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Adenylate Metabolism of Embryonic Axes from Deteriorated Soybean Seeds

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ABSTRACT

RNA and protein syntheses in axes excised from dry soybean (*Glycine max* L.) seeds at different levels of deterioration were assayed. Low rates of protein synthesis in slightly deteriorated seeds were not due to losses in ribosomal or soluble fraction activities. However, the lowered rates of RNA and protein syntheses of deteriorated seeds were associated with reduced ATP content of the tissues. Adenine and adenosine conversions to ATP were reduced in deteriorated axes, and these reductions were reflected in reduced incorporation of these compounds into RNA.

Seeds stored at high moisture and temperature rapidly deteriorate and lose germinability. Despite a number of studies which indicate that isolated embryos or axes from such seeds have lowered respiration rates (2) and lowered rates of protein (2, 18), polysaccharide (2), and RNA (18, 20) synthesis, the mechanism by which seeds lose viability is not known. Recently, deteriorated seeds and seeds of low vigor were shown to have low ATP levels (9, 10, 20). Since ATP is extremely important in controlling, regulating, and directing biosynthesis, alterations in its metabolism could contribute markedly to the deterioration and loss of viability of stored seeds. Thus, I compared the embryonic axes of deteriorated and sound seeds with respect to ATP levels, to conversion of adenine and adenosine to AMP, ADP, ATP, and RNA, and to synthesis of protein. The results support the concept that the inability to sustain ATP synthesis is probably the major cause of the low rates of biosynthetic activity in deteriorated seed and might cause loss of germinability.

MATERIALS AND METHODS

Soybean (*Glycine max.* L. cv. Kent) seeds were harvested at Beltsville in 1972 and stored in sealed containers at 4 C until used. The seeds were then transferred to cloth bags and stored at 3 C and 80% relative humidity for 2 to 3 weeks so that moisture content was increased to about 20%. Deterioration (rapid aging) was induced by storage of these seeds in sealed glass jars at 35 C. Samples were taken at 0- to 9-day intervals, depending on the experiment. For determination of germinability, seeds were incubated on wet germination paper in the dark at 22 C for 5 days.

The embryonic axes minus the plumules were excised from dry seeds and were generally used within 2 days. They were used either dry or after imbibing water containing 10 μg/ml chloramphenicol at 25 C.

Isolation of Ribosomes and Supernatant Fraction. Ribosomes and supernatant fractions were prepared in the cold from 50 embryonic axes (≈135 mg) of rapid aged soybean seeds. The axes were ground in 5 ml bicarbonate buffer (pH 6.8) (13) with a mortar and pestle, and the homogenate was centrifuged at 23,500*g* for 10 min. The supernatant fraction was made 10 mM in tris and 2 mM in Mg acetate, then layered over 3 ml of ribosomal buffer (13) containing 0.5 M sucrose (RNase-free). The layer was centrifuged 1 hr at 35,000 rpm in the No. 40 angle head rotor in a Spinco model L ultracentrifuge.[1] The top two-thirds of the supernatant was removed and stored at −20 C until used. The remaining supernatant and the sucrose layer were aspirated and the pellet was taken up in 0.5 ml of ribosomal buffer. The mixture was clarified by centrifugation at 10,000*g* and the supernatant held at −20 C until used.

For the removal of endogenous amino acids, supernatants to be used in *in vitro* phenylalanine-incorporation experiments were dialyzed 2 hr against a solution of 1 mM tris (pH 7.6), 50 mM KCl, 2 mM Mg acetate, and 4 mM 2-mercaptoethanol.

Phenylalanine Incorporation. Phenylalanine was incorporated in a modified wheat embryo system (12) as detailed in Table I. Assay mixtures were incubated for 30 min at 25 C. Addition of 5% trichloroacetic acid and heating of the mixture for 15 min at 90 to 95 C terminated the reaction. The mixtures were filtered under vacuum with Whatman GF/C filters. Dried filters were placed in glass scintillation vials with 5 ml of Liquifluor for counting.

