Plant Physiol. (1980) 65, 1062–1066
0032-0889/80/65/1062/05/$00.50/0

Role of Hydration State and Thiol-Disulfide Status in the Control of Thermal Stability and Protein Synthesis in Wheat Embryo[1]

Received for publication September 4, 1979 and in revised form December 26, 1979

ROBERT C. FAHEY, DEENA L. DI STEFANO, G. PATRICK MEIER, AND [2]ROBERT N. BRYAN
Departments of Chemistry and [2]Biology, University of California, San Diego, La Jolla, California 92093

ABSTRACT

Reduced (GSH), oxidized (GSSG), and protein-bound (PSSG) glutathione were determined in dry and hydrated wheat embryos. Dry embryos contained about 0.6 μmoles per gram dry weight each of GSSG and PSSG, and these levels declined 5- to 10-fold within minutes after the onset of imbibition. GSH declined from about 8 to 2 μmoles per gram over a period of 90 minutes. Similar changes occurred when embryos were hydrated by storage at 100% relative humidity. The decline in glutathione levels was not reversed upon redrying hydrated embryos. About 40% of the cysteine residues of embryo protein was found to be in the disulfide form in both dry and imbibed embryos. The ability of wheat embryos to withstand heat shock was shown to correlate with water content but not GSSG content. Incorporation of [^{35}S]methionine into protein was studied using a system based upon wheat embryo extract (S23). Incorporation rate was found to be sensitive to the nature of thiol added to the system and to be decreased by GSSG. S23 exhibited a substantial capacity to reduce GSSG and preparation of S23 having a GSSG content comparable to dry embryos required addition of large amounts of GSSG to the extraction buffer. S23 prepared in this fashion exhibited a marked decrease in ability to support protein synthesis. These results suggest that the early decrease in GSSG during germination is necessary for optimal protein synthesis in wheat embryo.

This is one of a series of studies undertaken to identify biological systems in which changes in the glutathione thiol-disulfide status (9) might be of potential importance in regulating biological activity. In an earlier study (6) we demonstrated that GSSG[3] levels are higher in the asexual spores (conidia) than in the vegetative cells of the fungus *Neurospora crassa* and that this difference disappears during the first minutes of conidial germination. Parallel changes occur in the level of PSSG, suggesting that reversible conversion of protein thiol groups to disulfide forms might be involved as part of a control process. The initial objective of the present studies was to ascertain whether similar changes in glutathione thiol-disulfide status occur during germination of seed embryos. The results obtained with wheat and barley embryos proved generally similar to those obtained with *N. crassa* conidia.

What role do such thiol-disulfide changes play in seed embryos? Since disulfide proteins generally exhibit greater thermal stability than proteins lacking disulfide bonds (12) one possible role would be stabilization of proteins through conversion to disulfide forms in the inactive or dormant state. However, dehydration can also confer thermal stability upon proteins. The second objective in the present study was to differentiate the effects of dehydration and thiol-disulfide changes upon thermal stability in seed embryos.

Another possibility is that thiol-disulfide reactions are involved in the control of protein synthesis. It has been shown that GSSG inhibits the initiation of protein synthesis in cell-free extracts from rabbit reticulocytes (4, 10). Wheat embryos lack polysomes in the dry, quiescent state but polysomes appear shortly after initiation of germination (15) as has also been found for *N. crassa* conidia (17). Endogenous ribosome activity is largely lost if embryos are desiccated after the early stages of imbibition but is regained upon reimbibition (3). It has been established that protein synthesis which occurs during the early stages of germination uses preexisting mRNA but the mechanism regulating the translation of the message has not been established (2–17). Indirect evidence presented by Weeks and Marcus (18) implicates a sulfhydryl protein present in a partially purified message fraction as potentially important to template activity. It seemed to us that thiol-disulfide reactions of such a protein with GSSG might be involved in the control of protein synthesis. The third objective of the present studies was to test this possibility through studies of the effect of GSSG upon *in vitro* incorporation using the wheat embryo system.

