Int. J. Biochem. Vol. 23, No. 10, pp. 1155–1159, 1991
Printed in Great Britain.

0020-711X/91 $3.00 + 0.00

REGULATORY PROPERTIES OF AMP DEAMINASES FROM RAT TISSUES

JOZEF SPYCHALA[1] and JAROSLAW MARSZALEK[2]

[1]University of Michigan, Department of Internal Medicine, Division of Rheumatology, MSRB I, Room 5520, Ann Arbor, MI 48109-0680, U.S.A. and [2]Gdansk University, Department of Molecular Biology, ul. Kladki 24, 80-822 Gdansk, Poland

(Received 19 November 1990)

Abstract—1. Phosphocelluse column chromatography under double gradient conditions (phosphate and KCl) revealed two forms of AMP deaminase in rat heart and brain and a single form in the liver and skeletal muscle.

2. Kinetically all purified AMP deaminases were classified into two categories: those, which elute from the column at lower KCl and P_i concentrations, display low $S_{0.5}$ value are only moderately affected by MgATP, MgGTP and P_i; and those which elute at higher KCl and P_i concentrations, display high $S_{0.5}$ values and are strongly regulated by allosteric effectors.

3. Physiological significance of the occurrence of two kinetic forms of AMP deaminase in some tissues is discussed.

INTRODUCTION

AMP deaminase (EC 3.5.4.6), which catalyzes hydrolytic deamination of AMP, is an ubiquitous enzyme occurring in all eukariotic cells (Purzycka, 1962). The enzyme is regulated by a number of modulators. The most important are ATP (activator) and GTP and inorganic phosphate (inhibitors) (Setlow *et al.*, 1966; Van den Berghe *et al.*, 1977; Spychala *et al.*, 1986). Its complex regulatory properties seem to be compatible with the control of adenine nucleotide breakdown. This homeostatic function, vital for each living cell, has been most extensively studied with isolated cells systems (Chapman and Atkinson, 1973; Chapman *et al.*, 1976). These studies revealed that under conditions of intensified ATP utilization AMP deaminase provides a mechanism which, together with adenylate kinase, stabilizes the relative concentrations of adenylates at the cost of a net loss in the adenine nucleotide pool. In the heart, this function may be shared with 5′-nucleotidase (Itoh *et al.*, 1986; Skladanowski and Newby, 1990).

However, the observation that the enzyme activity varies widely in animal tissues and the multiple molecular forms have been detected in some of them (Spychala *et al.*, 1986; Ogasawara *et al.*, 1972, 1978, 1982), suggests a significant degree of diversity in the regulation of AMP degradation. In human and rat liver a single form of AMP deaminase has been described (Ogasawara *et al.*, 1978, 1982). On the other hand, in chicken liver, the occurrence of two forms of AMP deaminase seems to be associated with the intensive metabolism of purine nucleotides due to uricotelism (Spychala and Makarewicz, 1983; Spychala and Van den Berghe, 1987). Similarly, two kinetically distinct forms of AMP deaminase were purified from rat small intestine and a detailed kinetic studies have revealed that the form exhibiting a lower K_m is less sensitive to activators and inhibitors. Compatible with our previous chromatographic and kinetic studies showing two forms of the enzyme in some tissues are genetic experiments showing the existence of two genes coding in for AMP deaminase in human and rat tissues (Sabina *et al.*, 1990).

Therefore, it seemed worthwhile in investigate, in addition to the previous studies which focused mainly on chromatographic and immunological differences (Ogasawara *et al.*, 1972, 1978, 1982), the tissue distribution of two previously described different AMP deaminases (Spychala and Makarewicz, 1983; Spychala *et al.*, 1986) and compare their regulation by $MgATP^{-2}$, $MgGTP^{-2}$ and inorganic phosphate.

MATERIALS AND METHODS

Chemicals

AMP (free acid) was purchased from Sigma Chemical Co., U.S.A. Phosphocellulose P-11 was supplied by Whatman, U.K. ATP (disodium salt) and GTP (disodium salt) were from Boehringer Mannheim, F.R.G. Sodium cocadylate and 2-mercaptoethanol were purchased from Loba Chem., Austria. All other reagents were of the highest purity available.

Purification of AMP deaminase

Male Wistar rats weighing 300–400 g were used throughout the study as a source of liver skeletal muscle, heart and brain. The animals were killed by decapitation and the tissues were immediately dissected, washed with ice-cold 5 mM Tris-HCl pH 7.4 containing 1 mM mercaptoethanol and 1 mM PMSF (buffer A), and then weighed. Ten grammes of each tissue were homogenized in 10 volumes of buffer A using a Waring-Blendor type homogenizer. The homogenate was then stirred for 10 min at 4°C and centrifuged at 18,000 *g* for 30 min.

