Phytochemistry, 1975. Vol. 14, pp. 99–101. Pergamon Press. Printed in England.

PURIFICATION AND PROPERTIES OF A POLYAMINE OXIDASE FROM *ZEA MAYS*

EIJI HIRASAWA* and YONEZO SUZUKI

Laboratory of Plant Physiology, Biological Institute, (Bunri-Gakubu), University of Toyama, Toyama, Japan

(*Received* 29 *May* 1974)

Key Word Index—*Zea mays*; Gramineae; maize; spermidine; polyamine oxidase; FAD; *p*-benzoquinone reduction.

Abstract—Polyamine oxidase, purified 260-fold from maize shoots, was light yellow in colour. Maximum light-absorption was at 450 nm and was decreased by the addition of either sodium dithionite or spermidine, but not by putrescine. Under aerobic conditions, the enzyme could use *p*-benzoquinone as an electron acceptor. Cu^{2+} inhibited the enzyme activity, while SO_3^- was stimulatory. Several metal-binding agents and thiol reagents were without effect.

INTRODUCTION

The polyamine oxidase which occurs in the seedling of barley [1] and maize [2] differs from the amine oxidase in pea [3], lupin [4], tobacco [5] and soybean [6]. We have previously reported [2] that a partially purified enzyme from maize shoots requires FAD, and in this paper, we report the purification and further properties of this enzyme.

RESULTS AND DISCUSSION

Purification of maize polyamine oxidase

A summary of the enzyme purification is given in Table 1. The specific activity at the final stage was *ca* 260× that of the original extract (recovery 13·8%). Ammonium sulphate could not be used for the concentration of the enzyme since it causes inactivation [1, 2]. However, polyethylene glycol is an efficient concentrating agent for this enzyme.

Spectrophotometric observations

The absorption spectrum of the purified polyamine oxidase of step 5 (225 μgN/ml) was determined from 350 nm to 600 nm. The enzyme exhibited a maximum at about 450 nm and a minimum at 410 nm (85% of max) but the absorption maximum between 350 nm and 400 nm which is usually observed in flavoprotein enzymes was absent [7]. Under aerobic conditions, the A at 450 nm was decreased by sodium dithionite (× 0·3) or spermidine (× 0·8), but not by putrescine. Addition of dithionite greatly increased A below 400 nm and the peak at 450 nm was lost. The flavin concentration, calculated [8] from E at 450 nm ($1·13 \times 10^4$ cm^2 mol^{-1}), was $5·5 \times 10^{-3}$ μmol per mg protein.

* Present address: Dept. of Agricultural Chemistry, Kyoto University, Kyoto 606, Japan.

Table 1. Purification of maize polyamine oxidase

Step	Fraction	Total vol. (ml)	Units/ml	Nitrogen (mg/ml)	Sp. act. (unit/mgN)	Recovery (%)
1	Crude extract	3060	3·8	0·89	4·3	100
2	Particles	—	6·3	1·49	4·2	—
3	KH_2PO_4(0·5 M) extract	220	11·3	0·43	26·3	21·4
4	Sephadex G-100 column eluates	60	23·6	0·062	380·6	12·2
5	Ca phosphate gel eluates	60	26·7	0·024	1112·5	13·8

A unit of activity is defined as the amount of enzyme which causes 10 μl O_2 uptake or a 0·0212 increase of A at 435 nm (equivalent to the amount of O_2 uptake) in 10 min under standard conditions using the *O*-aminobenzoldehyde assay [2].

Effect of the concentration of FAD on the apoenzyme

FAD but not FMN regenerated the holoenzyme from apoenzyme. Increasing the concentration of FAD up to 5×10^{-3} μmol stimulated holoenzyme formation but the increase stopped abruptly at this point and the enzyme activity became constant.

p-*Benzoquinone reduction by polyamine oxidase*

Maize polyamine oxidase differs from a spermidine dehydrogenase [9] of the bacterium *Serratia marcescens*, which requires FAD and an additional electron carrier, in that the maize enzyme—both aerobically and anaerobically—does not reduce added electron carriers such as 2,6-dichlorophenolindophenol, phenazine methosulphate and ferricyanide. However, we have now found that under aerobic conditions the maize enzyme can reduce *p*-benzoquinone. The coupled reduction of cytochrome *c* or 2,6-dichlorophenolindophenol could be effected by this system. With cytochrome *c* as reductant (see Experimental) A at 550 nm increased from 0·28 to 0·42 in 30 min with spermidine as substrate; activity with spermine was about 50% of that with spermidine. NADH-could not replace spermidine. Enzymatic reduction of *p*-benzoquinone was inhibited 70% by Cu^{2+}. With boiled enzyme or on omitting polyamine substrate no increase in A 550 nm was detected. Similarly with 2,6-dichlorophenolindophenol as reductant the A 600 nm was reduced from 0·23 to 0·15 in 30 min. (see Experimental).

