On the Location of Glutamate Decarboxylase in the Caryopsis of Hard Wheat (*Triticum durum*) and its Activity during Early Germination

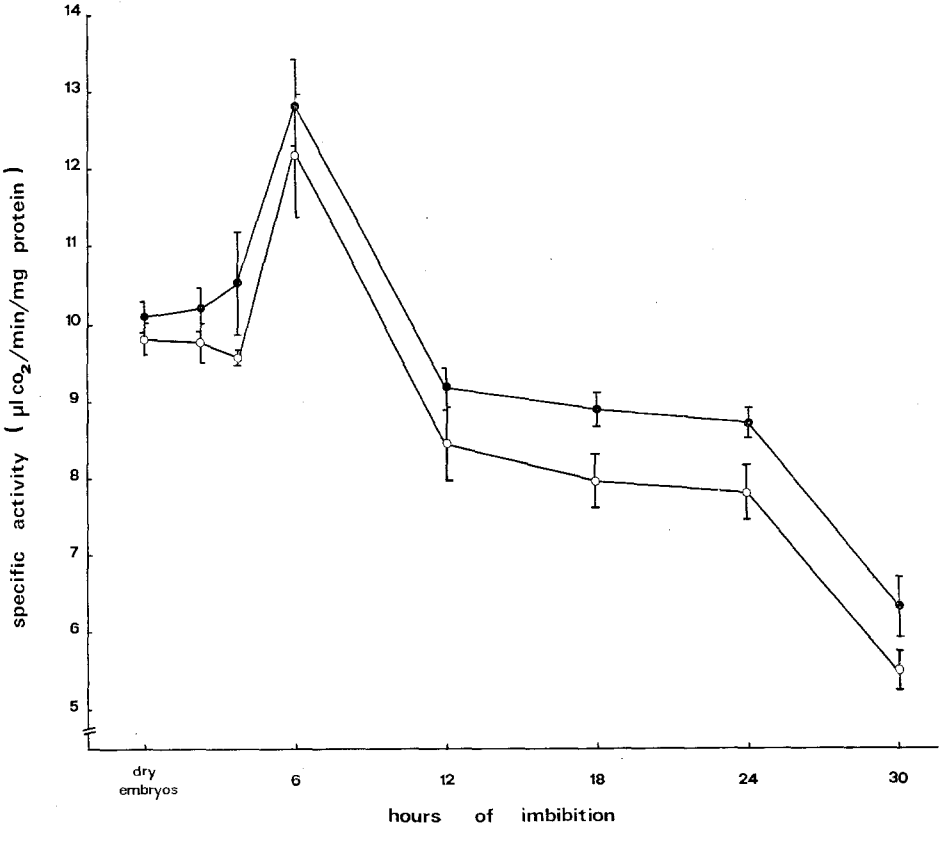
The presence of glutamate decarboxylase (L-glutamate 1-carboxylyase, EC 4.1.1.15) (GAD) in higher plants was first reported by OKUNUKI[1] and then detected in a number of plants[2–4]. However, no detailed information on the structural and functional properties of the plant enzyme is so far available, and its physiological role, as well as that of the better known bacterial enzyme, has not been elucidated[5].

High GAD activity was reported in wheat seeds[6], which appear appropriate material for purification of the enzyme and for studies on its function during plant development. We report here the location of GAD in the caryopsis of hard wheat and variations of its activity during germination. The furtherance of these studies is expected to contribute both to the knowledge of enzymatic processes occurring during the different phases of seed life-span, and to the elucidation of the role of GAD reaction in plant metabolism.