Wet phosphocellulose (20 ml prepared as recommended by the manufacturer and extensively washed with 100 mM phosphate buffer and buffer A) was added to the supernatant fraction of the homogenate and the batch adsorption was carried out at 5°C for 30 min under gentle stirring. The

phosphocellulose slurry was then washed three times with 0.25 M KCl by repeated centrifugation and placed in a column (Pharmacia K 16/20). The column was then washed with 0.4 M KCl (adjusted to pH 7.0 was imidazole) and subsequently the AMP deaminase activity was eluted using a double gradient of KCl (0.4–0.7 M) and potassium phosphate (0–0.1 M, pH 7.0, with KCl in the mixing vessel and KCl plus phosphate in the reservoir) at a flow of 20 ml/ hr.

The fractions corresponding to each peak of activity were pooled, diluted 5-fold with ice-cold 1 mM mercaptoethanol, adsorbed onto phosphocellulose (5 ml of bed volume) for 15 min and placed in the column. After washing with 0.4 M KCl, the enzymes were eluted with 1.0 M KCl, pH 7.0 (in the case of form I) or 1.2 M KCl, pH 7.0 (in the case of form II).

Enzyme assay

AMP deaminase was assayed by means of ammonia determination (Chaney and Marbach, 1962). The reaction mixture contained 50 mM cacodylate buffer pH 7.2, 150 mM KCl, 1 mM free $MgCl_2$, 1 mM mercaptoethanol, 1 mg/ml bovine serum albumin and AMP concentrations as indicated. The incubation was carried out at 25°C for 10–30 min and was terminated by the addition of phenol and hypochlorite reagents. The reaction was linear with protein and with time up to 60 min. During the enzyme purification the enzyme activity was measured in K^+/succinate buffer pH 6.5, 150 mM KCl and 10 mM AMP.

For the evaluation of kinetic parameters nonlinear regression method was employed. Initial estimates for kinetic parameters (h and $S_{0.5}$) were computed from the data (0.1–0.9 of V_{max}) using linear regression of the transformed Hill equation [$\log(v/V - v)$ vs $\log s$]. The experimental value of V_{max} was used for calculations at the initial stage. The final fit of experimental data to the kinetic function was performed using iterative, nonlinear least-squares data fitting. An algorithm developed by Marquardt (Marquardt, 1963) was employed for this purpose. To facilitate the calculations an overall computer program was written in BASIC for the IBM PC (Marszalek *et al.*, 1989). To obtain an equal distribution of experimental points (not less than 12, ranging from 0.1–24 mM of substrate concentrations), preliminary experiments were performed under each condition.

Protein assay

Portein concentration was determined by the method of Bradford (Bradford, 1976) with bovine serum albumin as the reference.

RESULTS

The redesigned chromatographic procedure with double phosphate/KCl gradient allowed reproducible and selective separation of two kinetic forms of AMP deaminase from chicken liver (Spychala and Makarewicz, 1983) and rat small intestine (Spychala *et al.*, 1986) and proved useful for the purpose of the present study. Therefore, this technique was used for the screening of rat tissues for the occurrence of multiple forms of this enzyme. A two-step procedure was employed. First, phosphocellulose column chromatography in a double gradient mode was used to separate two forms of AMP deaminase and second, phosphocellulose column chromatography in a step elution mode was used to further purify the enzyme, concentrate it and separate it from inorganic phosphate. The 0.4 M KCl wash during the first step did not elute any enzyme activity (Fig. 1). Subsequent elution with a double phosphate/KCl gradient revealed two forms of AMP deaminase in rat heart and brain and single form in rat liver and skeletal muscle. They were classified as form I or II according to the elution sequence. Additional minor peaks of AMP deaminase activity were also observed with brain and heart extracts. The double phosphocellulose column chromatography procedure yielded highly purified enzyme preparations with specific activities of 9.8, 2.5 and 177.9 μmol/min/mg for form I from the brain, heart and skeletal muscle and 20.0, 17.0 and 11.9 μmol/min/mg for form II from the liver, brain and heart respectively. Enzyme preparations were essentially free of adenylate kinase, 5′-nucleotidase, nonspecific phosphatase and adenosine deaminase activities.