In Vivo [^{14}C]Leucine and [^{3}H]Adenosine Incorporation. Axes were imbibed in a solution of 0.1 mM L-[^{14}C]leucine (0.3 μCi/ml) and 0.1 mM [^{3}H]adenosine (1 μCi/ml) containing 50 μg/ml each of streptomycin sulfate and penicillin G for 1, 2, and 3 hr. The axes were removed, washed with tap water, and kept at −20 C until extracted with 5% trichloroacetic acid containing 1 mM each of nonradioactive leucine and adenosine. Aliquots of acid-insoluble and -soluble materials were analyzed for radioactivity.

Adenine Nucleotide Determination. Axes were imbibed in H_2O containing 10 μg/ml chloramphenicol for 0 to 5 hr at 25 C, removed, frozen in liquid N_2, and then homogenized in 5 ml 0.25 N $HClO_4$ with mortar and pestle. The homogenate was centrifuged at 10,000*g* and the supernatant saved. The pellet was washed with $HClO_4$ and centrifuged. The combined $HClO_4$ extracts were neutralized with 2.5 M $KHCO_3$, and the potassium perchlorate precipitate was removed after centrifugation and washed with water. After centrifuging, the supernatant was added to the neutralized extract and the $KClO_4$ discarded.

Aliquots were analyzed for ATP, ADP, and AMP. AMP was determined by conversion to ADP with myokinase (8), and ADP by conversion to ATP with pyruvate kinase and phosphoenolpyruvate. Both enzymes were desalted by passage of the samples through a 6-ml Sephadex G-25 column (21).

ATP was determined with purified luciferin-luciferase (Du

[1] Mention of a trade name, proprietary product or specific equipment does not constitute a guarantee or warranty by the United States Department of Agriculture and does not imply its approval to the exclusion of other products that may also be available.

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Pont) in an Aminco microphotometer. The content of each vial of luciferin-luciferase was diluted in 3 ml of supplied buffer then with 9 ml of a solution that was 25 mM HEPES (pH 7.4) and 25 mM $MgSO_4$. Assays were run in tubes (6 × 50 mm) with 200 μl of luciferin-luciferase. The neutralized $HClO_4$ supernatant (100 μl) was injected with a syringe. Relative light intensity was integrated for 10 sec with a Vidar 260 voltage to frequency converter connected to a Monsanto counter timer, model 1500.

ATP was determined by constant addition (19) with samples diluted so that responses were in the linear range.

Adenine and Adenosine Determination. Aliquots of the neutralized $HClO_3$-soluble fraction were analyzed for adenine and adenosine with a high pressure liquid chromatograph from Waters Associates. Nucleosides and purine and pyrimidine bases were separated on a uBondapak C_{18} column using a 0.1% (w/v) $NH_4H_2PO_4$ and methanol gradient (0–30% methanol) with the No. 8 curve of the model 660 solvent programer. Detection of components was at 254 nm with a model 440 absorbance detector. Adenine was identified by co-chromatography and adenosine was identified by co-chromatography and sensitivity to adenosine deaminase. Quantitation was by peak height analysis.

Uptake of Adenine and Adenosine. Axes were incubated in [8-^{14}C]adenine or [8-^{14}C]adenosine for 1.5 and 3 hr at 25 C with 10 μg/ml chloramphenicol. Axes were frozen in liquid N_2 and homogenized as described above. Radioactivity in the acid-soluble and -insoluble fractions was determined. The distribution of radioactivity in the adenylate nucleotides of the soluble fraction was determined by PEI[2] chromatography (15). Aliquots were streaked on PEI sheets (5 × 20 cm) along with marker ATP, ADP, and AMP, and developed twice with H_2O to move adenine and adenosine to the top half of the plate. The chromatogram was then developed (10 cm) with 0.75 M NaH_2PO_4 buffer (pH 3.4) to separate the adenylate nucleotides.

