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ARGININE DECARBOXYLASE OF OAT SEEDLINGS

TERENCE A. SMITH

Research Station (University of Bristol), Long Ashton, Bristol, BS18 9AF, U.K.

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Key Word Index—*Avena sativa*; Gramineae; oat seedlings; cereals; arginine decarboxylase; enzyme purification; agmatine; putrescine; polyamines.

Abstract—Arginine decarboxylase activity in the shoots of seedlings was high in oats, intermediate in barley and low in rice, maize, wheat and rye. After partial purification, the arginine decarboxylase from the shoots of potassium deficient oat seedlings was separated into two fractions, A (MW 195 000) and B (MW 118 000), by gel chromatography. On gel electrophoresis, the mobilities of these fractions were respectively 0.12 and 0.55 relative to bromophenol blue at pH 9.5. Fraction A was twice as active as fraction B in extracts of seedlings grown with both normal and potassium deficient nutrition, despite the greater activity (×5) of the potassium deficient plants. The properties of the two fractions were similar with respect to pH optimum (7–7.5), K_m (3×10^{-5}M) and the effect of inhibitors. Fraction A was purified to apparent homogeneity by DEAE-cellulose chromatography. The enzyme was specific for L-arginine and it was strongly inhibited by NSD 1055, D-arginine and canavanine. Mercaptoethanol and dithiothreitol stimulated the enzyme by *ca* 50% and *p*-chloromercuribenzoate was an inhibitor. Pyridoxal phosphate stimulated activity by *ca* 30% and EDTA stimulated activity by 30%. Ca^{2+} and Mg^{2+} inhibited the enzyme by 50% at *ca* 20 mM. Putrescine and the polyamines showed only moderate inhibition at 10 mM, but agmatine reduced activity to 30% at this concentration.

INTRODUCTION

Arginine decarboxylase (L-arginine carboxy-lyase; EC 4.1.1.19) occurs widely in bacteria and plants, together with its product, agmatine [1, 2]. However, in animals, arginine decarboxylase has been found only in the parasitic worm *Ascaris* [3], and agmatine has only been detected in certain invertebrates [4, 5] in which it may be formed by transamidination of putrescine [5], and in herring milt in which it was first characterized [6].

In animals, bacteria and certain plants, putrescine is formed by ornithine decarboxylase. In animals this enzyme is now recognized as the rate-limiting factor in the biosynthesis of polyamines, substances which are implicated in the regulation of growth, protein synthesis and nucleic acid metabolism [1, 2]. In higher plants ornithine appears to be of lesser importance than arginine and agmatine as the precursor of putrescine [7–11]. In earlier work on *Escherichia coli*, both constitutive [12] and inducible [13] arginine decarboxylases have been characterized and purified. In higher plants arginine decarboxylase has been purified to homogeneity from the dicotyledonous plant *Lathyrus sativus* [14]. However amongst the monocotyledonous plants, arginine decarboxylase has been studied only in barley seedlings, from which it was purified about 10-fold [15]. In the present work the arginine decarboxylase from oat seedlings has been purified to apparent homogeneity and some of its properties investigated, using a new spectrophotometric assay [16].

RESULTS AND DISCUSSION

Distribution of arginine decarboxylase in cereals

The isotropic assay was used to survey the distribution of arginine decarboxylase in the leaves of 6 species of cereals grown in constant darkness or with diurnal illumination (Table 1). The effect of potassium (K) deficiency on arginine decarboxylase from oats and barley was also studied, since earlier work had shown that activity is considerably increased in these conditions [15]. Activity was quite low in rice, maize, wheat and rye, high in oats and intermediate in barley. In principle this agrees with the earlier qualitative results for various grass and cereal species [15]. No consistent effect of illumination was observed, though in potassium deficient (−K) oats grown in darkness the activity was only 6% of that in oats of the same age (21 days) grown with illumination. Activity of the leaves of oats grown in the light for 26 days was increased over 3-fold by K deficiency. Activity in −K oat seedlings appeared to reach a peak after 21–28 days growth, though in the +K plants activity was still increasing at this age.

A similar increase in arginine decarboxylase (2.2-fold) was found with −K barley, and in both species putrescine was increased 20- to 25-fold with K deficiency (Table 2). In barley, spermidine showed a lesser increase (7-fold) and spermine concentration was unaffected. In oats, spermidine was increased 2-fold and spermine 3-fold by K deficiency. In earlier experiments the effect of K deficiency on spermidine and spermine concentrations was inconsistent [17, 18], although putrescine content was invariably increased.