Materials and methods. Hard wheat, *Triticum durum* cv. Cappelli, harvest 1972, (18-month-old seeds, with 75% germination after 48 h) was employed. When the seeds were operated in dry state, whole embryo, embryo axis, scutellum and endosperm were isolated, following the general techniques previously described[7]. For experiments on germinating seeds, the seeds were imbibed on water-moistened filter paper for stated times under aerated conditions in the dark, then the whole embryos (axis plus scutellum) were isolated. All seed constituents were then processed as follows. The seed parts were ground separately in a mortar with double volume of alumina. The powder obtained was suspended with 0.2 *M* sodium acetate buffer pH 4.0 (1:5, w:v) and allowed to extract for 3 h at 4 °C with stirring. The solid particles were then removed by centrifugation. The protein concentration of the extracts was determined by the biuret method[8], after precipitation with trichloroacetic acid. GAD activity of extracts was evaluated manometrically, by measuring the CO_2 released from L-glutamate. The standard incubation mixture contained 2.0 ml 0.05 *M* pyridine HCl buffer pH 5.3, 0.3 ml of extract, 0.1 ml pyridoxal-5′-phosphate (PLP) solution (when required) and H_2O to the final volume of 3.0 ml in the main compartment of the Warburg vessel, and 0.3 ml 0.05 *M* sodium L-glutamate in the side arm. After temperature equilibration, the reaction was started and manometric readings were taken at 5 min intervals, at 37 °C, with air as gas phase.

Results and discussion. The comparative GAD activities of the constituents of the wheat caryopsis, isolated at dry state, are reported in the Table. Since the isolated

[1] K. OKUNUKI, Bot. Mag., Tokyo *51*, 270 (1937).
[2] O. SCHALES and S. S. SCHALES, Meth. Enzym. *2*, 190 (1955).
[3] B. M. GUIRARD and E. E. SNELL, Compreh. Biochem. *15*, 174 (1964).
[4] E. A. BOEKER and E. E. SNELL, Enzymes *6*, 217 (1972).
[5] I. COZZANI, Acta vitam., Milano *27*, 191 (1973).
[6] Y. Y. CHENG, P. LINKO and M. MILNER, Suomen Kemist. *31*, 333 (1958).
[7] P. MELETTI, G. Bot. ital. *66*, 438 (1959).
[8] T. E. WEICHSELBAUM, Am. J. clin. Pathol. *10*, 40 (1946).



GAD specific activities of extracts from dry embryos and from embryos at different germination times. Each point is the mean value (± SEM) of 6 independent assays, run either in the presence (●) or in the absence (○) of 10^{-4} *M* PLP.

endosperm is devoid of this enzyme activity and the embryo axis contains much higher enzyme level than the scutellum, it can be concluded that GAD of dry seeds is located in dry embryos. Consistent results were reported for GAD of barley seeds[9]. The GAD activities of embryos were then determined at different times of seed germination, both in the absence and in the presence of PLP. As shown in the Figure, during the first 3 h of germination, the enzyme activity maintains values close to those of the dry embryos. A significant increase is observed after 6 h of germination, when the GAD specific activity attains its maximum value. Then the enzyme levels progressively decrease during the following germination time. PLP enhances significantly the GAD activity throughout all the germination time, with higher effect in the late period. Although this activation cannot directly account for the variations of GAD activity during germination, as observed in the Figure, the possibility could be raised of a control of GAD level through the regulation of synthesis and breakdown of the apoenzyme, that was shown in mammalian organs to undergo preferential attack by group specific proteases[10]. An alternative or additional regulation mechanism, explaining the variations of GAD activity during the seed life-span, could be provided by the concentration of nucleotides and Pi. In fact, partially purified preparations of GAD from wheat embryos were found to be sensitive to 'physiologic' concentrations of these metabolites[11]. A deeper insight into these problems seems to be conditioned by further purification and characterization of the plant GAD, as well as by further investigations on amino acid and nucleotide metabolism in plants[12].

Riassunto. La glutammico decarbossilasi è localizzata nell'embrione di semi secchi di *Triticum durum*, mentre l'endosperma ne risulta praticamente privo. Durante la germinazione si ha un aumento dell'attività specifica GAD alla sesta ora di imbibizione, seguita da una lenta e progressiva diminuzione per le seguenti 24 ore. L'enzima è attivato dal piridossal-5'-fosfato, ma le variazioni di attività GAD durante la germinazione non sembrano dipendere direttamente dalla concentrazione del coenzima.