MATERIALS AND METHODS

Materials. All inorganic chemicals were of reagent or higher grade. Unless specified otherwise all organic chemicals and biochemicals were from Calbiochem. Wheat (*Triticum aestivum* L. var. Cajeme) and barley (*Hordeum sativum* L.) were obtained from the Desert Seed Company, El Centro, California, and Turkey wheat, a hard red winter type, was supplied by the Desert Supply Company, Redlands, California. The latter wheat variety was used except as indicated.

Preparation and Treatment of Embryos. Embryos were prepared according to the procedure of Johnston and Stern (8). Viability was determined from extent of germination in samples of 75–125 embryos after incubation in Petri dishes at room temperature for 4 days on filter papers saturated with a solution of 1% (w/v) glucose and 0.01% (w/v) streptomycin. Embryos were imbibed in Petri dishes on filter paper saturated with distilled H_2O. Mixtures of H_2SO_4-H_2O as specified in the International Critical Tables were utilized to establish varying RH in closed glass containers and embryos were exposed as a monolayer on a glass surface suspended above the humidifying solution. Thermal shock treatments were conducted in 3-ml test tubes containing about 50 embryos/tube. The open tubes were immersed in a preheated sand bath contained in an oven at 100 C for 30 min after which they were removed and cooled in water.

Determination of H_2O, GSH, GSSG, and PSSG. Water content of embryos was estimated by determining the weight loss upon heating for 1 h at 80 C; heating for longer periods or at higher temperatures was found to give only 1–3% lower values for the apparent water content. Embryos were weighed and then extracted

[1] Supported by United States Public Health Service Grants GM 22122 and NS 10111.

[3] Abbreviations: GSSG: oxidized glutathione; PSSG: protein-bound glutathione; GSH: reduced glutathione.

as previously described (6) except that extractions were conducted in Duall tissue grinders (Kontes) with thorough homogenization. Assay of GSH, GSSG, and PSSG was accomplished using a cycling enzyme assay (6). Internal standardization of each sample established the absence of factors interfering with the assay. Results are expressed in terms of dry weight as determined from the weight and water content of the embryo sample.

Determination of Protein Thiol and Disulfide Content. Total protein thiol plus half-disulfide groups were determined on approximately 25-mg samples of embryos. The embryos were mixed with two parts Bio-Rad AG 7 neutral Alumina and 40 μl extraction buffer, comprised of 0.5% (w/v) SDS (Pierce) in 50 mM phosphate buffer (pH 7.5) containing 2.5 mM EDTA, in a Duall tissue grinder (Kontes) and thoroughly homogenized. After preliminary grinding, 1.5 ml extraction buffer preheated to 90 C was added and the mixture heated at 90 C for 2 min with continued homogenization. Insoluble material was pelleted in a clinical centrifuge and washed with two 0.5-ml portions of extraction buffer. The volume of the combined supernatants was recorded and a 1-ml portion removed. To this was added 30 μl 100 mM DTT and the mixture allowed to stand 4 h under N_2. The mixture was dialyzed under N_2 three times against 100 ml deoxygenated 0.5% SDS in 5 mM phosphate (pH 7.5), the first and the last dialyses being for 2 h and the second overnight. After the last dialysis the volume was measured and the sample immediately titrated (5) with 5,5'dithiobis(2-nitrobenzoic acid), a sample of the last dialysate serving as a blank sample. For disulfide determination the procedure was identical except that 40 μl 100 mM *N*-ethylmaleimide was added prior to grinding and 40 μl 100 mM DTT was used for reduction prior to dialysis. Titration of the final samples for protein by the method of Lowry (13) showed that the protein present amounted to 34 ± 4% of the original sample dry weight.

For determination of Tris-soluble and Tris-insoluble protein thiol and disulfide values the embryos were first homogenized at 20 C in 2 ml of 25 mM Tris buffer (pH 7.5), containing 3 mM $MgCl_2$ and 50 mM KCl for determination of protein thiol plus half-disulfide values. For determination of protein half-disulfide values 40 μl 100 mM *N*-ethylmaleimide was added to the Tris buffer. After centrifugation SDS extraction buffer was added to the pellet and the supernatant was made 0.5% (w/v) in SDS. Both were then treated as described for the extract in the preceding paragraph.

