ENERGY BUDGETED FOR PROTEIN SYNTHESIS IN CULTURED PLANT CELLS[1]

SHEPLEY S. C. CHEN[2]

Abstract: It is estimated that the growing cell culture of sycamore consumes approximately 50% of its metabolic energy for protein synthesis. This finding indicates that (1) the plant cell culture is a highly efficient system in which most of energy obtained from respiration can be accounted for; (2) protein synthesis is indeed an expensive process and needs to be regulated.

An ardent advocate of unhampered creativity, Dr. R. A. Good (America's leading cancer immunotherapist) encourages his students and colleagues to try a wide variety of approaches in their search for answers. "Hypotheses," he tells them, "are instruments. It doesn't matter if they are right or wrong as long as they stimulate thought." Thus, he reasons, no one need feel chagrined when his pet theory is shot down. "Right now, our theories are widely accepted," says he, "but I'm sure that some young bastard will come along and make us mad as hell with some intellectual leap that postulates a completely new theory. Whether he's right or wrong doesn't matter. Just trying to find out if he is or isn't should force us to think, to examine, to do new experiments. That's what science is—or should be—all about."—Time, March 19, 1973.

The cells isolated from the cambium of sycamore plant (*Acer pseudoplatanus* L.) (Lamport & Northcate, 1960) can be cultured in a modified White medium containing sucrose, NO_3^- (as a nitrogen source), minerals, B vitamins, and 2,4-D (White, 1963). They have a fast growth rate (the generation time is 2 days), and form a friable culture in a liquid suspension. This culture can be handled like a bacterial culture, and thus is particularly suitable for biochemical and growth studies.

Associated with the growth of plant cells are biosynthetic processes and respiration (Steward, 1968). It is generally conceived that the living cells often conserve the energy by synthesizing only those proteins which are needed in a particular time and space (Lwoff, 1968). It should be important to know the extent to which the metabolic energy is consumed in biosynthesis of proteins, a major class of macromolecules in the living system. This could be accomplished by simultaneously measuring the respiration and the amount of amino acids incorporated into proteins.

The respiratory quotient of the sycamore cultured cells in the logarithmic phase of growth is close to unity (it was measured to be 1.02), suggesting sugars being the substrate for respiration. For each mole of hexose sugar respired, 6 moles of O_2 is used, resulting in production of approximately 36 moles of ATP from ADP (Mabler & Cordes, 1971).

Meanwhile, protein synthesis can be measured by incorporation of ^{14}C-leucine in presence of a swamping concentration of the carrier. Assuming that the leucine in the protein precursor pool of the cells equilibrates readily with that of the

(1) Supported by a University of Illinois at Chicago Circle Research Board Grant and in part by the National Science Foundation Grant GB 30750.

(2) Department of Biological Sciences, University of Illinois at Chicago Circle, Chicago, Illinois, 60680.

medium, and its specific radioactivity is represented by the specific activity of the leucine in the medium, the amount of this amino acid incorporated can be calculated. Furthermore, an amino acid analysis of the gross protein in the sycamore cells would reveal how many moles of other amino acids are incorporated into proteins in conjunction with one mole of leucine. Therefore, the total amount of amino acids incorporated could be obtained.

Since these cells are grown in a medium devoid of amino acids, they have to synthesize the building block of proteins from sugar and nitrate (Steward & Bidwell, 1958; Bidwell *et al.*, 1964) (when ^{14}C-sucrose was fed, radioactivity readily appeared in trichloroactic acid-insoluble material which could be hydrolyzed by pronase, data not shown). Fifteen moles of ATP are required for synthesis of one mole of amino acid: 4 mole of $NADPH_2$ (equivalent to 12 moles of ATP) for reduction of NO_3^- to NH_3, and 1 mole of $NADPH_2$ for amination of α-keto acids. Elaboration of the carbon skeleton of amino acids involves little net energy change (Mable & Cordes, 1971). It is estimated that about 4 moleculses of ATP are needed to make one peptide link (Watson, 1970). Therefore, a total of 19 moles of ATP would be required for synthesis and addition of one mole of amino acids to the polypeptide chains. The total amount of ATP used in protein synthesis is thus obtained by multiplying the moles of amino acids incorporated into proteins by 19.

The sycamore cells were cultured in the liquid modified White medium (made of instant tissue culture powder, General Biochemicals, Chargrin Falls, Ohio), harvested in the logarithmic phase, and suspended in a fresh medium to a concentration of 50 mg fresh weight (or 5 mg dry weight) per ml. Two ml of the cell suspension were pipetted to a Warburg vessel and the amount of O_2 absorbed over 1 hr period was measured by means of a Gilson differential respirometer. One hundred mg fresh weight of the cells respired 43.8 μl (equivalent to 1.78 μ moles at S. T. P.) of O_2 at 28°C in 1 hr. Theoretically, this should result in phosphorylation of $1.78 \div 6 \times 36 = 10.68$ μ moles of ADP to ATP.