Adenosine Kinase. Excised axes [25] were ground in 2.5 ml of cold grinding buffer (0.1 M tris-HCl [pH 8], 1 mM $MgCl_2$, 50 mM 2-mercaptoethanol, and 5% glycerol) with a mortar and pestle. The homogenate was centrifuged at 6,500*g* for 10 min, and the supernatant, filtered with Miracloth, was used as enzyme. Assay mixtures (100 μl) for adenosine kinase were 0.7 mM ATP and 0.1 mM [^{14}C]adenosine (0.1 μCi) and contained 0.01 ml grinding buffer plus enzyme (0.1-0.2 mg protein). Reactions were started by the addition of 10 μl 7 mM ATP. Aliquots were taken at various time intervals, spotted on sheets (2.5 × 5 cm) coated with PEI cellulose, and developed in H_2O as soon as the drop was absorbed. After the front had moved to the top, the plate was dried. Nucleotides remained at the origin while nucleosides and free bases were mobile (17). The origin was cut from the sheets, and radioactivity determined.

Assay mixtures for adenine phosphoribosyltransferase were the same as for adenosine kinase except that 0.1 mM PRPP was used instead of ATP, and 0.1 mM [^{14}C]adenine (0.1 μCi) was used instead of labeled adenosine. Conversion of adenine to nucleotides was determined by chromatography as described above.

Protein Determination. Protein content of 10% trichloroacetic acid-precipitated material was determined by fluorescence (5).

RESULTS AND DISCUSSION

Induction of Deterioration. The induction of deterioration, loss of vigor and germinability, occurred rapidly in seeds stored at 35 C and 20% seed moisture. Seed samples stored for 2 or 3 days under these conditions lost some germinability (Figs. 1 and 2). Longer periods of storage resulted in a higher percentage of seeds that were unable to germinate. The rate of deterioration varied between experiments (Figs. 1 and 2).

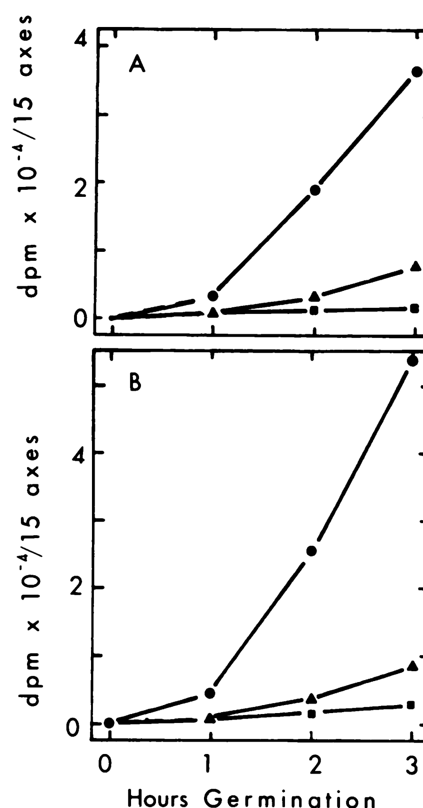

FIG. 1. Incorporation of [^{14}C]leucine (A) and [^{3}H]adenosine (B) into trichloroacetic acid-insoluble material by imbibed soybean embryonic axes from rapid aged seeds of different germinability (circles, triangles, and squares represent seed lots rapid aged for 0, 3, and 9 days with 99, 90, and 0% germinability, respectively). Each point is the average of two replicates with 15 axes/replicate.