The activity of the polyamine oxidase in the seedlings of cereals [19] was low in rice, rye and wheat, intermediate in barley and high in oats. The similar pattern found for arginine decarboxylase in the present study indicates that oats may be a relatively active source of other enzymes associated with di- and polyamine metabolism. It is also of interest that the growth of oats

Table 1. Arginine decarboxylase in the shoots of cereal seedlings grown with or without potassium (K) in the dark or in diurnal illumination (light). Activity was determined by the release of $^{14}CO_2$ from L-arginine-[U-^{14}C] (see Experimental)

Plant	Nutritional status	Illumination	Age (days)	pkat/g fr. wt
Rice	+K	greenhouse	35	0.1
Maize	+K	light	23	4
Wheat	+K	light	14	7
Wheat	+K	dark	13	6
Rye	+K	light	14	26
Rye	+K	dark	13	27
Barley	+K	light	14	98
Barley*	+K	light	26	189
Barley*	−K	light	26	416
Oats	+K	light	14	252
Oats	+K	light	18	353
Oats	+K	light	21	625
Oats*	+K	light	26	833
Oats	−K	light	18	761
Oats	−K	light	21	2750
Oats*	−K	light	26	2700
Oats	−K	dark	8	134
Oats	−K	dark	21	173

Polyamines in the plants marked (*) are shown in Table 2.

Table 2. Polyamine content of normal (+K) and potassium deficient (−K) 26-day-old barley and oat leaves in nmol/g fr. wt. Arginine decarboxylase activity for these plants is shown in Table 1 (marked *)

Plant	Nutritional status	Putrescine	Spermidine	Spermine	Wt of one unit (g)
Barley	+K	242	64	10	0.40
Barley	−K	4540	471	10	0.20
Oats	+K	107	78	21	0.56
Oats	−K	2570	157	71	0.42

appears to be less affected by K deficiency than barley. K deficiency reduced growth of barley by 50% but oats showed only 25% growth reduction in the same conditions (Table 2).

Purification

With the procedure described in Experimental, but using direct precipitation of the enzyme with 2 vols. of acetone, 12-fold purification was achieved (Table 3) with a specific activity of 0.7 to 1.3 nkat/mg protein. Fractionation with acetone (0.5 to 2 vols.) gave considerably improved purity (2 to 3.7 nkat/mg). On gel filtration with BioGel A-1.5 m (step 4), two arginine decarboxylase fractions were obtained, fraction A (MW 195000) having a specific activity *ca* 6-fold higher than fraction B (MW 118000). Similar results were obtained by separation on Sepharose 6B (Fig. 1). BioGel A-1.5 m was used for most of the preparations in this study. Attention was concentrated on fraction A, which on one occasion had a specific activity of 96 nkat/mg protein at step 4. However, in general, the purification factor for this step was 10–15× for fraction A and 1.4–2× for fraction B on gel filtration (Table 3). The proportion of fraction A to fraction B arginine decarboxylase in plants supplied with normal concentrations of K was similar to that found in the −K plants, even though the total arginine decarboxylase activity was 5-fold greater in the latter (Table 3). The enzyme was further purified on DEAE-cellulose (step 5) from which both fractions were eluted with 0.33 M KCl. The bulk of the protein was eluted after the enzyme. At this stage, enzyme activity of fraction A, demonstrated by the immersion technique (see Experimental) and by estimating activity in individual segments of the gel, was coincident with the main Coomassie blue staining band (Rb 0.12), but other faint protein bands were found at Rb 0.2–0.45. The band at Rb 0.12 represented *ca* 80 to 90% of the total protein. The specific activity of this preparation was *ca* 250 nkat/mg protein and represents a 3500-fold purification. This specific activity is 10-fold greater than that of the homogeneous arginine decarboxylase obtained from *Lathyrus* [14], and is similar to the specific activity of the homogeneous constitutive arginine decarboxylase of *Escherichia coli* [12] (Table 4).