Amino Acid Incorporation Studies. The system described by Marcus *et al.* (14) was employed using either L-[4,5-^{3}H]leucine (New England Nuclear), 30–50 Ci/mol or L-[^{35}S]methionine (New England Nuclear), 0.5 Ci/mmol, and 100 μl total volumes in each experiment. Specially prepared S23 was substituted for standard S23 containing GSSG, the standard procedure was modified by incorporation of GSSG into the homogenization buffer and conducting the dialysis in two stages, first for 30 min and second for 1 h, against buffer with 2-mercaptoethanol omitted and GSSG added.

Several different lots of rabbit globin mRNA (Miles Laboratories) were utilized in 1- to 2-μg quantities/experiment. Crude wheat embryo mRNA was prepared by the procedure of Weeks and Marcus (18) except that DTT was omitted from the buffer for resuspension of the 23,000*g* pellet as it had little effect upon the activity of the preparation; 20 μl of the preparation was used in each experiment. Rabbit reticulocyte mRNA was prepared by a modification of the method of Lingrel (11). After reticulocytes were lysed, debris was removed by centrifugation and the supernatant was adjusted to pH 5 with acetic acid. The resulting precipitate was collected by centrifugation and dissolved in binding buffer for oligo(dT)-cellulose affinity chromatography isolation of mRNA (1). Preparations of S23 and mRNA were frozen and stored at −70 C until used. Cajeme wheat embryos were used for all wheat preparations.

Measurements of GSH and GSSG were made on S23 preparations and on complete incorporation systems prepared with unlabeled amino acid. For determination of total glutathione (GSH plus GSSG) samples were removed and quenched by dilution in 0.1 mM HCl. For determination of GSSG samples were diluted in 100 mM phosphate (pH 7.5) containing 5 mM each of ethylenediamine tetraacetate and *N*-ethylmaleimide. After 2 min at 30 C the sample was made 10 mM in 2-mercaptoethanol to react excess *N*-ethylmaleimide. The samples were stored on ice until assayed. The procedure was checked with standard samples of GSH and GSSG.

RESULTS

Glutathione Levels in Dry and Imbibed Embryos. The GSH, GSSG, and PSSG levels in several preparations and two different varieties of wheat embryos were determined in the dry state and after imbibing for varying periods (Fig. 1). Considerable variability was found in the GSH content and values tended to decline during storage of the embryos. Embryos having values greater than 2 μmol/g dry weight exhibited a decrease in GSH content during imbibition, the value dropping to 2 μmol/g dry weight during the 1st h and then remaining constant. The total soluble thiol content was determined in some experiments and found to be comparable to the GSH content in dry embryos having high GSH content. In embryos that had lower GSH content as the result of imbibing or storage the soluble thiol level exceeded the GSH level by as much as 100%. In contrast to the slow change in GSH level, the GSSG and PSSG levels exhibited a sharp decrease during the first 10 min of imbibition (Fig. 1).

One variety of barley embryo was also examined to test the generality of the results with wheat embryo. The sample examined exhibited a GSH level at the lower end of the range found for wheat embryo and this did not drop during imbibition. The GSSG