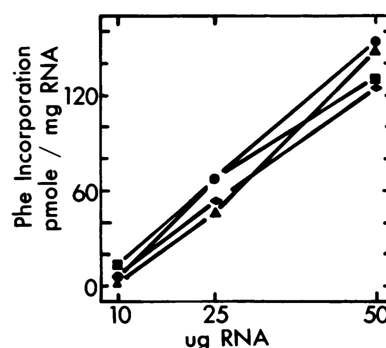

FIG. 2. Dependence of poly U-directed incorporation of phenylalanine on ribosomes. Ribosomes extracted from embryonic axes of rapid aged seeds differing in germinability (circles, triangles, squares, and diamonds represent seed lots rapid aged for 0, 2, 4, and 9 days with 85, 76, 36, and 0% germinability, respectively). Each point is the average of two replicate assays.

Incorporation of Amino Acids and Adenosine in Axes of Deteriorated and Sound Seeds. During imbibition, rates of incorporation of labeled leucine (Fig. 1A) and adenosine (Fig. 1B) into acid-insoluble materials increased significantly in axes of control seeds. Ribosomes isolated from axes of soybean seeds at various stages of deterioration (0–85% germinability) did not differ greatly in their ability to support *in vitro* poly U-directed phenylalanine-incorporation (Fig. 2). All of the corresponding supernatants effectively supported incorporation of phenylalanine in the presence of added soybean ribosomes (Table I).

[2] Abbreviations: PEI: polyethyleneimine; PRPP: Na-phosphoribosylpyrophosphate; EC: energy charge; poly U: polyuridylic acid.

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However, the incorporation for supernatants from seeds of 0% germinability was about 33% lower than that for any of the other supernatants. Thus, the ribosomes and water-soluble components of partially deteriorated soybean seeds appear to be as effective as those of sound seeds in supporting protein synthesis. These data on soybean axes differ somewhat from those on rye embryos (18).

ATP Levels in Rapid Aged Seeds. ATP content of germinating axes increased severalfold during the first hours of imbibition (Fig. 3). In axes from control seeds of 85% germinability, ATP increased from a level of about 0.3 nmol/dry axis to 3.6 nmol/axis in the 1st hr of imbibition. Concurrently, AMP decreased from 1.2 nmol/dry axis to a level barely detectable by the method employed. The ADP level was fairly constant (0.3–1 nmol/axis) during this time period. The increase in ATP is not accounted for by depletion of the AMP and ADP pools as it is in other seeds (6, 9, 14, 15). Total content of adenylate nucleotides of soybean axes increased during germination regardless of extent of deterioration (Fig. 4). In control seeds, the net 5-hr increase, which was due primarily to ATP, amounted to more

Table I. Incorporation of labeled phenylalanine into protein. Support of poly U-directed protein synthesis by supernatants from embryonic axes of seeds differing in germinability. Ribosomes were from axes of the 85% germinability seeds. Assays contained 18 μg protein per reaction

Rapid-Aged	Seed Germination	^{14}C-phenylalanine incorporated
days	%	p moles/mg protein/30 min
0	85	332
2	76	300
4	36	343
9	0	216

Assay mixture (400 μl) was 19 mM tris (pH8), 25 mM KCl, 5 mM Mg acetate, 2 mM dithiothreitol, 0.7 mM ATP, 6 mM creatine phosphate, and 2 mM GTP with 30 μg creatine kinase, 100 μg poly U, 27 μg wheat germ tRNA (12), 0.1 μCi phenylalamine (418 mCi/nmole), ribosomes, and supernatant.

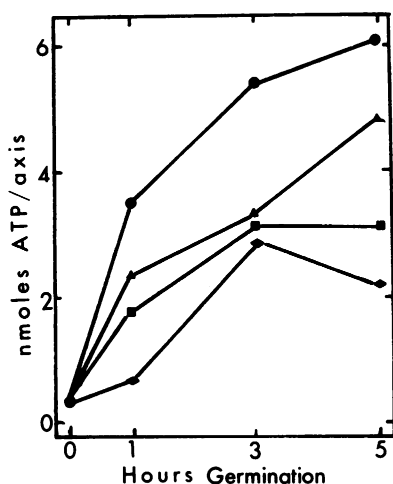

FIG. 3. ATP content of germinating soybean axes from rapid aged seeds differing in germinability (symbols same as Fig. 2). Each point is the average of two replicates with 10 axes/replicate.