In *Escherichia coli* the constitutive and inducible arginine decarboxylases are composed of subunits, and are respectively a tetramer and a decamer [12, 20]. Similarly the arginine decarboxylase from *Lathyrus* is a

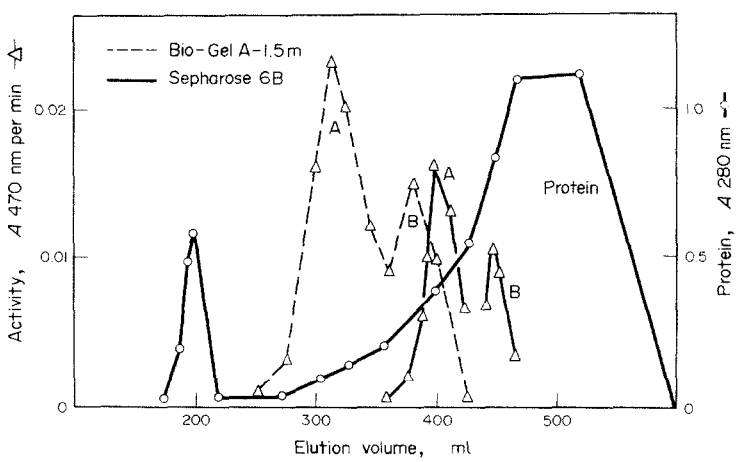

Fig. 1. Gel chromatography of arginine decarboxylase (acetone precipitate) on BioGel A-1.5 m and Sepharose 6B. Enzyme activity (fractions A and B), △. Protein, ○.

Table 3. Purification of arginine decarboxylase from potassium deficient or normal oat seedlings

Purification step*	Vol. (ml)	Total protein (mg)	Total activity (nkat)	Sp. act. (nkat/mg)	Purification† factor	Yield %
From plants with potassium deficiency:						
Experiment 1						
Crude extract (1)	2050	10500	697	0.066	1	100
$(NH_4)_2SO_4$ fractionation (2)	170	1750	765	0.43	6.7	106
Precipitation with 2 vols. acetone (3)	74	880	606	0.69	11.5	85
Experiment 2						
Acetone fractionation (3)	10	60	170	2.8	1 (40)	100
BioGel A-1.5 m (4) Fraction A	83	4.9	161	32.5	12 (464)	95
Fraction B	51	15.3	71	4.6	1.6 (65)	41
Experiment 3						
BioGel A-1.5 m (4) Fraction A	53	0.8	26.5	33.3	1 (475)	100
DEAE-Cellulose Fraction A	66	0.04	9.9	247	7.4 (3530)	37
From plants with normal nutrition:						
Experiment 4						
Acetone fractionation (3)	10	100	24	0.24	1 (4)	100
BioGel A-1.5 m (4) Fraction A	80	2	16	8.0	33 (1320)	67
Fraction B	40	1.5	3.2	2.1	9 (360)	13

* Purification step numbers, in parentheses, correspond to those in Experimental.
† Purification factors, in parentheses, are values relative to the crude extract (sp. act. 70 pkat/mg protein).

hexamer of similar subunits [14]. However the ratio of the MWs of fractions A and B of the oat leaf arginine decarboxylase does not indicate a simple polymeric system.

Gel electrophoresis of fraction B (step 4) showed activity at Rb 0.55 with both the immersion technique and on sectioning the gel. The region of activity coincided with a protein band. After preparative gel electrophoresis, re-electrophoresis of fractions A and B gave single protein bands at Rb 0.12 and 0.55, respectively, together with an inert band at Rb 1.0 in each case. This inert band therefore appears to be associated with the arginine decarboxylase during the preparative electrophoresis.

Gel electrophoresis of samples taken in the earlier stages of purification indicated that the immersion technique (see Experimental) could cause artefacts. Using this method, activity in samples from step 3 appeared to be associated with a protein at Rb 0.20, although analysis of the sections showed peak enzyme activity at Rb 0.1–0.15. It appears that proteins in the gel may adsorb the guaiacol oxidation products differentially in the immersion technique and the results obtained may be difficult to interpret in impure preparations.

Isoelectric focusing of fractions A and B showed peaks of activity with pI at 4.95 and 4.65, respectively. The inducible arginine decarboxylase of *Escherichia coli* had pI of 4.44 [20]. Isoelectric focusing caused protein precipitation with the oat leaf preparations, as had been found in previous work with the enzyme from *E. coli* [20]. Gel electrophoresis of the active peak of fraction A after isoelectric focusing gave a single protein band at Rb 0.12 and a thin band at Rb 1.0. Only *ca* 10 to 20% of the applied activity was recovered after isoelectric focusing.