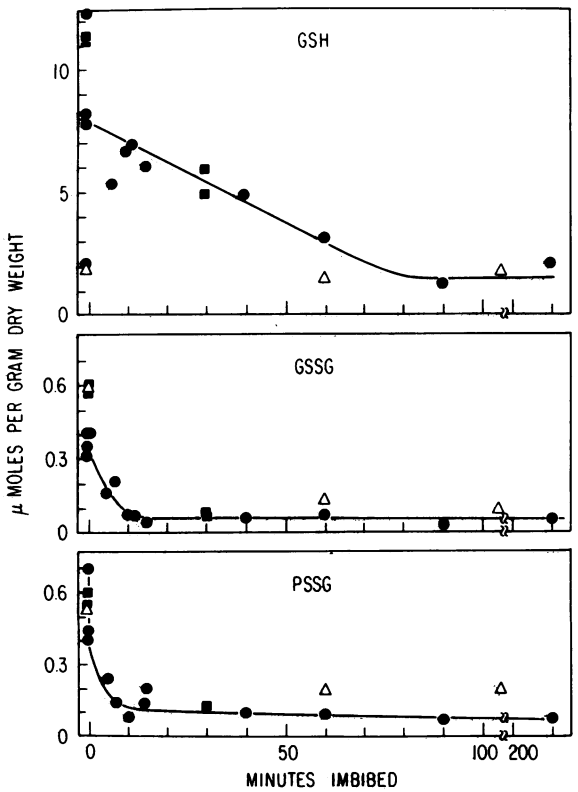

FIG. 1. Effect of germination upon GSH, GSSG, and PSSG levels of seed embryos. (●): Turkey wheat embryos; (■): Cajeme wheat embryos; (△): barley embryos.

and PSSG levels were slightly higher but decreased during imbibition in a fashion analogous to that found for wheat embryo (Fig. 1).

Redrying of Imbibed Embryos. The water content and glutathione content was determined for embryos imbibed for 1 h, and then air dried for various periods after removal of excess moisture by suction filtration. The results (Table I) show that most of the water gained during imbibition was lost within the first 6 h. This is not accompanied by a return of the GSSG and the PSSG levels to the values for untreated embryos, although some increase in GSSG and PSSG values appears to occur over longer periods for embryos imbibed 1 h.

Effects of Varying Relative Humidity. Freshly harvested embryos contained 10–13% water and this level was maintained during storage in closed containers. Such embryos exhibited substantial resistance to heating at 100 C and this appeared to be enhanced when the water content was lowered by storage at 0% RH (Table II). Storage at 100% RH, with resulting increase in water content, led to a complete loss of thermal resistance but this loss could be reversed by redrying of the embryos. Hydrating the embryos at 100% RH results in changes similar to those occurring during imbibition, *i.e.* a marked decrease in GSSG level and lesser declines in GSH and PSSG levels. As with imbibed embryos these changes were not reversed upon redrying. Storage at intermediate RH for 5 days resulted in intermediate values of water content. Significant GSH loss was encountered under all conditions but GSSG and PSSG levels declined only at the higher RH.

Embryos maintained under 100% RH remained viable and slowly increased their water content, a value of 55% being reached after 7 days. The longer embryos were maintained at 100% RH the more rapidly they germinated when transferred to germination medium and after 7 days they began to germinate within the humidity chamber itself.

Protein Thiol-Disulfide Status. The thiol and disulfide content of embryo protein was examined in order to determine whether marked changes occur during the 1st h of germination. For this purpose extracts of whole embryos were prepared in hot SDS to solubilize the protein. Alternatively, embryos were first extracted with Tris buffer and the resulting soluble and insoluble fractions treated with hot SDS so that differences between soluble and insoluble protein fractions could be identified. The results (Table III) show that a substantial fraction (40%) of the protein cyst(e)ine residues are present in the disulfide form and that this disulfide content does not change significantly during the 1st h of germination. The fraction of groups in the disulfide form is greater in the Tris-insoluble fraction (67%) than in the Tris soluble fraction (27%).

Effect of Thiols and Disulfides upon the Wheat Embryo Amino Acid Incorporation System. A series of studies using the amino acid incorporation system derived from wheat embryo with several different sources of mRNA indicated a complex dependence of this system upon the thiols and disulfides present. The standard incorporation system contains 2 mM DTT (16). Since DTT rapidly reduces GSSG it was necessary to replace DTT with GSH in order to test the effects of GSSG upon the system. Replacement of DTT by GSH caused a marked decrease in the rate of incorporation with rabbit globin mRNA (Fig. 2). A further decrease occurred upon addition of 0.5 mM GSSG. The results presented in Figure 2 are illustrative of the incorporation *versus* time plots obtained, an initial lag period being generally observed and the incorporation rate declining after 60 min. Further results are summarized here in terms of the net incorporation at 60 min. A given experimental series (simultaneous experiments using the same source of materials) is identified by an experiment number. Experiments having different numbers cannot be directly compared since different mRNA and S23 preparations may be involved, and different amounts of radiolabeled amino acid were sometimes used.