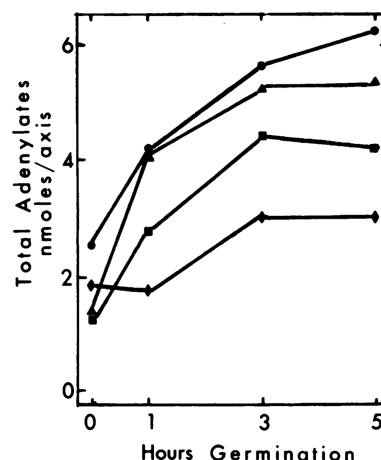

FIG. 4. Total content of adenylate nucleotides in soybean axes from rapid aged seeds differing in germinability (symbols same as Fig. 2).

Table II. Adenine and adenosine content of dry and imbibed soybean axes

Hours of Imbibition	Adenine	Adenosine
	nmoles/axis	
0	0.28	2.27
1	N.D+	0.44++
3	N.D	0.10

+Not detected

++Includes the amount of adenosine found in the extracts and in the imbibition media.

Each datum represents the means of 2 to 4 replicates.

Table III. Uptake of adenosine-8-^{14}C by germinating soybean embryonic axes at different stages of deterioration.

Rapid-Aged	Germination	Imbibition	Uptake	RNA	ATP	ADP	AMP	Specific activity of ATP
days	%	hours	cpm x 10^{-3}/10 axes		% of uptake			cpm/nmoles
0	85	1.5	154	1.7	28.9	13.0	9.2	1677
		3.0	253	4.7	37.1	13.1	6.9	2264
2	78	1.5	172	0.9	16.3	7.7	7.7	1090
		3.0	232	1.8	19.6	9.3	11.2	2553
4	40	1.5	151	0.8	10.2	8.2	12.6	800
		3.0	167	1.4	16.7	10.3	9.3	1448
9	0	1.5	204	0.5	8.0	7.5	9.7	1990
		3.0	207	0.6	4.9	6.0	9.7	1170

Axes were incubated in adenosine-8-^{14}C (17,940 cpm/nmole). Each datum represents the mean of 2 experiments with 10 axes per experiment.

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Table IV. Uptake of adenine-8-^{14}C by germinating soybean embryonic axes at different stages of deterioration

Rapid-Aged	Germination	Imbibition	Uptake	RNA	ATP	ADP	AMP	Specific activity of ATP
days	%	hours	cpm x 10^{-3}/10 axes	% of uptake				cpm/nmoles
0	85	1.5	249	4.1	43.1	9.1	4.4	2254
		3.0	386	8.4	44.8	7.7	4.0	2148
2	78	1.5	246	2.6	26.8	4.4	4.1	1523
		3.0	331	6.0	35.7	10.4	4.5	2592
4	40	1.5	167	2.0	21.5	8.8	6.0	1345
		3.0	225	4.9	30.1	11.3	6.7	1855
9	0	1.5	180	1.2	9.5	7.1	8.7	821
		3.0	216	1.8	15.5	7.6	6.3	1433

Axes were incubated in adenine-^{14}C (17,400 cpm/nmole). Each datum represents the mean of 2 experiments with 10 axes per experiment.

than three times the total adenylate nucleoside phosphates of the dry axes (Fig. 4) and is similar to what happens in crimson clover seeds (10). This increase in total adenine nucleotides during these early hours of germination can be accounted for, in part, to their synthesis from the endogenous pools of adenine and adenosine.

Data in Table II show that there is sufficient adenine and adenosine present in the dry axis to double the adenylate nucleotides. The rapid decline of these endogenous pools and their incorporation (Tables III and IV) into adenylate nucleotides strongly suggest that adenine and adenosine can and do serve as substrates for adenylate nucleotides in soybean axes. Nucleosides have been regarded as nucleotide reserves in seeds (7, 11). The increase in adenylates not accounted for by adenine and adenosine probably arises from *de novo* synthesis of nucleotides and conversion of 3′-AMP from RNase activity to adenosine by 3′-nucleotidase.