Affinity chromatography

L-Arginine bound to AH- or CH-Sepharose 4B was used in an attempt to purify the acetone precipitate (step 3). Arginine AH-Sepharose 4B gave *ca* 10×

Table 4. Activity of arginine decarboxylase in extracts of various species

Species	Tissue	Assay temp.	pH optimum	Specific activity nkat/mg protein: Crude extract	Specific activity nkat/mg protein: Final preparation	Ref.
Aeromonas shigelloides		37°	5.5	—	—	[21]
Escherichia coli						
constitutive		37°	8.4	0.18	280	[12]
inducible		37°	5.2	220	7000	[13]
Pseudomonas spp.		37°	8.1	0.05	1.9	[22]
Panus tigrinus	fruit bodies	37°	5.2	3.0	165	[23, 24]
Avena sativa	shoots	30°	7.0	0.07	250	[16] and present work
Cucumis sativus	cotyledons	40°	8.3	0.005	—	[25]
Glycine max	axis	30°	7.0	—	—	[26]
Hordeum vulgare	shoots	25°	7.0	0.01	0.1	[15, 27]
Lathyrus sativus	seedlings	45°	8.5	0.03	25	[14]

— = Not determined.

purification with 30% recovery and arginine CH-Sepharose 4B gave 4 × purification with 50% recovery.

Stoichiometry

In earlier work [16], stoichiometry of the acetone precipitate for CO_2 was shown to be *ca* 97%. Since agmatine iminohydrolase has been detected in extracts of oat leaves [28], the hydrolysis of agmatine by the acetone precipitate was studied. After incubation with agmatine for 3 hr at pH 7.5, only a trace of *N*-carbamylputrescine and no putrescine could be detected, suggesting the absence of this enzyme. However a very active *N*-carbamylputrescine amidohydrolase was demonstrated, confirming the presence of this enzyme in oat leaves [29].

Effect of inhibitors

Amines. Since the amines are oxidized by the diamine oxidase, the isotopic method was used for assay. Agmatine inhibited the arginine decarboxylase to 91 and 33% of the control at 1 and 10 mM, respectively. Putrescine had no significant effect on the activity at 1 or 10 mM. Spermidine, spermine, diaminopropane and diaminodipropylamine at 10 mM reduced activity to 74, 68, 77 and 40% of the control, respectively, but activity was not significantly affected at 1 mM. The content of spermidine and spermine in the oat leaves (Table 2) was not high enough to suggest that these amines would inhibit *in vivo*, though local concentrations inside the cell may be quite high. Agmatine has not been estimated in oats, but the highest concentrations in K deficient barley (600 nmol/g fr. wt) [18, 36] indicate that the product may not exert direct control over arginine decarboxylase activity. Polyamine oxidase, which is very active in extracts of oat seedlings [31], could deplete spermidine and spermine added as potential inhibitors. However, this enzyme is probably eliminated by gel chromatography (MW of polyamine oxidase 85000), and was not detected in these preparations at step 4. For the *Lathyrus* arginine decarboxylase [14], 10 mM putrescine, spermidine, spermine and agmatine reduced activity to 80, 68, 45 and 73%, respectively. The oat seedling arginine decarboxylase therefore appears to be inhibited less by the first three amines, but it shows greater inhibition with agmatine.

Amino acids. All amino acids, except lysine which is a substrate for the amine oxidase, were tested initially by the spectrophotometric assay (Table 5). Arginine decarboxylase activity was unaffected by L-lysine at 1 or 10 mM. L-Ornithine, L-homoarginine and 4-guanidinobutyric acid did not show more than 50% inhibition at 5 mM, and L-citrulline gave no inhibition at this concentration. However, D-arginine inhibited by *ca* 60% at 1 mM in both the spectrophotometric and isotopic assays. D-Arginine has been shown to inhibit the arginine decarboxylase from a *Pseudomonas* sp. [22] and barley [15]. *Lathyrus* arginine decarboxylase was relatively resistant, with 40 mM D-arginine causing only 35% inhibition [14]. L-Canavanine was also a very effective inhibitor of the oat enzyme, 1 mM causing *ca* 50% inhibition. For the *Lathyrus* enzyme, 40 mM canavanine was needed for 50% inhibition [14]. Canavanine was not a substrate for the arginine decarboxylase from oats, unlike the inducible enzyme from *E. coli* [13]. Indeed L-arginine was apparently the only substrate for the oat enzyme, like the enzymes from *Pseudomonas* [22] and the constitutive enzyme from *E. coli* [12]. Activity of the oat enzyme with arginine as substrate was at least 20000 times greater than activity with L-ornithine-[1-^{14}C]. The inducible enzyme from *E. coli* also attacks L-canavanine at 40% of the rate with L-arginine [13]. The *Lathyrus* enzyme showed no activity with L-homoarginine, L-lysine or L-ornithine, though other unrelated substrates were apparently decarboxylated by the preparation studied [14].

