SULFHYDRL STATUS AND THE GERMINATION OF NEUROSPORA CRASSA CONIDIA

D. V. Richmond and E. Somers

Long Ashton Research Station,
University of Bristol, England

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Plant and animal cells have the ability to maintain thiols - particularly glutathione (GSH) - in the reduced state and there is evidence that the protein SH - disulfide transformation has an important function in cell division (Mazia, 1959; Stern, 1959). The germination of pea seeds is characterized by a rapid reduction of GSSG to GSH with a concomitant increase of protein SH (Spragg et al., 1962) and Mortenson & Beinert (1953) showed that the SH content of bacteria increased during the early log phase of growth. Changes in the SH status of fungal spores on germination have not previously been investigated and we have found the SH content of Neurospora crassa to increase on germination at the same rate as the dry weight and nitrogen content. The conidia can rapidly reduce the disulfide dithiodiglycol giving an increase in the intracellular soluble SH content but this treatment had little effect on the subsequent germination of the spores.

MATERIALS AND METHODS

Conidia of N. crassa, macroconidial wild-type Em 5297a, were harvested and washed from 7 day cultures then incubated with the reagents at 25° using the techniques previously described (Richmond & Somers, 1962, 1966). Spore germination was induced by incubation in Fries medium at 30° (Ryan, 1948; Richmond & Somers, 1966). Dry weights were determined by heating the washed conidia at 102° to constant weight: total nitrogen was determined by the micro-Kjeldahl method.

The SH content of the spores, on a fresh weight basis, was determined by amperometric titration with silver nitrate in ammonia-ammonium nitrate-ethanol

(Richmond & Somers, 1966). Soluble thiols were extracted by immersing the spores in boiling water for 1 min.

RESULTS AND DISCUSSION

The changes in total SH content and dry weight of washed N. crassa spores after incubation in Fries medium are shown in Fig. 1. In the early stages of germination dry weight increased more rapidly than SH but after 10% germination the SH/dry wt. ratio remained constant: the percentage increase in total nitrogen (not shown) followed the dry weight curve very closely. Batches of N. crassa examined had different SH contents ranging from 6.4 to 12.0 μ moles/g. yet in no instance was the rate of spore germination affected by the initial SH level.

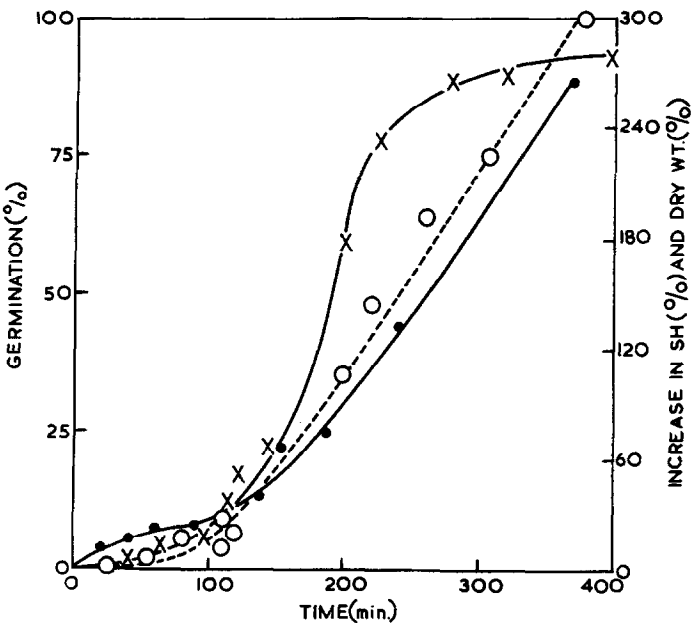

Fig. 1. Increase in total SH content and dry weight of Neurospora crassa conidia (20 million/ml) on germination in Fries medium at 30°. X - X, germination; O —— O, SH content; ● - ●, dry weight.