Effect of inhibitors

Massey *et al.* [7] have reported that those flavoprotein enzymes which react readily with oxygen also react readily with SO_3^-. It was therefore of interest to find that maize polyamine oxidase is not inhibited but, on the contrary, is stimulated, showing a 230% increase in activity over the control in 10 min at 20 mM conc of sodium sulphite. Enzyme activity, as measured by oxygen uptake was inhibited about 66% by 0·1 mM $CuSO_4$(2-mercaptoethanol-free preparation was used). However, the activity is completely unaffected by $AgNO_3$ or $CdSO_4$ (each at 0·1 mM). With *p*-hydroxymercuriphenylsulphonate, *N*-ethylamaleimide (each 1 mM) and phenylmercuric acetate (0·5 mM) no inhibition was found either. These results suggest that Cu^{2+} is probably reacting with a flavin component of the enzyme. Preincubation (30°, 5 hr) with metal-binding agents (cyanide, azide, bathocuproine, bathophenanthroline, xanthogenate (each 1 mM), diethyldithiocarbamate (0·1 mM) and cupferron (0·5 mM)) had no effect on the enzyme activity. The additon of quinacrine or rivanol (each at 2 mM) resulted in inhibitions of 49% and 88%, respectively after 20 min of preincubation.

EXPERIMENTAL

Plant material. Maize seedlings (*Zea mays* L. Goldencross Bantam T51) were grown in moistened vermiculite at 25° in the dark for 7–8 days.

Purification of enzyme. 220 ml of partially purified enzyme [2] (step 3 in Table 1) in dialysis tube was concentrated by contact with solid polyethylene glycol (MW = 15000). 20 ml of the conc enzyme soln was applied to a Sephadex G-100 column (3·4 × 65 cm) equilibrated with a buffer containing 0·5 M KH_2PO_4, 1 mM EDTA and 10 mM 2-mercaptoethanol and eluted with the same buffer (flow rate 20 ml/hr; 10 ml fractions). Fractions from tube 23–28 were pooled (60 ml), then diluted on 120 ml with deionized H_2O containing 1 mM EDTA and 10 mM 2-mercaptoethanol. The resulting soln was treated with calcium phosphate gel [10] (dry wt 1·56 g). The suspension was stirred for 10 min and the gel recovered by centrifugation. The enzyme was then eluted from the gel by stirring for 10 min with 60 l of M·KH_2PO_4 soln, containing 1 mM EDTA and 10 mM 2-mercaptoethanol. After centrifugation, the supernatant (*ca* 60 ml) was conc to 15 ml (90 μg N/ml) by using polyethylene glycol as above. Enzyme preparation (70 μg N/ml) free from 2-mercaptoethanol was prepared by passing an enzyme soln through a Sephadex G-25 column (1·8 × 50 cm), equilibrated with 0·5 M KH_2PO_4 and 1 mM EDTA. All operations were carried out at 0–4°.

Preparation of apoenzyme. Details are similar to those described in ref. 2.

Determination of enzyme activity was given in ref. 2. Experiments to determine the effect of inhibitors were based on estimation of O_2 uptake. Protein was determined by ref. 11 and N determinations were carried out according to ref. 12.

The coupled reduction of cytochrome c. The reaction system contained, in a total vol. of 4 ml: 0·63 μmol cytochrome *c*, 0·5 μmol *p*-benzoquinone, 200 μmol phosphate buffer (pH = 7), 250 μg catalase, 1·2 mg bovine serum albumin and 50 μmol substrates, in addition to 0·2 ml of enzyme soln eluted from Sephadex G-25. Reaction mixtures were incubated for 20 min at 30° before the addition of substrate.

The coupled reduction of 2,6-dichlorophenolindophenol. Experimental conditions similar to the case of cytochrome *c* reduction. Bovine serum albumin was omitted. 0·1 μmol 2,6-dichlorophenolindophenol was used.

Acknowledgements—The authors are greatly indebted to Professor B. J. D. Meeuse of the University of Washington, Seattle, U.S.A. for correcting the English manuscript, and also to Professor K. Fukui of Hiroshima University for his preparation of *o*-aminobenzaldehyde.

REFERENCES

1. Smith, T. A. (1972) *Phytochemistry* **11**, 899.
2. Suzuki, Y. and Hirasawa, E. (1973) *Phytochemistry* **12**, 2863.

3. Hill, J. M. and Mann, P. J. G. (1964) *Biochem. J.* **91,** 171.
4. Schütte, H. R., Knöfel, D. and Heyer, O. (1966) *Z. Pflanzenphysiol.* **55,** 110.
5. Mizusaki, S., Tanabe, Y., Noguchi, M. and Tamaki, E. (1972) *Phytochemistry* **11,** 2757.
6. Suzuki, Y. (1973) *Plant Cell Physiol.* **14,** 413.
7. Massey, V., Müller, F., Feldberg, R., Schuman, M., Sullivan, P. A., Howell, L. G., Mayhew, S. G., Matthews, R. G. and Foust, G. P. (1969) *J. Biol. Chem.* **244,** 3999.
8. Gomes, B., Igaue, I., Kloepfer, H. G. and Yasunobu, K. T. (1969) *Arch. Biochem. Biophys.* **132,** 16.
9. Tabor, C. W. and Kellogg, P. D. (1970) *J. Biol. Chem.* **245,** 5424.
10. Tiselius, A., Hjerten, S. and Levin, Ö. (1956) *Arch. Biochem. Biophys.* **65,** 132.
11. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) *J. Biol. Chem.* **193,** 265.
12. Katsumata, I. (1953) *Chiba Igaku-Kai Zashi* **29,** 207.