Miscellaneous inhibitors and promoters. These were initially tested by the spectrophotometric technique, but when a significant effect was detected, further studies were made by the isotopic method (Table 6). Pyridoxal and guanidine showed little inhibition. With the peroxidase assay, arcain gave considerable inhibition, though with the isotopic assay less inhibition was found. A similar discrepancy occurred with MGBG (methylglyoxal bis-(guanylhydrazone)) and NSD 1055 (4-bromo 3-hydroxybenzyloxyamine dihydrogen phosphate). NSD 1055 was a powerful inhibitor of arginine decarboxylase in both assays, indicating that this enzyme is pyridoxal phosphate dependent [32]. It was subsequently shown that

Table 5. Effect of amino acids on activity of arginine decarboxylase (fractions A and B) using isotopic assay (C) and spectrophotometric assay (S)

Compound	Assay	Concentration (mM)	Fraction A (%)	Fraction B (%)
L-Ornithine	S	5	67	—
L-Homoarginine	S	5	58	61
D-Arginine	S	0.1	105	94
	S	1	47	44
	C	1	37	11
	S	5	13	—
4-Guanidino butyric acid	S	5	79	83
L-Canavanine	S	1	47	72
	C	1	52	—
	S	5	0	0
	C	10	0	—

— = Not determined.

Table 6. Effect of miscellaneous substances on arginine decarboxylase (fractions A and B) using the isotopic assay (C) and the spectrophotometric assay (S)

Compound	Assay	Concentration (mM)	Fraction A (%)	Fraction B (%)
Pyridoxal	S	1	105	94
Guanidine	S	5	108	—
Arcain	S	0.01	92	82
	S	0.1	50	37
	C	0.1	98	—
	S	1	0	0
	C	1	89	—
MGBG	S	0.1	46	—
	S	1	13	—
	C	1	93	—
	S	5	0	—
	C	10	79	—
NSD 1055	S	0.01	84	78
	S	0.1	11	17
	C	0.1	68	—
	S	1	0	6
	C	1	34	—
p-Chloromercuribenzoate	C	0.1	78	—
	C	1	43	—
Mercaptoethanol	C	0.1	156	—
	C	1	151	—
Dithiothreitol	C	0.1	156	—
	C	1	155	—
Na ethylene diamine tetra-acetic acid	S	1	138	—
	C	1	122	—

— = Not determined.

Table 7. Effect of various metal ions as chloride salts on arginine decarboxylase (fractions A and B) using the isotopic assay (C) and the spectrophotometric assay (S)

Metal ion	Assay	Concentration (mM)	Fraction A (%)	Fraction B (%)
Mn	C	0.1	103	—
	C	2	94	—
	C	40	77	—
Ca	S*	5	50	—
	S*	20	—	50
	C	10	76	—
Mg	S*	10	50	—
	S*	5	—	50
	C	10	67	—

* Obtained by interpolation. — = Not determined.

arcain, MGBG and NSD 1055 are effective inhibitors of pea seedling diamine oxidase. Arcain at 1 mM inhibited this enzyme to 16%, and MGBG at 1 mM inhibited to 32%. NSD 1055 at 1 and 0.1 mM inhibited to 0 and 48%, respectively. *p*-Chloromercuribenzoate inhibited the oat leaf arginine decarboxylase to 43% at 1 mM, indicating that the enzyme is sulphydryl dependent, like the arginine decarboxylase from *Lathyrus* [14]. This was also suggested by the stimulation of activity shown by mercaptoethanol and dithiothreitol. Ethylenediaminetetra-acetic acid stimulated activity significantly in both assays, indicating that the enzyme was inhibited by contaminating metal ions (Table 6).

