SOD, P and C values in normal rats; effect of chronic H_2O_2 drinking

Organs	SOD Units/g w.t.wt Controls	SOD H_2O_2-treated	P Units/g w.t.wt Controls	P H_2O_2-treated	C Units/g w.t.wt Controls	C H_2O_2-treated
Liver	4000 ± 600	10,408 ± 750	0	0	4.80 ± 0.43	25.250 ± 2.455
Kidney	1120 ± 151	1692 ± 240	361 ± 33	1200 ± 105	0.36 ± 0.03	3.300 ± 0.295
Spleen	560 ± 50	470 ± 240	963 ± 91	4250 ± 418	2.40 ± 0.21	0.730 ± 0.062
Testes	960 ± 63	1100 ± 53	407 ± 41	–	0.41 ± 0.03	0.150 ± 0.011
Whole brain	240 ± 24	397 ± 38	120 ± 10	140 ± 13	0.04 ± 0.004	0.012 ± 0.001
Lung	210 ± 20	895 ± 85	872 ± 86	2020 ± 201	0.26 ± 0.02	0.520 ± 0.050
Pancreas	310 ± 31	352 ± 33	136 ± 13	480 ± 46	0.195 ± 0.010	0.255 ± 0.023
Muscles						
Heart	480 ± 47	950 ± 93	2690 ± 300	1615 ± 155	0.245 ± 0.023	0.725 ± 0.065
Skeletal	300 ± 27	530 ± 50	105 ± 10	750 ± 69	0.11 ± 0.009	0.092 ± 0.009
Haemolysate*	696 ± 68	787 ± 80	11,666 ± 1000	17,900 ± 1340	4.12 ± 0.04	6.98 ± 0.07

* Units/ml. W.t.wt, wet tissue weight; n = 10; C-activity-values are given in B.units/g w.t.wt. All values are X ± SD.

The first 2 columns compare the SOD activities in control rats and rats which have been drinking H_2O_2 solution for 2 months.

The data reveal that, with the exception of the spleen, drinking H_2O_2 solution increases the SOD activity to a considerable extent. For example, this increase is more than 250% of the control value in the liver.

The second 2 series of columns compare the tissue peroxidase activity changes. The P activities are also increased, with the one exception of heart muscle.

However, the largest activity changes are observed in catalase as shown in the table. For instance, after prolonged H_2O_2 intake, the C-activity increase is about 6 times the control value in liver, and more than 9 times in kidney. In contrast, the C-activity decreases in spleen (to about $^1/_3$), testis, brain and skeletal muscle.
In general, therefore, it can be said that prolonged intake H_2O_2 solution brings about an induction of the PME in most organs. However, no explanation can be given of why catalase in spleen, testis, brain tissue and skeletal muscle do not fit this general picture.

Variation of glutamate decarboxylase activity and γ-amino butyric acid content of wheat embryos during ripening of seeds[1]

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Summary. GAD activity and γ-ABA content of wheat embryos at 7 ripening stages were verified with the aim of studying the metabolic activity of embryo during dehydration and quiescence of caryopsis. Data showed that in the early stage of ripening GAD activity is very low, increases rapidly at dough-stage, remaining constant up to waxy-stage, and decreases in the last fully-ripe embryos. γ-ABA content appears to be roughly parallel to the variations of GAD activity.

Studies on the metabolism of γ-aminobutyric acid (γ-ABA) in plants demonstrated that this amino acid is formed from L-glutamic acid by way of L-glutamate-1-carboxylyase, EC 4.1.1.15 (GAD). γ-ABA, in turn, enters the dicarboxylic acid cycle as succinic acid, after the action of the 2 succeeding enzymes γ-aminobutyrate-α-ketoglutarate aminotransferase (EC 2.6.1.19) and succinic semialdehyde dehydrogenase (EC 1.2.1.16)[3,4]. Some data are also available on the variations of GAD activity and γ-ABA content in plants[5–7]. A parallel increase of both were reported to occur in leaves during senescence[8–10]. In the present paper we report the variations of GAD activity and of γ-ABA content occurring in wheat embryos during the development of the seed. The aim was to study some of the metabolic processes which occur during the ripening of wheat caryopsis and to obtain preliminary information correlating these processes with the onset of seed quiescence that is associated with the cessation of mitotic activity and with the increasing dehydration during ripening.

