interest that Siegel and Weintraub (9) have found that the inhibitory effect of hydrogen peroxide on the activity of IAA in the Avena test could be almost completely reversed by subsequent incubation of the IAA-peroxide mixture with catalase, prior to its application to the coleoptile. (Catalase alone has no effect on IAA response, nor causes curvature in its absence.) Consequently, it must be assumed that the loss of IAA activity in the presence of hydrogen peroxide is not due to auxin destruction, but to some type of interference of the growth response itself. These investigators also noted that organic peroxides from ether acted in the same manner. The removal of these substances however, had to be accomplished by dilution rather than by catalase. Thus we find here a parallel case in which peroxides negate the activity of an auxin without destroying it.