Other properties. Pyridoxal phosphate at 0.8 mM stimulated activity by 34% (fraction A) and 28% (fraction B) using the spectrophotometric assay. With the isotopic assay, pyridoxal phosphate at 0.8 mM stimulated fraction A by 25 and 30% (duplicate assay). No stimulation of activity could be detected with Mn^{2+}, unlike the *Lathyrus* enzyme which was stimulated by 35% with 0.1 mM Mn^{2+} [14]. Ca^{2+} and Mg^{2+} were inhibitory at 10 mM (Table 7). The Michaelis constants of fractions A and B were similar, *ca* 3.3×10^{-5} M at pH 7.5. This is lower than the K_m for the acetone precipitate (8×10^{-5} M). The pH optimum of the acetone precipitate is 7.0 [16]. Using the isotopic assay, activity relative to pH 7 (=100%) for pH 6 and 8 was 66 and 106% for fraction A and 66 and 69% for fraction B.

EXPERIMENTAL

Plants. Seedlings of rice (*Oryza sativa* L.), maize (*Zea mays* L.), wheat (*Triticum vulgare* L.), rye (*Secale cereale* L.), barley (*Hordeum vulgare* L. cv Zephyr) and oats (*Avena sativa* L. cv Black Supreme) were grown in sand in polyethylene pots in diurnal illumination (16-hr day, 10 klx, 24° light, 19° dark) (light-grown) or in the dark (21°). The plants were watered daily with a nutrient medium containing (with concentration in mM) K_2SO_4 (2), $MgSO_4$ (1.5), $CaCl_2$ (4), Na_2HPO_4 (0.33), $NaNO_3$ (4), $(NH_4)_2SO_4$ (4), with Fe EDTA and micronutrients. K deficient plants were grown in a medium in which Na_2SO_4 was substituted for K_2SO_4. Intact oat and barley leaves stored at −15° for 8 months retained 11 and 31% of their arginine decarboxylase activity, respectively.

Isotopic assay was conducted by a modification of the method of ref. [16]. The incubates (2.5 ml) contained L-arginine (1 mM), 0.1 μCi L-arginine-[U-^{14}C] and arginine decarboxylase (10 μl–1 ml, 50–250 pkat) in Tris–HCl buffer (0.1 M, pH 7.5, unless stated otherwise). To determine the activity of the arginine decarboxylase in the cereal seedlings (Table 1), leaves were blended with 2 vols. 50 mM Na_2HPO_4, the extract centrifuged (3000 *g*, 10 min) and dialysed against pH 6.3, 0.1 M Pi buffer. Samples (1–2 ml) were used in the isotopic assay in Pi buffer (pH 6.3, 0.1 M).

Spectrophotometric assay was conducted essentially by the method of ref. [16]. In an optically pre-matched glass tube (1 × 12 cm) was placed 2 ml of Tris–HCl buffer (pH 7.5, 0.1 M), 0.1 ml of pea seedling diamine oxidase (10 nkat), 0.1 ml peroxidase (20 purpurogallin units), 0.1 ml guaiacol (25 mM) and 0.1 ml arginine decarboxylase preparation (50–250 pkat). The mixture was incubated at 30° for 1 min and then placed in a spectrophotometer with a cuvette holder at 30°. The reaction was initiated by adding 0.1 ml 25 mM L-arginine (mono HCl). Increase in *A* at 470 nm was followed on a logarithmic recorder. This technique could not be used to assay the activity of crude preparations prior to step 2, since *A* was high and substrates of diamine oxidase were present [16]. Sulphydryl activators also interfere [31].

Putrescine and the polyamines were estimated by the method of ref. [33] and *protein* was estimated by the method of ref. [34] with BSA as standard. To eliminate interference from phenolics, the protein was precipitated with 10% TCA before estimation. *N*-Carbamylputrescine and putrescine were separated by TLC according to the method of ref. [27].

