ne synthetase from rat brain is highly sensitive to heavy metal ions. Cu^{2+}, Cd^{2+}, Hg^{2+} and Zn^{2+} completely inhibited the enzymatic synthesis of carnosine and homocarnosine. This characteristic property resembles that of sulfhydryl enzymes, e.g. brain L-glutamate decarboxylase[12]. 0.1 mM of Li^{+}, Ni^{2+} and Ca^{2+} also inhibited the enzyme but to a lesser extent.

Among the CNS agents tested at 1 mM, norepinephrine, α-methyl DOPA and chlorpromazine appeared to inhibit the enzyme approximately 50% while hydroxylamine, epinephrine, dopamine and DOPA had no significant effect. In our earlier study[13], the enzyme was prepared in the absence of dithioerythritol and we found that 1.5 mM of hydroxylamine, α-methyl DOPA, dopamine, norepinephrine and epinephrine inhibited the enzyme activity 44% or greater while DOPA had no effect. The inhibition by hydroxylamine, epinephrine and dopamine observed previously may be involved with the availability of free sulfhydryl groups on the enzyme since the enzyme activity is very labile in the absence of a sulfhydryl reagent. Chlorpromazine, although stimulatory at low concentration (10^{-4} M) in earlier study, inhibited the enzyme 45% at 10^{-3} M. These contradictory effects have also been reported in other enzyme systems, e.g., the mitochondrial ATPase is stimulated by chlorpromazine below 1×10^{-4} M but is inhibited by chlorpromazine above 1×10^{-4} M[14]. Our present finding that chlorpromazine is capable of inhibiting the synthetase in vitro may explain the in vivo finding by MARSHALL[10] that chlorpromazine lowered the levels of carnosine and homocarnosine in rat brain but had no effect on the level of histidine.

[12] J.-Y. WU and E. ROBERTS, J. Neurochem. *23*, 759 (1974).
[13] R. H. NG and F. D. MARSHALL, Trans. Am. Soc. Neurochem. *5*, 170 (1974).
[14] H. LÖW, Biochim. biophys. Acta *32*, 11 (1959).

Auxin Dependence and Auxin Oxidase of Cultured Sycamore Cells

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Summary. Quantitative and qualitative differences in auxin-oxidases extracted from auxin-dependent (S) or auxin-independent (MB) sycamore cells were analyzed. MB auxin-oxidases have a higher activity, but the molecular weight of this enzymatic complex is lowered by freeze-drying, without loss of the activity. Correlations with auxin-independence are discussed in this context.

Cultured sycamore tissues are represented by 2 strains, an auxin-independent phenotypic variant (MB)[1] and the original auxin-dependent strain (S)[2]. This auxin-independence might be at least explained by 2 hypotheses: 1. the amount of 3-indolyl-acetic acid (IAA) synthesized via tryptophan is higher in MB than in S; 2. the inactivation of IAA by enzyme systems is lower in MB than in S. The first explanation was in fact not true; as previously demonstrated[3], both strains producing a similar level of IAA after incubation with labelled tryptophan. The aim of the present paper was to test the last hypothesis, analyzing the amount of IAA destroyed in vitro by extracts of tissues from both strains (S and MB).

Preliminary experiments indicated that crude extracts did not destroy IAA, even when cofactors such as $MnCl_2$ and 2,4-dichlorophenol[4,5] were added. The tissues probably contained endogenous inhibitors liberated during

[1] A. M. LESCURE and C. PEAUD-LENOËL, C. r. Acad. Sci., Paris *265*, 1803 (1967).
[2] D. T. A. LAMPORT and D. H. NORTHCOTE, Nature, Lond. *188*, 665 (1960).
[3] A. M. LESCURE, Soc. bot. fr. Mémoires *1970*, 353 (1970).
[4] P. E. PILET, Experientia *13*, 35 (1957).
[5] R. FOX and W. K. PURVES, Plant Physiol. *43*, 454 (1968).