Eldjarn et al., (1962) have shown that intact erythrocytes can reduce low molecular weight disulfides probably via exchange reactions with intracellular GSH. With yeast cells, however, Maw (1963) found that L-cystine does not enter the cell and L-cysteine penetrates only very slowly. In fact the slow intra-

cellular reduction of disulfides is often due to a low rate of penetration into the cell (Skrede et al., 1965). This effect is illustrated in Table 1: GSH in the oxidized or reduced form has a negligible influence on the SH content of the spores. Dithiodiglycol (DTDG), however, is rapidly reduced by N.crassa - presumably to 2-mercaptoethanol, perhaps catalyzed by GSSG reductase (Eldjarn et al., 1962). The resulting increase in intracellular SH content occurs almost wholly in the soluble thiol pool (Table 2) and there was no evidence of DTDG reduction in the ambient solution; repeated washing of DTDG - treated spores did not alter their SH content. The ability of N. crassa to accumulate and reduce DTDG requires aerobic conditions. There was no change in the SH status of the spores when the incubation was carried out in an atmosphere of nitrogen. This is in accord with the link proposed by Marre & Arrigoni (1957) between SH reduction and oxidative metabolism.

TABLE 1. Effect of glutathione and disulfides on the SH content of N. crassa conidia

Spores, at 20 million/ml, were incubated with the reagents for various time periods then centrifuged, washed twice and their total SH content determined.

Reagent	Concn (mM)	Time of Incubation (min.)	SH content (μ moles/g) Initial	Final
GSH	1	30	10.6	11.3
	1	105	11.0	11.8
	2	30	10.6	11.5
GSSG	1	30	10.9	11.4
Dithiodiglycol	1	30	10.6	12.7
	1	60	10.1	13.5
	2	30	10.6	14.4
	2	120	10.8	17.7
	2	230	10.8	23.4

TABLE 2 Effect of dithiodiglycol on the intracellular SH of *N. crassa* conidia

Spores, at 40 million/ml, were incubated with 2mM DTDG for 60min. then washed twice before analysis. Control spores were incubated with water for 60 min.

Treatment	SH content (μ moles/g)		
	Soluble	Insoluble	Total
Control	4.6	8.0	12.6
DTDG	15.1	8.4	23.5

These results show no evidence of a SH-oxidizing enzyme on the surface of *N. crassa* conidia as has been reported by Mandels (1956) for *Myrothecium verrucaria* spores.

Although DTDG can increase the intracellular SH content of *N. crassa* when DTDG or GSH, at 1mM, were added to Fries medium the rate of spore germination was unchanged from that of control spores (Fig. 1). Even pre-incubation with DTDG or GSH, at 1mM, did not alter the rate of spore germination. Earlier studies have shown that the alkylating agent iodoacetic acid, acting as the anion, can markedly reduce the soluble SH content of *N. crassa* conidia (Richmond & Somers, 1966). This treatment also reduces the rate of germination of the spores in Fries medium but although the SH content of iodoacetate-treated spores can be increased by incubation with DTDG their germination capacity cannot be restored (Table 3).

Stern (1959) has shown that the GSH concentration must be above a critical level for cell division to occur and Toyoda (1965) found the rate of seed germination to be related to GSH content. Although the germination of *N. crassa* was retarded by the iodoacetate treatment it is apparent that the SH content of these conidia is sufficiently high for germination to proceed even when a large proportion of the SH has been irreversibly alkylated; presumably because of rapid *de novo* synthesis of GSH. Conversely increasing the spore SH content by DTDG

TABLE 3 Effect of dithiodiglycol on the SH content and germination of iodoacetate-treated conidia of N. crassa

Spores, at 20 million/ml, were incubated with 0.3mM iodoacetic acid for 30 min., washed twice, and re-incubated for a further 30 min. in either water or 2mM DTDG and their SH content determined. As a control untreated spores were incubated in water for 30 min. then re-incubated in water or DTDG. Spores from a duplicate set of all treatments were incubated for 4.75hr in Fries medium for germination counts.

First Treatment	Second Treatment			
	Control		DTDG	
	Germination (%)	SH content (μ moles/g)	Germination (%)	SH content (μ moles/g)
Control	70	9.8	58	15.0
Iodoacetic acid	36	7.1	30	9.8

treatment has no effect on a germination process which can operate within a wide SH margin. However, the ability of conidia to rapidly reduce a permeable disulfide may aid the design of new fungicides if this reduction occurs more rapidly in the fungal spore than in the host plant.

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