Purification of the arginine decarboxylase of oats. Step 1. Leaves of 21-day-old K-deficient light-grown oat seedlings were macerated in 2 vols. 50 mM Na_2HPO_4, the extract filtered under pressure through nylon cloth and frozen for 18 hr (crude extract). *Step* 2. On thawing, $(NH_4)_2SO_4$ was dissolved (200 g/l.) and the ppt. discarded after centrifugation (2000 *g*, 15 min). $(NH_4)_2SO_4$ was added to increase the concn to 500 g/l. The ppt. (2000 *g*, 15 min) was dispersed in Tris–HCl (0.1 M, pH 6.7) and dialysed against this buffer for 18 hr ($(NH_4)_2SO_4$ ppt.).
Step 3. Me_2CO at −15° (50 ml/100 ml enzyme) was then added with stirring at 0°, the extract centrifuged (2000 *g*/10 min) and the ppt. discarded. Further Me_2CO at −15° was added (150 ml/100 ml enzyme) with stirring at 0° and the ppt. recovered by centrifugation (5000 *g*/5 min) was dissolved in Tris–HCl buffer (0.1 M, pH 7). The extract was dialysed against this buffer and centrifuged at 15000 *g* for 5 min (Me_2CO ppt.).
Step 4. This enzyme preparation (usually 10 ml) was applied to a column (3.2 × 70 cm, bed vol. 570 ml) of BioGel A-1.5 m, equilibrated with 50 mM Tris–HCl buffer (0.1 M, pH 7) containing 0.1 M KCl. The column was eluted with this buffer and fractions (4–5 ml) collected at 10 min intervals. The active fractions were combined and concd by ultra-filtration (Diaflo, PM 30). The preparation was stable for 3 months at 4°. For estimation of MW the column was calibrated with thyroglobulin, catalase, fibrinogen, alcohol dehydrogenase and bovine serum albumin.
Step 5. The enzyme (fraction A) was applied to a column (1.9 × 7 cm) of DEAE-cellulose (Whatman DE 23) equilibrated with Tris–HCl (50 mM, pH 6.7). The enzyme was eluted with a linear gradient of KCl (0–1 M), total vol. 400 ml, with fractions (4–5 ml) collected at 10 min intervals. Active fractions were pooled and concd in Minicon B 15 concentration cells (Amicon Ltd.). At this stage 50% activity was lost on storage for 1 week at −15°.

Gel electrophoresis was effected by the method of ref. [35]. Small pore gels (0.5 × 7 cm) with 7.5% acrylamide (pH 9.5) and Tris–glycine buffer (pH 8.4) were prepared according to the method of ref. [35]. Sucrose (5%) was added to the enzyme and samples (150 μl) were applied to each tube (cathode end) without stacking gel. Tracking dye was bromophenol blue (BPB). After stacking for *ca* 20 min at 1 mA/tube current was increased to 3 mA/tube (*ca* 200 V). After 1–2 hr when the BPB was *ca* 1 cm

from the anodic end, the tubes were removed. For the detection of protein the gels were fixed in 12% TCA for 30 min, and stained in Coomassie blue (0.1% in 12% TCA) [37]. For the detection of the arginine decarboxylase, an adaptation of the spectrophotometric method was developed (immersion technique), similar to that used for the detection of polyamine oxidase [31]. The gel was immersed in a soln composed of 2 ml Tris–HCl buffer (0.1 M, pH 7.5), 0.1 ml diamine oxidase (10 nkat), 0.1 ml peroxidase (20 purpurogallin units), 0.1 ml guaiacol (25 mM), and 0.1 ml L-arginine (25 mM). On incubation at 30°, the arginine decarboxylase produced a brown band after an interval depending on the amount of arginine decarboxylase activity. The colour was stable on storage of the gel in H_2O. For the detection of the arginine decarboxylase in sections of the gel, these were individually eluted with Tris–HCl buffer (0.1 M, pH 7.5) for 18 hr. prior to estimation of the enzyme by the spectrophotometric method. Mobilities of the bands were measured relative to the BPB (Rb = 1.0).

Isoelectric focusing was performed by the method of ref. [38]. A sucrose density gradient containing 1% of carrier ampholytes (pH 3–6) was used in a 110 ml LKB 8100 apparatus. After separation for 20 hr at 500 V (current: initial 1.5 mA; final 0.6 mA), the gradient was fractionated and assayed for protein, enzyme and pH.

Affinity chromatography. Arginine AH or CH Sepharose 4B were prepared according to manufacturers' instructions. 1-Ethyl-3(3-dimethylaminopropyl)carbodiimide was used for coupling. The mixture was adjusted to pH 4.5 and shaken 18 hr at room temp. After applying the arginine decarboxylase, the affinity column was washed with increasing concns of KCl (0.05–3 M).

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