The effects of thiols and GSSG were further examined, including studies employing crude wheat embryo mRNA, as summarized in Table IV. Lower incorporation is observed with GSH than with DTT using wheat mRNA (experiment 1c *versus* 1d). Sensitivity of the wheat mRNA preparation to exogenous thiol was explored by addition of DTT to the extraction buffer used to prepare the

Table I. *Effect of Air Drying upon Imbibed Turkey Wheat Embryos*

Treatment	Water Content	Content of		
		GSH	GSSG	PSSG
	% wet wt	*μmol/g dry wt*		
Untreated	10	5	0.5	0.4
Imbibed 1 h and dried 0 h	69	2.7	0.07	0.14
6 h	11	1.6	0.07	0.18
3 days	12	0.8	0.16	0.14
7 days	12	0.6	0.15	0.23
11 days	11	0.3	0.12	0.22
Imbibed 2 h and dried 0 h	65	1.2	0.05	0.10
6 h	12	1.0	0.06	0.13
11 days	ND[a]	0.2	0.05	0.13

[a] ND: not determined.

Table II. *Effect of RH during Storage upon Thermal Resistance and Glutathione Levels of Wheat Embryos*

Treatment	Water Content	Viability		Content of		
		Unheated	Heated[a]	GSH	GSSG	PSSG
	% wet wt	*% germination*		*μmol/g dry wt*		
Untreated	10	90 ± 10	50 ± 10	2.1	0.35	0.4
24 h at 0% RH	3	95 ± 5	95 ± 5	ND[b]	ND[b]	ND
24 h at 100% RH	36	95 ± 5	<5	0.8	0.05	0.2
24 h at 100% RH then 24 h at 0% RH	3	85 ± 10	65 ± 10	1.0	0.03	0.16
5 days at 61% RH	13	ND	ND	0.3	0.4	0.5
5 days at 81% RH	16	ND	ND	0.4	0.2	0.4
5 days at 91% RH	24	ND	ND	0.3	0.03	0.14

[a] Embryos heated 30 min at 100 C.
[b] ND: not determined.

Table III. *Protein Thiol and Disulfide Content of Wheat Embryos*

Component[a]	Time Imbibed (h)	
	0	1
μmol/g dry wt		
Thiol plus half disulfide		
total	22	24
Tris-soluble	15	ND[b]
Tris-insoluble	9	ND
Half disulfide		
total[c]	9	10
Tris-soluble[c]	4	ND
Tris-insoluble[c]	6	ND
Thiol		
total	13	15
Tris-soluble	11	ND
Tris-insoluble	3	ND

[a] Uncertainty is ±2 except as noted.
[b] ND: not determined.
[c] Uncertainty is ±1.

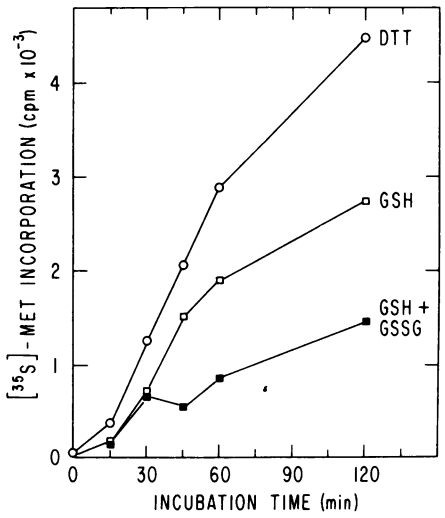

FIG. 2. Representative incorporation *versus* time curves with rabbit globin mRNA and the wheat embryo incorporation system. (DTT): standard system containing 2 mM DTT; (GSH): DTT replaced by 2 mM GSH; (GSH + GSSG): DTT replaced by 2 mM GSH and 0.5 mM GSSG.

