Acta Biologica Hungarica 66(2), pp. 242–245 (2015)
DOI: 10.1556/018.66.2015.2.10

DEGRADATION OF GLUTATHIONE IN *ASPERGILLUS NIDULANS*

SHORT COMMUNICATION

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(Received: July 28, 2014; accepted: September 15, 2014)

Relative transcriptions of *Aspergillus nidulans dug1-3* (orthologes of *Saccharomyces cerevisiae* DUG – deficient in utilization of glutathione – pathway genes) and *ggtA* encoding γ-glutamyl transpeptidase were studied under conditions inducing glutathione degradation. G*gtA* was induced in all cases when glutathione levels decreased, but addition of yeast extract, which moderated glutathione degradation, enhanced its induction. Although *dug2* showed constitutive transcription, *dug1* and *dug3* were induced by carbon and nitrogen starvation and yeast extract did not caused significant changes in their relative transcription. The *in silico* reconstructed DUG pathway of *A. nidulans* is a promising candidate for cytosolic GSH degradation induced by carbon/nitrogen stress.

Keywords: Glutathione degradation – γ-glutamyl transpeptidase – DUG pathway – carbon stress – *Aspergillus nidulans*

Glutathione (GSH, γ-L-glutamyl-L-cysteinyl-glycine) is a γ-glutamyl type, low molecular weight thiol occurring in almost all eukaryotic cells. GSH contributes not only to different stress responses (*e.g.* oxidative, heat and osmotic stress, desiccation, starvations) but as an essential compound it is also important in maintenance of membrane integrity, cell differentiation and development [8]. Understanding the GSH metabolism (including its degradation) is crucial either if we want to produce GSH by microbes or when we want to modify the physiology (*e.g.* stress tolerance) and/or morphology of industrial strains. Originally, γ-glutamyl transpeptidase (γGT) was described as the only enzyme responsible for degradation of GSH [8]. Recent developments in this field demonstrate that GSH can be degraded by different biochemical pathways. In mammalian cells, the ChaC γ-glutamyl cyclotransferase represents the cytosolic way of GSH degradation [6], while in *Saccharomyces cerevisiae,* the DUG (deficient in utilization of glutathione) system degrades cytosolic GSH [4]. The gene *dug1* encodes a Cys-Gly metallo-di-peptidase, while *dug2* and *dug3* encode the two subunits of a glutamine amidotransferase complex [4]. In contrast, γGT degrades

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extracellular and vacuolar GSH, GSH S-conjugates, oxidized glutathione or other γ-glutamyl compounds not related to GSH in several species [1–3, 5, 7–9]. Here, we present data on the relative transcription of *Aspergillus nidulans dug1-3* orthologes (as well as of certain other genes potentially involved in the hydrolysis of γ-glutamyl bound) under culturing conditions inducing GSH degradation.

GSH degradation was induced by carbon starvation (carbon free cultures), carbon limitation (lactose containing cultures) as well as nitrogen starvation (nitrogen free cultures) and GSH degradation was moderated by addition of yeast extract to the cultures (Table 1). Relative transcription of the following genes was tested: AN3459, AN1879, AN1092 *(dug1-3),* orthologes of *S. cerevisiae* dug genes (*Aspergillus* Genome database; http://www.aspergillusgenome.org); AN10444 *(ggtA)* encoding γGT; AN5658 encoding a γ-glutamyl transpeptidase domain containing protein; AN4809 *(gtaA)* encoding glutaminase A; AN3150 *(ggcsA)* encoding the key enzyme of GSH synthesis.

Among the DUG orthologes, *dug2* showed constitutive transcription. In contrast, *dug1* and *dug3* were induced by carbon and nitrogen starvation, *dug1* was also induced by carbon limitation, and yeast extract did not cause significant changes in their relative transcription (Table 1). Moreover, good negative correlation was found between the GSH content and the relative transcription of *dug1* or *dug3* (Table 1). Relative transcription of *ggtA* was induced in all cases when GSH degradation was observed, but surprisingly yeast extract even enhanced its induction (Table 1). Our preliminary results with loss-of-function *ggtA* mutants also suggest that gGT is not necessary for bulk degradation of GSH in carbon stressed cultures. The relative transcription of AN5658 did not change significantly (Table 1). *GtaA* encoding an extracellular glutaminase A was induced by carbon and nitrogen starvation, and addition of yeast extract to carbon starved cultures decreased its relative transcription (Table 1). AN3150 *(ggcsA)* was induced by yeast extract only. This induction could be important in the moderate GSH degradation observed in these cultures, but does not explain GSH degradation in carbon starved, carbon limited and nitrogen starved cultures.