The pattern of ATP increase during germination (or imbibition) was similar for axes from seeds of different germinability, but the average rate of increase, and hence, the total level of ATP, varied inversely with degree of seed deterioration (Fig. 3). Deteriorated axes accumulated less adenylate nucleotides than control axes. After 3 hr of imbibition, the axes from the most deteriorated seeds declined in ATP content (Fig. 3).

The low rate of incorporation of adenosine into RNA (Fig. 1B) by deteriorated seeds suggested poor absorption of adenosine or poor conversion of this substrate to ATP. To test these possibilities, I incubated [^{14}C]adenine and [^{14}C]adenosine with axes and analyzed the $HClO_4$-soluble material for total radioactivity and for ATP, ADP, and AMP. The radioactivity of the RNA fraction was also determined. Generally, the uptake of adenine and adenosine, the conversion to ATP and ADP, and the incorporation of the substrates into RNA decreased with increased seed deterioration (Tables III and IV). However, the per cent conversion of these substrates to AMP tended to increase, with seed deterioration, suggesting a block between AMP and ADP or ATP. The correlations between the total ATP pool and the amount of adenine (Fig. 5A) and adenosine (Fig. 5B) converted to ATP were significant. These data support the concept that the incorporation of adenine and adenosine into RNA is directly related to ATP pool size.

The low amounts of radioactive adenine nucleotides in deteriorated axes (Fig. 4) could have been caused by inactivation of the enzymes that catalyze nucleotide synthesis. However, crude extracts of axes from seeds of control and 9-day rapid aged (85 and 0% germinability) did not differ in the activities of adenosine kinase and adenine phosphoribosyltransferase enzymes that convert adenosine and adenine to AMP (Table V). Activity of the former enzyme was found to depend on ATP concentration

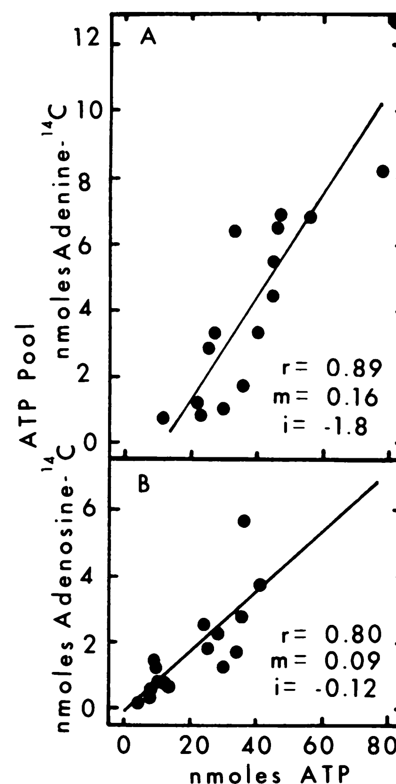

FIG. 5. Correlation between total ATP pool in soybean axes (10) from rapid aged seeds and amount of radioactivity from [^{14}C]adenine (A) and [^{14}C]adenosine (B) in the ATP pool. Axes were germinated for 1.5 or 3 hr in 0.1 mM solutions of ^{14}C-labeled adenine or adenosine. Each datum point represents one determination, r = correlation coefficient, m = slope, and i = intercept.

Table V. Adenosine kinase and adenine phosphoribosyltransferase activities from soybean embryonic axes

Rapid Aged	Germinability	Adenosine Kinase Exp. 1	Adenosine Kinase Exp. 2	Adenine phosphoribosyltransferase Exp. 1	Adenine phosphoribosyltransferase Exp. 2
days	%	nmoles*/mg protein/min			
0	85	5.89	3.33	0.62	1.59
9	0	5.40	3.16	0.76	1.45

* substrate converted to product. Each datum presents the mean of 2 to 4 different extracts assayed at 2 different enzyme levels.