Table IV. *[35S]Methionine Incorporation with Rabbit Globin (RG) and Crude Wheat Embryo (WE) mRNA Preparations*

Experiment No.	mRNA	Variations in Assay System	Incorporation
			cpm
1 a	RG	2 mM DDT	24,100
b		2 mM GSH	15,400
c	WE	2 mM DDT	9,300
d		2 mM GSH	6,360
e	WE-DTT extracted[a]	2 mM DTT	9,320
f		2 mM GSH	4,770
2 a	RG	preincubate[b]; add 1 mM GSH plus 1 mM DTT	4,030
b		preincubate[b] with 0.5 mM GSSG; add 1.5 mM DTT	0
3 a	RG	preincubate[b]; add 1 mM GSH plus 1 mM DTT	1,590
b	WE-DTT extracted[a]	preincubate[b]; add 1 mM GSH plus 1 mM DTT	1,370
c		preincubate[b] with 0.5 mM GSSG; add 1.5 mM DTT	485
4 a	RG	2 mM GSH	5,130
b		2 mM GSH; S23 prepared with GSSG[c, d]	0
c	WE-GSSG extracted[c]	2 mM GSH	2,500
d		2 mM GSH; S23 prepared with GSSG[c, d]	0

[a] Three mM DTT added to the extraction buffer.

[b] Preincubation of all components except thiol and mRNA for 5 min at 30 C after which thiol and mRNA were added.

[c] Three mM GSSG added to extraction buffer.

[d] Three and 0.3 mM GSSG in first and second dialysis buffers, respectively. Final S23 contained 4 mM GSH and 0.4 mM GSSG.

message. No effect was observed when the resulting message was assayed in a system containing DTT (experiment 1e *versus* 1c) and at best a minor effect was seen when measured with GSH as the thiol (experiment 1f *versus* 1d). The effect of preincubation with GSSG upon the system was also examined. With rabbit globin mRNA a complete loss of ability to support incorporation resulted (experiment 2a *versus* 2b) whereas with wheat mRNA only a partial loss occurred (experiment 3b *versus* 3c).

Assays of the initial extracts made in the preparation of crude wheat mRNA and S23 showed that the GSSG content was low (< 0.01 mM). This suggested that the extraction process was activating the system for reduction of GSSG. This was confirmed by adding exogenous GSSG and demonstrating its conversion to GSH. To test whether such reduction of GSSG is essential to obtain active amino acid incorporation, sufficient GSSG was added to the extraction buffer to overwhelm the reducing system and maintain a high GSSG level. The resulting extract was then dialyzed *versus* buffer containing GSSG in order to maintain this level. Tests of the incorporating ability of the S23 fraction prepared in this fashion showed that it lacked activity when measured with rabbit globin mRNA (experiment 4a *versus* 4b). Wheat embryo mRNA prepared in extraction buffer containing GSSG and assayed with normal S23 exhibited typical activity compared to rabbit globin mRNA (experiment 4c *versus* 4a) but no activity when measured in S23 prepared from buffer containing high GSSG concentrations (experiment 4d).

A series of experiments analogous to those of Table IV was conducted using the total mRNA from rabbit reticulocyte. Qualitatively similar results were obtained. Thus, the sensitivity of S23 and of the final incorporation system to GSSG does not appear to depend upon the source or extent of purification of the mRNA used.

DISCUSSION

In the earlier study of *N. crassa* conidia (7) a three state model was advanced to simplify analysis of hydration state effects and this model is useful in considering the present results. The three states were defined as: (a) "dry" state (<5% water content, attained at <10% RH, only tightly bound water present); (*b*) "semidry" state (5–15% water content, attained at intermediate RH, only bound water present); and (*c*) "wet" state (40–70% water content, attained in water or at high RH, bound plus bulk water present). Freshly prepared wheat embryos are in a semidry state and are converted to a dry state upon desiccation or to a wet state upon imbibing or exposure to 100% RH (Tables I and II).