Over the same period of time, incorporation of ^{14}C-leucine was measured, using another flask containing the same amount of cells. Two ml of the cell suspension was made 1 mM with respect to leucine and 200,400 cpm of uniformly labeled ^{14}C-letucine was added. The culture was shaken at 28°C for 1 hr. The cells were then collected by centrifugation, washed in ice-cold leucine solution, suspended in 0.2 M NaCl+0.1 mM leucine, and sonicated with a Branson Sonifier Cell Disruptor (with a cooling jacket) at half the maximal output for 1 min. The cell wall was spun down quickly at 500×g for 5 min. One fifth of the supernatant fraction was precipitated by an equal volume of 15% trichloroacetic acid. The precipitate was collected on a Millipore filter by filtration, and the radioactivity was determined in a Beckman liquid scintillation system. The amount of radioactivity added to the medium was determined by spreading 1/1000 of the medium on a Millipore filter, which was then dried and counted similarly.

Table 1 shows the result of a representative experiment. The cells incorporated 1386 cpm of leucine into acid-insoluble material. Pronase released at least 60% of the label associated with acid-insoluble material (data not shown). Cycloheximide, a potent protein synthesis inhibitor (Kerridge, 1958; Siegel & Sisler, 1964), exerted 80% inhibition of incorporation.

In one treatment, the cells were exposed to ^{14}C-leucine for 1 hr, then briefly washed, transferred to a medium containing 1 mM non-radioactive leucine, and further incubated for 1 hr. The result shows that the label incorporated into acid-insoluble

material, instead of decreasing, increased after the chase. This suggests that there was little or no degradation of newly synthesized proteins within 1 hr period, and some labeled precursor which had been previously taken up was further incorporated during that period (Chen & Varner, 1970).

Table 1. Incorporation by Sycamore Cultured Cells of ^{14}C-leucine into Acid-insolube Material

Treatment	cpm incorporated per 10 mg dry weight
1. 1 mM leucine	1386
2. 1 mM leucine, 10μg/ml cycloheximide	267
3. 1 mM leucine, chased in ^{12}C-leucine	2012
4 0.1 mM leucine	11791

The cells were exposed to 200,400 cpm of ^{14}C-leucine in 2 ml of the medium containing 1 mM leucine (except for treatment 4) for 1 hr at 28°C, then disrupted and the radioactivity incorporated into trichloroacetic acid-insoluble material in cytoplasm was determined. Treatment 3 was further chased in 1 mM leucine for 1 hr. See text for experimental details.

In another treatment, incorporation of ^{14}C-leucine was carried out in the presence of 0.1 mM (instead of 1 mM) leucine, this resulted in a nearly 10 times increase in the radioactivity incorporated, suggesting that the leucine in the medium does get in quickly and overwhelm the endogenous pool.

From the foregoing discussions, it is highly probable that (1) the incorporation observed here does represent protein synthesis; (2) there is little turn-over of newly synthesized proteins within 1 hr period; and (3) the specific radioactivity of leucine in the protein precursor pool must be close to that in the medium. It is thus possible to estimate the amount of leucine incorporated into proteins in that 1 hr period. This amouts to 2000 mμ moles $\times 1386/200{,}400 = 13.83$ mμ moles.

An amino acid analysis of gross protein extracted from the sycamore cells reveals that leucine comprises approximately 1/20 of the amino acids in this protein. Assuming that the amino acid composition of the gross protein is indicative of that of newly synthesized proteins, for each mole of leucine incorporated there should be a total of 20 moles of amino acids incorporated into proteins. That is to say. $13.83 \times 20 = 276.6$ mμ moles, or 0.2766 μ moles, of amino acids were incorporated. This would require $0.2766 \times 19 = 5.26$ μ moles of ATP. Thus the percent of energy used for synthesis of proteins would be $5.26/10.68 = 50\%$ of the total released in respiration!

It is noteworthy that cycloheximide at 10 μg/ml inhibited 80% of amino acid incorporation within 1 hr; it had, however, no immediate effect on O_2 uptake. The cells treated with cycloheximide respired at the same rate as did the control. Oxygen uptake slowed down only 2 hr after addition of the inhibitor (Fig. 1). This seems to suggest that respiration and protein synthesis are not so tightly coupled.

In conclusion, it is estimated that about 50% of the energy released from respiration is utilized in protein synthesis. A preliminary experiment based on the similar technique and assumptions also showed that *ca.* 10% of total energy is consumed in cell wall synthesis and somewhat less in nucleic acid synthesis. Plant cultured cells appear to be a highly efficient system: more than 60% of the metabolic energy can be accounted for the known biological processes.

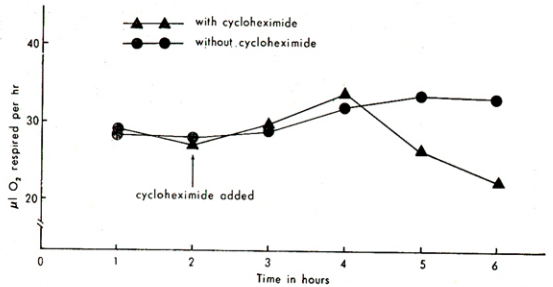

Fig. 1. Time course of O_2 uptake at 28°C in the cultured sycamore cells in presence or absence of 10 μg/ml cycloheximide.

ACKNOWLEDGEMENTS

The author wishes to thank Drs. J. E. Varner, D. T. A. Lamport, and P. Filner, all of Michigan State University/Atomic Energy Commission Plant Research Laboratory in East Lansing, for valuable discussions, and Mr. Charles N. Steele for amino acid analysis of the Sycamore cell protein.

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