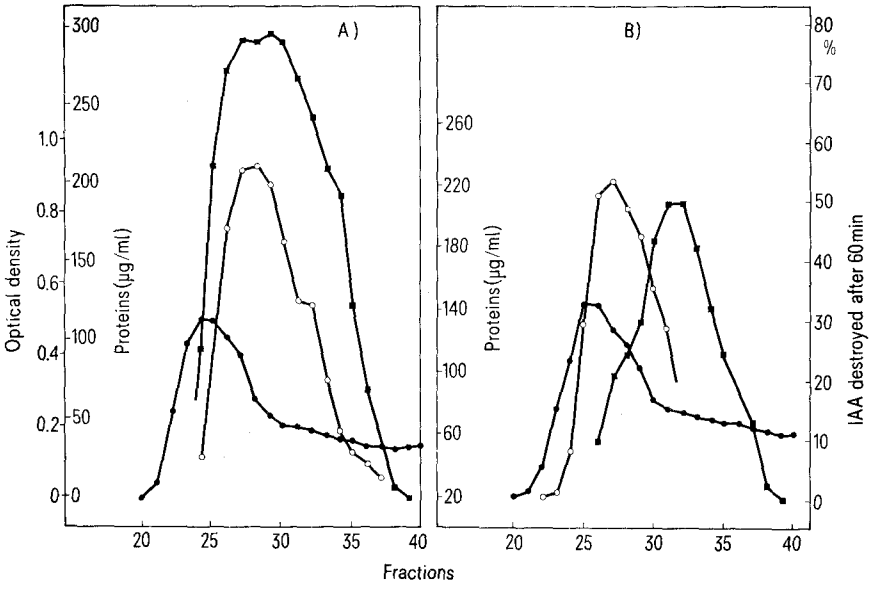

Fig. 1. Elution patterns of G 25 prepurified extracts from 5 g fresh (A) or 500 mg freeze-dried (B) strain MB tissues on Sephadex G 100. Fraction volume: 4.2 ml. Elution speed: 24 ml/h, temp.: 4 °C ± 0.5. Open circles, proteins; plain circles, optical density; squares, IAA destroyed.

homogenization[6]. Therefore, extracts were purified by gel filtration on Sephadex columns. Explants were grown on synthetic agar medium[7] containing (S) or not (MB) 1 mg of 2,4-dichlorophenoxyacetic acid/l. Suspension cultures were grown on identical medium, without agar. Homogenization was conducted from 5 g FW (or 500 mg DW) at 1 °C in *Tris*-EDTA buffer pH 8.1 by means of a glass beads homogenizator[8] during 3 min after a pre-homogenization in a Potter. Mercaptoethanol (0.57 μmoles) and Na disulfite (1.26 mmoles) were added to reduce oxidative reactions. Glass beads were eliminated by sieving and 50 mmoles/ml of $CaCl_2$ were added to eliminate pectins. The extract was then centrifuged at +1 °C for 30 min at $40{,}000 \times g$.

The supernatant was collected and placed on a 30×2.5 cm column of Sephadex G 25. Elution was made by borax-phosphate buffer 5 m*M*, pH 8.2[9]. The first peak of proteins was immediately refiltered on a G 100 column under the same conditions. Each fraction was assayed for protein content by the Folin technique[10] and IAA-oxidase activity[11] by the modified Salkowski reagent[12], with 10^{-4} *M* $MnCl_2$ and 10^{-5} *M* *p*-coumaric acid.

IAA-oxidase activity of Sephadex G 100 purified extracts from freeze-dried S and MB tissues

	Strains	
	S	MB
Maximal activity of IAA destroyed per h/μg protein of the same fraction (%)	21.2	43.8
Mean IAA-ox activity/mean protein content (μg)	12.3	23.1
Mean IAA-ox activity/total protein content of the whole extract (μg)	1.5	2.6