The present results exclude thiol-disulfide interconversion as a major, general mechanism for stabilization of proteins toward heat in dry and semidry embryos. Thermal resistance was not found to be associated with GSSG or PSSG content (Table II) and a generalized conversion of protein from disulfide to thiol form was not observed to accompany hydration (Table III). The results show that dry and semidry embryos exhibit thermal resistance whereas wet embryos do not. Similar results were obtained with *N. crassa* conidia and were attributed to the documented effects of dehydration in stabilizing macromolecules toward heat denaturation (7). Thus, it seems that dehydration suffices as a general mechanism for stabilizing macromolecules in the dormant or quiescent state and that operation of other mechanisms, such as thiol-disulfide interconversion, is restricted to a limited number of proteins at best.

The changes in glutathione levels observed to accompany hydration of wheat embryo are similar in some respects to the changes found with *N. crassa* conidia, but important differences exist. Whereas GSSG and PSSG levels in freshly prepared conidia and embryos are similar, and both decline upon hydration, the disulfide levels of conidia become elevated again during redrying whereas those of embryos did not. Also, the slow decline in GSH level found with embryos did not occur in conidia (6). For conidia it could be demonstrated that loss of GSSG is accompanied by a corresponding increase in GSH implying that glutathione reductase activity is present in hydrated conidia (7). This was not possible with wheat embryo, although glutathione reductase activity could be demonstrated in extracts of wheat embryo. The reason

for the large net loss in glutathione from embryos during imbibition is not clear but may reflect utilization of glutathione as a pool of cysteine needed in protein synthesis. Alternatively, GSH may be degraded or converted to another form, thus explaining the finding that the ethanol-soluble thiol level often exceeded the GSH level by as much as 100% and suggesting that some low mol wt thiol other than GSH may be important in germination.

The finding that a large fraction of the cyst(e)ine residues of embryo protein is in the disulfide form but that this undergoes no major change during germination demonstrates that extensive conversion of disulfides to thiols is not involved in control functions during germination. Changes affecting a limited number of thiol or disulfide groups would not have been detected so that selective thiol-disulfide control is not excluded by these results.

The postulated involvement of GSSG in the control of protein synthesis seemed compatible with this conclusion and was therefore tested in *in vitro* studies. Two difficulties were encountered which greatly complicated these studies. First, although GSSG declines during hydration, the process is not reversible, *i.e.* restoration of GSSG to the original level does not occur upon redrying (Table I). Thus, if protein synthesis is inhibited in fresh, semidry embryos by elevated GSSG then a different phenomenon, perhaps simply the decrease in water activity, must be responsible for inactivating protein synthesis when imbibed embryos are redried. This irreversibility limits studies of the relationship between GSSG and protein synthesis to the activation process. The second experimental difficulty stems from the fact that hydration, whether promoted by high humidity, imbibing, or preparation of aqueous extracts, activates the system which reduces GSSG and PSSG. This reduction system must be blocked or, as in the present studies, exhausted with added GSSG if extracts containing disulfide levels comparable to the dry embryo are to be prepared, but this necessarily perturbs other metabolite levels.

Keeping these difficulties in mind we turn to the results of the incorporation studies. When message fraction and S23 obtained from wheat embryo in the presence of added GSSG were tested (experiment 4d) no incorporation was obtained. Incorporation is obtained when GSSG-treated message fraction is used with normal S23 (experiment 4c) so that any GSSG sensitive, essential protein in this fraction must be reducible by the thiol present in the final incorporating system. These results do not support the view that the sulfhydryl-sensitive protein found (17) associated with wheat message can be influenced by GSSG in a way that effects translational activity. The inactivating effect of GSSG upon S23 could result from: (*a*) blocking the necessary reduction of one or more essential proteins; (*b*) inactivation of essential proteins by oxidation in the presence of elevated GSSG levels; and (*c*) secondary effects resulting from perturbed levels of NADPH, other cofactors, and substrates produced by the excess GSSG.

In conclusion, we find that wheat embryo is similar to *N. crassa* conidia with regard to changes in the glutathione thiol-disulfide status during germination, although some differences were found. The results exclude thiol-disulfide interconversion as a general mechanism for inactivation of enzymes or for stabilization of proteins toward heat in dry and semidry embryos. They are consistent with the view that most enzymes are inactivated as a result of the restricted molecular diffusion which accompanies dehydration and that most proteins can be stabilized toward thermal denaturation as a direct consequence of the absence of bulk water in the dry and semidry states (7). The results do not exclude limited involvement of thiol-disulfide control mechanisms and are compatible with control of protein synthesis by GSSG providing that the site of such control resides in the soluble fraction (S23) rather than the mRNA fraction obtained from wheat embryo.