Materials and methods. In the period from May to June 1976, seeds of durum wheat c.v. Cappelli at different stages of ripening were collected and their relative humidity was measured with a hygrometric balance (Ultrax-Simplex). Embryos (embryo axis plus scutellum) were isolated from ice-cold seeds, sterilized with 1% NaClO,

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GAD activity with or without PLP, γ-ABA content of embryos and percent relative humidity of seeds from ripening durum wheat

Ripening stages of seeds	Relative humidity (%)	GAD activity (μl CO_2/min/mg protein) − PLP	+ PLP	γ-ABA (μg/g dry wt)
Milky-ripe	75.5	4.85 ± 0.927	5.02 ± 0.433	125.93 ± 0.20
Mealy-ripe	68.5	9.25 ± 0.694	9.95 ± 0.759	170.52 ± 0.04
Mealy-dough-ripe	63.0	13.41 ± 0.592	14.25 ± 0.427	180.28 ± 1.35
Dough-ripe	50.5	13.13 ± 0.096	14.72 ± 0.014	178.18 ± 0.14
Dough-waxy-ripe	38.5	13.32 ± 0.315	15.16 ± 0.041	177.97 ± 0.77
Waxy-ripe	35.5	13.98 ± 0.221	14.55 ± 0.108	179.92 ± 1.10
Fully-ripe	11.5	12.03 ± 0.150	13.60 ± 0.176	146.80 ± 0.60

Values are means ± SD.

and their GAD activity and γ-ABA content measured. GAD was extracted as described in a previous paper[11] and the enzyme activity was measured in the presence or absence of 10^{-4} M pyridoxal-5'-phosphate (PLP). γ-ABA determination was carried out on protein-free extracts prepared as follows: batches of 200 embryos were boiled in 80% ethanol, homogenized with twice their weight of alumina and extracted for 2 h in the cold room (2–3 °C) with 80% ethanol acidified to pH 3 with glacial acetic acid. After centrifugation of the extract (about 2 ml), glacial acetic acid (30 drops) was added to the supernatant and the precipitate so formed spun down by a second centrifugation. The supernatant was dried with a rotatory evaporator, solubilized with about 0.1 ml of 10% isopropanol acidified with 37% hydrochloric acid (1 drop per 100 ml) and then analyzed by thin layer chromatography. 2dimensional chromatography was effected using the following systems: first dimension, 1-butanol/acetic acid/water (80:20:20); second dimension, ethanol 70%/water (70:30). Quantitative determinations of γ-ABA, extracted from ninhydrin-staining spots with 1.2 ml of acetone/glacial acetic acid (8:2), were performed spectrophotometrically at 505 nm using a calibration curve obtained with standard γ-ABA solutions. Total protein was measured by biuret method[12].

Results and discussion. The table summarizes the values of GAD activities and γ-ABA levels of wheat embryos at the different stages of ripening, as defined by morphological aspects[13] and by the percent relative humidity of the seeds. The GAD activity had a very low level in milky-ripe seeds and increased significantly in mealy-ripe and dough-ripe embryos, remaining constant up to the waxy-ripe stage of ripening. Finally, it decreased significantly in fully-ripe embryos reaching values close to those observed in after-ripe non-dormant embryos[14]. The addition of PLP to the embryo extracts enhanced the GAD activity at all stages of ripening but the relative changes in activity during ripening appeared unchanged. The variations of γ-ABA content closely paralleled the changes of GAD activity at most ripening stages. However, significantly lower values of γ-ABA were observed at both the beginning and the end of seed ripening. The variations of GAD activities during the ripening of wheat seeds could be explained in at least 3 ways: a) inhibition by nucleotides; b) interconversion between forms with different activities; c) differences in the rates of synthesis and breakdown of the enzyme molecule. It appears that the first hypothesis could be ruled out on the basis of the ATP values measured in developing wheat caryopsis[15].

Our data on GAD levels in dry seeds and the variation of GAD activity during early germination are more compatible with the hypothesis that the increase in enzyme level results from 'de novo' synthesis[11]. Moreover, preliminary data on the RNA content of wheat seeds during the ripening period indicated a marked increase of total RNA at the early phases of the process (Grilli et al., unpublished data).

On the other hand, the drop in GAD activity during the last stage of ripening could be explained by breakdown of the enzyme molecule as a consequence of an enhancement of proteolytic activities under waterstress conditions[16]. In this context, the occurrence, in the last stages of wheat ripening, of endogenous inhibitors of germination[17] which might contribute to the quiescence of seeds in relation to the increasing dehydration, could determine the reduction of GAD activity. Alternatively, the presence of less stable enzyme forms has been proposed[18].

The changes of γ-ABA content during the ripening could be directly linked to the parallel variation of GAD activity. It should be considered, though, that the γ-ABA level is likely to be the resultant of its rate of production by GAD and its utilization through the 'γ-ABA-shunt'[3,4].

Whatever the mechanism regulating the γ-ABA concentration may be, it is interesting that the highest level of γ-ABA is observed during the slowing down of the growth processes related to seed dehydration. Similar observations were reported for aging leaves of legouminous plants[19] and for ripening mango fruits[20]. The exciting hypothesis could be raised that γ-ABA (which does not enter protein synthesis) could play a regulatory role in determining protein turnover rates. Attempts are in process to provide experimental support for this hypothesis.

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