TOWARD AN ESTIMATE OF ATP CONSUMPTION FOR PROTEIN SYNTHESIS IN GERMINATING SEEDS[1]

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Abstract: By simultaneously measuring amino acid incorporation and respiration, it is estimated that in germinating barley seeds, only 1% of the energy released from respiration is used for protein synthesis. This estimate is based on the assumption that (1) all O_2 absorbed by the seeds is used for respiration; combustion of 1 glucose with the aid of 6 O_2 yields 36 ATP; (2) protein synthesis involves assembly of preformed amino acids; the main energy expenditure is for making peptide bonds; formation of each peptide link requires 4 ATP; (3) specific radioactivity of the amino acid in the incubation medium reflects that of the precursor pool inside the seeds. The first and the third assumptions could be incorrect, therefore, the actual energy consumption for protein synthesis might be much higher.

Radicle protrusion marks a distinct stage in seed in seed germination: it is the sign indicative of subsequent normal development (and hence the criterion for germination); it is accompanied by a marked increase of metabolic activities, such as respiration and protein synthesis (Abdul-Baki, 1969; Chen, 1970a). It is often assumed that an increase of oxygen uptake is necessary to cope with increased energy demand for synthetic and other processes characteristic of growth. This study attempts to estimate the extent to which the energy released from respiration is consumed for biosynthesis of proteins. The technique involves simultaneously measuring respiration and incorporation of an isotopically-labeled amino acid into proteins (Chen, 1970b).

MATERIALS AND METHODS

Barley seeds—*Hordeum vulgare* cv. Himalaya seeds were pruchased from Wshington State University, Pullman, Washington.

^{3}H-leucine—L-leucine-4,5-^{3}H (S. A. 5.0 C/mmole) was pruchased from Schwarz Bio Research, Waltham, Massachusetts.

Measurement of oxygen uptake—O_2 uptake was measured with a Gilson differential respirometer at 27°C, using 10 seeds per flask.

Incorporation of ^{3}H-leucine into proteins—Barley seeds (grains) were surface-sterilized in 1% NaOCl for 10 min, washed and incubated at 27°C for 14 hr (they just began to germinate at this time). Lots of 10 seeds were exposed to ^{3}H-leucine for 2 or 4 hr, then quickly washed, frozen, ground in 0.2 M NaCl+1 mM leucine with sand. The homogenate was spun at 500×g for 3 min to remove sand, starch grains, and cell wall. One fiftieth of the supernatant fraction was precipitated with

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trichloroacetic acid and the precipitate was collected on Millipore filters, and the radioactivity incorporated determined in a Beckman liquid scintillation detector with a counting efficiency of 22% for tritium.

RESULTS

Lots of 10 barley seeds were exposed to ^{3}H-leucine in 2 ml of the media with varyidg amounts of the tracer and the carrier as indicated in Table 1. There was no difference in the amount of the label incorporated between #1 and #2, suggesting the concentration of leucine inside the seeds was probably higher than 0.1 mM. Although the specific activities (S. A.) of leucine in#2, #3, and #4 were the same, the amount of the label incorporated differed greatly: for each 10-fold increase of the carrier (normal leucine) concentration, there was 5-fold increase of incorporation. Furthermore, more label was incorporated during the second 2 hr period than the first 2 hr (the difference between cpm incorporated in 4 hr and those in 2 hr was regarded as the incrporation during the second 2 hr period; there was little or no degredetion of newly synthesized proteins within 2 hr period). These observations suggeted that the precursor pool (PP), that amount of amino acids readily available for incorporation into proteins (Holleman & Key, 1967), in barley seeds did not readiy equilibrate with the medium. Longer incubation in high concentration of leucine seemed to have helped PP to attain a S. A. closer to (and hopefully equal to) that of the medium.

Table 1. Incorporation of ^{3}H-leucine into proteins by germinating barley seeds

No.	μC of ^{3}H-leucine in 2 ml of medium	Conc. in mM of leucine in medium	cpm incorporated × 1/50		
			2 hr	4 hr	2nd 2 hr
#1	2	0	12.4	30.2	17.8
#2	2	0.1	12.0	35.4	23.4
#3	20	1	81.9	193.3	111.4
#4	200	10	524.1	1140.4	616.3

In this experiment, a computation based on the label incorporated during the second 2 hr in high concentration #4 medium should best represent the reality. That is to say, 30,800 cpm of the label associated with ^{3}H-leucine was incorporated into proteins. The S. A. of leucine was 4.8×10^6 cpm/μ mole. Therefore, 30,800 cpm should represent 308/48,000 μ mole of leucine incorporated, assuming that the S. A. of leucine in PP had reached that of the medium.