L. Galleschi, C. Floris, P. Meletti and I. Cozzani[13]

Institutes of Botany and Biochemistry, University of Pisa, Via Luca Ghini 5, I-56100 Pisa (Italy), 16 July 1974.

Location of GAD activity in the isolated dry constituents of the caryopsis of *Triticum durum*

Material	GAD specific activity (μl CO_2/min/mg protein)	Relative percentages
Embryo	9.8	100.0
Embryo axis	7.3	74.4
Scutellum	2.6	26.7
Endosperm	Not detectable	—

[9] K. Inatomi and J. C. Slaughter, J. exp. Bot. *22*, 561 (1971).
[10] N. Katunuma, E. Kominami, S. Kominami and K. Kito, Adv. Enzym. Reg. *10*, 289 (1972).
[11] I. Cozzani, F. Sgarrella and L. Galleschi, Boll. Soc. ital. Biol. sper. *48*, 20 (1972).
[12] This work was supported by a grant from the Consiglio Nazionale delle Ricerche.
[13] Institute of Biochemistry, University of Pisa, Italy.

Effects of DHEA or its Sulfoconjugates upon c-AMP Phosphodiesterase

Only recently the interrelationship between DHEA (dehydroepiandrosterone, 3β-hydroxy-5-androsten-17-one), G-6-PDH (glucose-6-phosphate dehydrogenase, EC 1.1.1.49) and c-AMP (cyclic adenosine-3',5'-monophosphate) under physiological conditions has been reported[1]. According to these investigations, decreased plasma levels of sulfoconjugated DHEA may be at least in part responsible for the elevated activity of red blood cell G-6-PDH in psoriasis, hyperlipoproteinemia, or the menopausal syndrome, as well as for lowered concentrations of c-AMP in plasma or erythrocytes. In order to substantiate the influence of DHEA or its sulfoconjugates upon the concentrations of c-AMP, the erythrocyte-rich corpuscular fraction from blood of normal subjects was incubated with DHEA, its sulfate or sulfatide (dipalmitoyl glycerosulfate) and the intracellular content of c-AMP determined. Furthermore, the hydrolysis of 8-^{14}C-c-AMP in protein fractions from hemolysates in the presence of DHEA as well as the components of the G-6-PDH system was studied.

Material and methods. The corpuscular, erythrocyte-rich fraction from normal subjects – hence-forth called 'erythrocytes' – was prepared as previously described[2]. Duplicate samples, consisting of 3.0 ml of the 'erythrocyte' suspension in 0.9% sodium chloride/0.025% EDTA and 0.02 ml dioxane with 7α-^{3}H-DHEA, its sulfate, or synthetic sulfatide were incubated for 30 min at 37 °C, the final concentration of steroid substrate corresponding to a 10^{-6} to 10^{-4} M solution. The 'erythrocytes' were washed, hemolyzed, centrifuged, and the amount of intracellular substrate measured by its ^{3}H-activity. In the second series of experiments, the labelled substrates were replaced by corresponding concentrations of non-labelled compounds and the concentration of intracellular c-AMP determined by the protein-binding assay of Gilman[3].

In addition, hemolysates from washed 'erythrocytes' were submitted to fractionate ammonium sulfate precipitation and the protein fractions, obtained at 25%, 50%, and 75% saturation, incubated with 8-^{14}C-c-AMP in the absence or presence of DHEA and the components of the G-6-PDH system as indicated in the Table. The incubates were extracted with chloroform-methanol, the aqueous methanolic extracts lyophilized, and the residues submitted to thin layer chromatography on PEI-cellulose in 0.25 M lithium chloride for separation of

[1] G. W. Oertel, P. Benes, M. Schirazi, H. Holzmann and G. Hoffmann, Experientia *30*, 872 (1974).
[2] G. Hoffmann, B. Morsches, U. Döhler, H. Holzmann and G. W. Oertel, Arch. Derm. Sypb. *243*, 18 (1972).
[3] A. G. Gilman, Proc. natn. Acad. Sci. *67*, 305 (1970).