According to these data we assume that γGT of *A. nidulans* may have versatile physiological functions but not necessary for cytosolic degradation of GSH like in several other species [2, 4, 6, 7). In contrast, the *in silico* reconstructed DUG pathway of *A. nidulans* is a promising candidate for degradation of cytosolic GSH.

Table 1
Effect of culturing conditions on intracellular GSH content and relative transcription of certain genes in *Aspergillus nidulans*

Culturing conditions	GSH (nmol/mg)	Relative transcription (DCP)						
		AN3459 *(dug1)*	AN1879 *(dug2)*	AN1092 *(dug3)*	AN10444 *(ggtA)*	AN5658	AN4809 *(gtaA)*	AN3150 *(ggcsA)*
20 g/l glucose, 6 g/l $NaNO_3$ (control)	7.6 ± 0.9	–2.1 ± 0.8	–4.2 ± 1	–8.2 ± 1.2	2.5 ± 0.5	–5.2 ± 1.2	–4.6 ± 0.8	3.7 ± 0.6
20 g/l lactose, 6 g/l $NaNO_3$	5.1 ± 0.5[a]	1.6 ± 0.8[a]	–4.1 ± 1	–6.9 ± 1.2	4.8 ± 0.6[a] *	–5.1 ± 1	–4.1 ± 0.8	3.5 ± 0.5
20 g/l lactose, 4 g/l yeast extract, 6 g/l $NaNO_3$	6.5 ± 0.8[b]	1.7 ± 0.8[a]	–4.5 ± 0.8	–8.3 ± 1.8	6.1 ± 0.8[a,b]	–5.2 ± 1	–4.2 ± 0.5	4.4 ± 0.6[b]
Carbon free, 6 g/l $NaNO_3$	4.5 ± 0.5[a]	2.2 ± 0.5[a]	–4.7 ± 0.8	–5.1 ± 1.5[a]	5.2 ± 0.8[a]	–3.6 ± 1	–2.1 ± 0.5[a]	3.6 ± 0.5
4 g/l yeast extract, 6 g/l $NaNO_3$	6.4 ± 0.8[b]	2.3 ± 0.5[a]	–4.6 ± 1	–7.2 ± 1.5	6.7 ± 0.8[a,b]	–4.2 ± 1	–3.1 ± 0.6[a,b]	4.6 ± 0.6[b]
20 g/l glucose, nitrogen free	6.2 ± 0.8[a]	1.2 ± 0.8[a]	–4.4 ± 1	–5.8 ± 1[a]	3.8 ± 0.7[a]	–5.1 ± 0.8	–2.5 ± 0.6[a]	3.7 ± 0.5
Correlation coefficient (relative transcription vs. GSH)	Not relevant	–0.74[d]	0.19	–0.79[d]	–0.39	–0.35	–0.52	0.41

A. nidulans tNJ36 was grown up in complex medium and the 18 h mycelia were washed and transferred into minimal media containing carbon and nitrogen sources described in the table [10]. Samples were taken at 8 h (relative transcription) and at 24 h (GSH content) after transferring. GSH content was measured as described earlier [10] and was given in nmol GSH/mg dry cell mass. (GSH content continuously decreased up to 110 h.) Relative transcription was characterized by DCP. $DCP = CP_{housekeeping\ gene} - CP_{tested\ gene}$ [10].
CP values stand for the qRT-PCR cycle numbers of crossing points. The AN6542 gene *(actA)* was used as "housekeeping" gene. Figures represent mean ± S.D. values calculated from 4 independent experiments.
[a]Significant difference in compare to the control cultures (Student's *t*-test, $p < 5\%$, $n = 3$).
[b]Significant difference between the yeast extract containing and the appropriate yeast extract free cultures (Student's *t*-test, $p < 5\%$, $n = 3$).
[c]Pearson's correlation coefficients calculated between relative transcription and GSH concentration using the original data sets are presented.
[d]Correlation coefficient significantly differs from zero ($p < 1\%$, $n = 18$).

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