An amino acid analysis of barley proteins shows that there is 1 leucine for every 15 amino acids (FAO, 1970). If this frequency of leucine holds for newly-synthesized proteins, for each leucine incorporated, there must be some 15 amino acids incorporated. Therefore, the total amount of amino acids incorporated into proteins would be $308\times15/48{,}000$ μ moles.

It is estimated that 4 ATP,s are required to build one peptide link (Watson, 1970). Hence the total amount of ATP required for protein synthesis would be $308\times15\times4/48{,}000=0.38$ μ mole.

In the mean time, the culture of 10 seeds respired 161 μl of O_2 at 27°C. This

is equivalent to 6.4 μ moles of O_2. The R. Q. was closed to unity, suggesting that carbohydrate was the substrate. According to the formula $6\ C_6H_{12}O_6+6\ O_2=6\ CO_2+6\ H_2O+36$ ATP, 38.4 μ moles of ATP shold be produced (phosphoryated).

Hence the percent of metablic energy consumed for protein synthesis was approximately 0.38/38.4=1%!

DISCUSSION

A similar study was made using the sycamore (*Acer pseudoplatanus*) cell suspension culture (Chen, 1973). It was estimated that the log phase cells spent *ca.* 50% of the energy obtained from respiration for reduction of nitrate, synthesis of amino acids, and assembly of polypeptides.

The barley seeds differ from the cell culture in two respects: (1) it is a multicellular system, permeation of ^{3}H-leucine may be more difficult, therefore the S. A. of PP may not attain that of the medium as easily as it would in the cell culture; (2) the cll culture was grown on sucrose, NO_3^-, $SO_4^=$, minerals, B vitamins, and auxin. No amino acids were added. A tremendous amount of energy had to be spent on synthesizing amino acids. While barley seeds obtained their supply of amino acids from the reserve protein; the main expenditure of energy was for building peptide bonds. These two facts may in part explain why the barley seeds use much less energy for protein synthesis. Besides, a growing cell culture may indeed be a far more efficient system than the barley seeds are.

Nevertheless, that puny 1% for the barley seeds is unbelievably low. One would expect that a sizable portion of the energy released from respiration should be budgeted for protein synthesis. Several sources of errors may have encountered; (1) Complete oxidation of a glucose yields 36 ATP's. This is a theoretical value based on a P/O ratio of 3. In real life, the P/O ratio might be lower, hence there is less ATP phosphorylated. Furthermore, the cereal grains, such as oat and barley, are known to carry out some oxidative process other than respiration during germination; it would be wrong to assume that all the O_2 absorbed is consumed for respiration. (2) Although a pulse/chase experiment showed that there was little or no turnover of newly synthesized proteins (NSP) (data not shown), it is possible that some degredation might have occurred, which had escaped detection. (3) The frequency of leucine in NSP might be different from that of the proteins in whole dry barley grains. (4) The S. A. of leucine in PP might not have approached that of the medium; it could be considerably lower.

If corrections are made for the factors (1), (2), and (4), the percent of energy consumed for protein synthesis should become greater. The factor (3) could alter the figure either way.

Let us figure out the correction factors by considering two somewhat extreme cases: (A) assume that the P/O ratio was 2, 20% of proteins was degraded, the S. A. of leucine in PP reached only 1/2 of that of the medium; (B) assume that the P/O ratio was 1, 20% of NSP was degraded at the end of the measurement, the leucine frequency in NSP was 1/2 of that of the available data, and the S. A. of leucine in PP was only 1/5 of that of the assumed value (Table 2). Then a carrection factor of 3.6 or/to 36 must be applied to the 1% figure derived earlier. One is stuck with an answer just as non-commital as the title of this article itself—3.6 to 36%!

Table 2. Possible correction factors factors for calculation of the energy spent on protein synthesis

	(A)	(B)
(1) the P/O ratio	×1.5	×3
(2) protein turnover	×1.2	×1.2
(3) leucine frequency	×1	×2
(4) the S. A. of PP	×2	×5
Correction factors	×3.6	×36

LITERATURE CITED

Abdul-Baki, A. A., 1969. Metabolism of barley seed during early hours of germination. Plant Physiol., **44**: 733-738.

Chen, S. S. C., 1970a. Influence of factors affecting germination on respiration of *Phacelia tanacetifolia* seeds. Planta, **95**: 330-335.

———, 1970b. Thoughts on problems related to plant development. Taiwania, **15**(2): 223-226.

———, 1973. Energy budgeted for protein synthesis in cultured plant cells. Taiwania, **18**(1): 1-4.

FAO., 1970. Amino-Acid Content of Foods and Biological Data on Protins. Food and Agriculture Organization of the United Nations. Rome, Italy.

Holleman, J. M., & J. L. Key, 1967. Inactive and protein pools of amino acids in the soybean hypocotyl. Plant Physiol., **42**: 29-36.

Watson, J. D., 1970. Molecular Biology of the Gene. 2nd ed. W. A. Benjamin, Inc., New York.