Acknowledgments—We thank Drs. S. Brody, M. Chrispeels, E. M. Kosower, N. S. Kosower, A. Marcus, and H. Stern for helpful advice and discussion.

LITERATURE CITED

1. BANTLE JA, IH MAXWELL, WE HAHN 1976 Specificity of oligo(dt)-cellulose chromatography in the isolation of polyadenylated RNA. Anal Biochem 72: 413–427
2. BROOKER JD, M TOMASZEWSKI, A MARCUS 1978 Preformed messenger RNAs and early wheat embryo germination. Plant Physiol 61: 145–149
3. CHEN D, S SARID, E KATCHALSKI 1968 The role of water stress in the inactivation of messenger RNA of germinating wheat embryos. Proc Nat Acad Sci USA 61: 1378–1383
4. CLEMENS MJ, B SAFER, WC MERRIC, WF ANDERSON, IM LONDON 1975 Inhibition of protein synthesis in rabbit reticulocyte lysates by double-stranded RNA and oxidized glutathione: indirect mode of action on polypeptide chain initiation. Proc Nat Acad Sci USA 72: 1286–1290
5. ELLMAN GL 1959 Tissue sulfhydryl groups. Arch Biochem Biophys 82: 70–77
6. FAHEY RC, S BRODY, SD MIKOLAJCZYK 1975 Changes in the glutathione thiol-disulfide status of *Neurospora crassa* conidia during germination and aging. J Bacteriol 121: 144–151
7. FAHEY RC, SD MIKOLAJCZYK, S BRODY 1978 Correlation of enzymatic activity and thermal resistance with hydration state in ungerminated *Neurospora* conidia. J Bacteriol 135: 868–875
8. JOHNSTON FB, H STERN 1957 Mass isolation of viable wheat embryos. Nature 179: 160–161
9. KOSOWER EM, NS KOSOWER 1974 Manifestations of changes in the GSH-GSSG status of biological systems. *In* L Flohe, M Ch Benohr, H Sies, HD Waller, A Wendel, eds, Glutathione. Thieme Verlag, Stuttgart, pp 287–295
10. KOSOWER NS, GA VANDERHOFF, EM KOSOWER 1972 Glutathione VIII. The effects of glutathione disulfide on initiation of protein synthesis. Biochim Biophys Acta 272: 623–637
11. LINGREL JB 1972 Preparation and assay of hemoglobin mRNA. *In* JA Last, AL Laskin, eds, Protein Biosynthesis in Nonbacterial Systems. Marcel Dekker, New York, pp 231–263
12. LIU TY 1977 The rule of sulfur in proteins. *In* H Neurath, RL Hill, eds, The Proteins, Ed III. Academic Press, New York, pp 239–402
13. LOWRY OH, NJ ROSEBROUGH, AL FARR, RJ RANDALL 1951 Protein measurement with the Folin phenol reagent. J Biol Chem 193: 265–275
14. MARCUS A, D EFRON, DP WEEKS 1974 The wheat embryo cell-free system. Methods Enzymol 30: 749–754
15. MARCUS A, J FEELEY, T VOLCANI 1966 Protein synthesis in imbibed seeds. III. Kinetics of amino acid incorporation ribosome activation, and polysome formation. Plant Physiol 41: 1167–1172
16. MIRKES PE 1974 Polysomes, ribonucleic acid, and protein synthesis during germination of *Neurospora crassa* conidia. J Bacteriol 117: 196–202
17. SPIEGEL S, A MARCUS 1975 Polyribosome formation in early wheat embryo germination independent of either transcription or polyadenylation. Nature 256: 228–230
18. WEEKS DP, A MARCUS 1971 Preformed messenger of quiescent wheat embryos. Biochim Biophys Acta 232: 671–684