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(Fig. 6). Although adenine phosphoribosyltransferase does not require ATP, the formation of PRPP from ribose-5-PO_4 is ATP-dependent (16). Possibly, the high correlation between ATP content and the *in vivo* conversion of [^{14}C]adenine to ATP indicates PRPP formation.

These data support the concept that energy charge (6, 8) ([ATP] + 1/2 [ADP])/([ATP] + [ADP] + [AMP]) is an important control of metabolic activity. In general, the less deteriorated the sample, the higher was the energy charge. This was true whether the EC calculation was based on the total adenine nucleotide pool or on the distribution of radioactivity in AMP, ADP, and ATP from adenine or adenosine (Table VI). It is not known whether the low EC of deteriorated axes is a cause or an effect of deterioration. The difference in EC between adenine- and adenosine-incubated tissues is being investigated.

The low conversion of AMP and ADP to ATP in deteriorated axes could also have resulted from impaired oxidative phosphorylation. Respiration rates of axes decrease with seed deterioration (2).

The synthesis of ATP by oxidative phosphorylation, as well as continuous uptake of substrates rely on intact membrane systems. Leaching experiments have shown that deteriorated soybean axes have altered membrane permeability (2). Possibly, the lower accumulation of ATP, the lower EC, and the lower uptake of radioactive substrates of deteriorated axes are all related to degraded membrane systems in the deteriorated tissues.

The low rates of biosynthetic activity in deteriorated seeds are probably related to the loss in ability to synthesize and accumulate ATP rather than to the loss of a specific enzyme in protein or RNA synthesis. Enzymes which require high ATP concentrations for activity (*e.g.* those involved in protein synthesis (4), RNA synthesis, and nucleotide systems) would be the first diminished or inactivated by decreases in ATP concentrations. Many of the observations on lowered rates of metabolic activity of deteriorated seeds can be explained on this basis, and yet

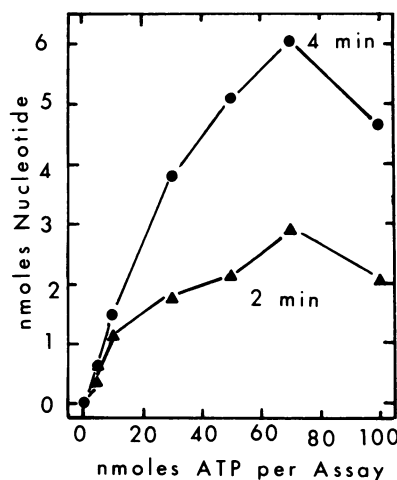

Fig. 6. Activity of adenosine kinase as a function of ATP concentration. Triangles and circles represent activity at 2 and 4 min, respectively, from ATP addition. After appropriate incubation times, 5-μl aliquots were removed, spotted on PEI sheets (1.5 × 2.5 cm), and developed in H_2O. After air-drying, the origins were cut from the sheets, placed directly into vials containing 5 ml of scintillation fluid, and counted so that the immobile nucleotides could be quantified.

Table VI. Energy charge (EC) of soybean axes incubated in either adenine-^{14}C or adenosine-^{14}C. The EC was determined for total pool by the luciferin-luciferase method and from the radioactivity by nucleotides separated on PEI cellulose.

Germination	Time	ADENINE		ADENOSINE	
		Total Pool	Radioactivity	Total Pool	Radioactivity
%	hours				
85	1.5	0.70	0.84	0.67	0.70
	3.0	0.89	0.87	0.75	0.77
78	1.5	0.62	0.84	0.75	0.62
	3.0	0.81	0.82	0.76	0.58
40	1.5	0.68	0.72	0.69	0.47
	3.0	0.80	0.75	0.60	0.60
0	1.5	0.69	0.58	0.57	0.45
	3.0	0.77	0.66	0.50	0.37

Each datum represents the average of 2 experiments with 10 axes per experiment.

allow for high activity of other reactions, *e.g.* acetate metabolism (1, 3).