IAA-ox, IAA-oxidase

IAA oxidase was first compared for S and MB. As can be seen in the Table, the auxin-independent strain (MB) possesses a higher activity. Consequently, the second hypothesis cannot account for the independence of MB. It has to be noticed that, on different auxin-independent materials such as crown-galls or habituated cultures, this fact was also reported[13–15]. Then, the elution diagrams of MB extracts obtained from fresh (Figure 1 A) or freeze-dried (Figure 1 B) material were analyzed. When fresh tissues were extracted, the peaks of maximal IAA-oxidase activity and maximal protein content were eluted simultaneously. By contrast, when freeze-dried tissues were analyzed, the 2 peaks were distinct. This phenomenon did not occur for S, as indicated in Figure 2. From its elution pattern, it was possible to determine the molecular weight (MW) of IAA-oxidase[16]. When strain MB was freeze-dried and stored, the MW of auxin-oxidase (11,400 ± 3,000) was lowered to the half of its value in fresh material (27,000 ± 5,000). During the freeze-drying procedure, a degradation of the enzymatic protein took place, without inducing a loss of the activity. Such a degradation did not occur when S was freeze-dried (MW: 33,800 ± 5,000). It is well established that many enzymes destroy IAA. Consequently, the activity reported on Figure 1 A might be in fact the summation of the activities of several enzymes. Moreover, the width of the IAA-oxidase peak might indicate that it is a mixture of different proteins, which are not properly separated on Sephadex G 100. During freeze-drying, some high MW enzymes of the IAA-oxidase complex could be altered. The smaller IAA-oxidase peak which can be noticed in Figure 1 B might be due to enzymes – not destroyed by freeze-drying – with a lower MW. The MW values presented for intact IAA-oxidase complex correspond to those found for lentil root peroxidase[17] and polyphenol-oxidase[18]. Therefore, it can be supposed that these two enzymes are destroyed by freeze-drying, in *Acer* cultures. For a similar material, an allosteric type of enzyme regulation was postulated[19], in relation to the possible existence of enzyme subunits. Our present data do not indicate any direct correlation between IAA-oxidase activity and the auxin independence.

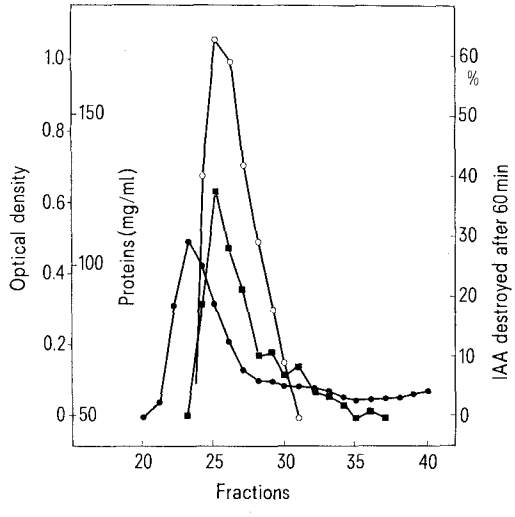

Fig. 2. Elution pattern of a G 25 prepurified extract from 500 mg freeze-dried strain S tissues on Sephadex G 100. See Figure 1.

[6] W. D. LOOMIS and J. BATAILLE, Phytochemistry *5*, 423 (1966).
[7] R. STUART and H. E. STREET, J. exp. Bot. *20*, 556 (1969).
[8] W. ZILLIG and H. HOLZEL, Hoppe-Seylers Z. physiol. Chem. *312*, 140 (1958).
[9] P. E. PILET, J. P. ZRYD and P. LAVANCHY, Physiol. vég. *5*, 327 (1967).
[10] O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR and R. J. RANDALL, J. biol. Chem. 193, 265 (1951).
[11] P. E. PILET, Phytochemistry *2*, 617 (1964).
[12] P. E. PILET and R. CHOLLET, C. r. Acad. Sci., Paris *271*, 1675 (1970).
[13] R. S. PLATT, Année Biol. *58*, 361 (1964).
[14] J. LIPETZ and A. W. GALSTON, Am. J. Bot. *46*, 193 (1959).
[15] E. I. KOVACZ and P. MALIGA, Acta bot. hung. *18*, 315 (1973).
[16] H. DETERMANN, *Chromatographie sur gel* (Ed. Masson, Paris 1969), p. 104.
[17] P. LAVANCHY, Thèse Faculté des Sciences, Université de Lausanne (1970).
[18] S. SEVHONKIAN, Thèse Faculté des Sciences, Université de Lausanne (1972).
[19] A. M. LESCURE, Bull. Soc. Chim. biol., Paris *52*, 953 (1970).