Acknowledgments—I thank T. Johnson for his excellent technical help.

LITERATURE CITED

1. Abdul-Baki AA 1971 Biochemical differences between embryonic axes from green and sun-bleached lima bean seeds. Synthesis of carbohydrates, proteins and lipids. J Am Soc Hort Sci 96: 266–270
2. Abdul-Baki AA, JD Anderson 1973 Vigor determination in soybean seed by multiple criteria. Crop Sci 13: 630–633
3. Anderson JD 1973 Metabolic changes associated with senescence. Seed Sci Technol 1: 401–416
4. Ayuso-Parrilla MS, R Parrilla 1975 Control of hepatic protein synthesis. Differential effect of ATP levels on the initiation and elongation steps. Eur J Biochem 55: 593–599
5. Böhlen P, S Stein, W Dairman, S Udenfriend 1973 Fluorometric assay of proteins in the nanogram range. Arch Biochem Biophys 155: 213–220
6. Bomsel JC, A Pradet 1968 Study of adenosine 5′-mono, di- and triphosphates in plant tissues. IV. Regulation of the level of nucleotides, *in vivo*, by adenylate kinase: theoretical and experimental study. Biochim Biophys Acta 162: 230–242
7. Brown EG 1965 Changes in the free nucleotide and nucleoside pattern of pea seeds in relation to germination. Biochem J 95: 509–514
8. Chapman AG, L Fall, DE Atkinson 1971 Adenylate energy charge in *Escherichia coli* during growth and starvation. J Bacteriol 108: 1072–1086
9. Ching TM 1973 Adenosine triphosphate content and seed vigor. Plant Physiol 51: 400–402
10. Ching TM 1975 Temperature regulation of germination in crimson clover seeds. Plant Physiol 56: 768–771
11. Grzelczak Z, J Buchowicz 1975 Purine and pyrimidine bases and nucleosides of germinating *Triticum aestivum* seeds. Phytochemistry 14: 329–331
12. Marcus A, D Efron, DP Weeks 1974 The wheat embryo cell-free system. Methods Enzymol 30: 749–754
13. Marcus A, SN Seal, DP Weeks 1974 Protein chain initiation in wheat embryo. Methods Enzymol 30: 94–101
14. Moreland DE, GG Hussey, CR Shriner, FS Farmer 1974 Adenosine phosphates in germinating radish (*Raphanus sativus* L.) seeds. Plant Physiol 54: 560–563
15. Obendorf RL, A Marcus 1974 Rapid increase in adenosine 5′-triphosphate during early wheat embryo germination. Plant Physiol 53: 779–781
16. Price CE, AW Murray 1969 Purine metabolism in germinating wheat embryos. Biochem J 115: 129–133
17. Reyes P 1972 PEI-cellulose thin-layer chromatography: a highly versatile system for separating purine and pyrimidine nucleotides from nucleosides and free bases. Anal Biochem 50: 35–39
18. Roberts BE, PI Payne, DJ Osborne 1973 Protein synthesis and the viability of rye grains. Loss of activity of protein-synthesizing system *in vitro* associated with a loss of viability. Biochem J 131: 275–286
19. St John JB 1970 Determination of ATP in *Chlorella* with the luciferin-luciferase enzyme system. Anal Biochem 37: 409–416
20. Van Onckelen HA, R Verbeek, AA Khan 1974 Relationship of ribonucleic acid metabolism in embryo and aleurone to α-amylase synthesis in barley. Plant Physiol 53: 562–568
21. Walter TJ, RJ Mans 1975 A rapid technique for the estimation of polynucleotide adenylyl transferase and ribonucleic acid polymerase in plant tissues. Plant Physiol 56: 821–825

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