Kinetic properties of the obtained enzyme preparations were studied at pH 7.2 in the presence of various combinations of effectors including: ATP, inorganic phosphate and GTP: all at 1 mM $MgCl_2$ in excess of nucleoside triphosphates. Preliminary experiments showed no effect of magnesium ions on AMP deaminase preparations tested. The results shown in Table 1 indicate that all the enzymes tested fall into two categories. The first category includes forms which elute at a lower P_i and KCl concentrations from the phosphocellulose column, have substrate half-saturation values of controls in the range of 0.2 to 0.7 mM and are activated by MgATP only moderately (form I), and the second includes forms which elute at higher KCl and phosphate concentrations, have $S_{0.5}$ values in the range of 3.2–5.7 mM and are strongly activated by MgATP (form II). In all cases, MgATP at 1 mM concentration activated purified AMP deaminases and inorganic phosphate and MgGTP inhibited them. However, in the presence of MgATP alone, the $S_{0.5}$ values of all AMP deaminases were almost identical. The combined effect of modulators brought about enzyme activation when compared with controls in the case of all enzymes tested except for skeletal muscle AMP deaminase (Table 1). The maximal velocity values changed no more than 25% of control (data not shown).

MgATP caused a shift in the kinetics from sigmoidal to hyperbolic (Table 1). On the other hand, MgGTP and inorganic phosphate increased the sigmoidicity in all enzymes tested. In addition, MgATP efficiently counteracted the effect of inhibitors on the Hill coefficient, except for AMP deaminase I from heart and skeletal muscle.

DISCUSSION

Multiple molecular forms of AMP deaminase have already been reported in rat and human tissues (Ogasawara *et al.*, 1972, 1978, 1982). On the basis of immunological and chromatographic properties three parental isoforms were proposed for rat: A (muscle), B (liver) and C (heart). Four isoforms have been proposed for humans: E1 and E2 (erythrocytes), M (muscle), and L (liver). It was postulated that other tissues possessed various combinations of these isozymes. However, the kinetic properties of some of the isozymes

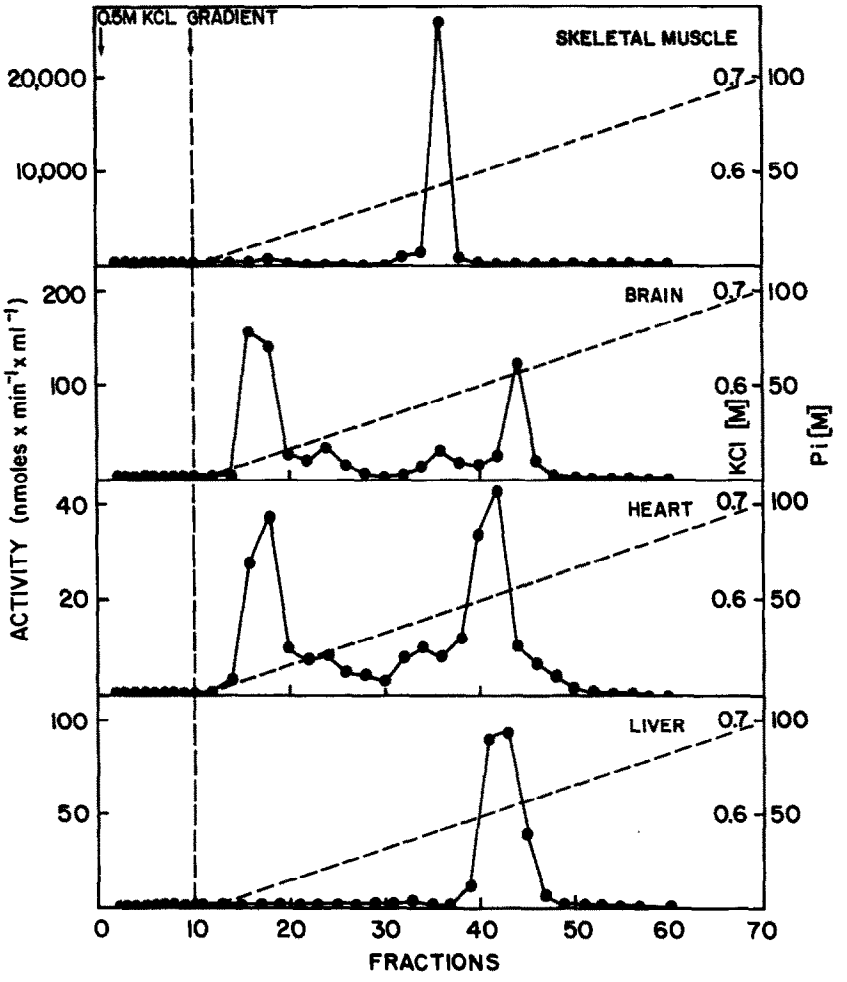

Fig. 1. Phosphocellulose column elution profiles of AMP deaminase from rat liver, brain, heart and skeletal muscle.

were only briefly presented and no conclusion could be drawn as far as their function is concerned (Ogasawara *et al.*, 1978, 1982).

In contrast to previous reports, which showed five peaks in rat brain (Ogasawara *et al.*, 1972) and a single peak of AMP deaminase in rat heart (Ogasawara *et al.*, 1978), we were able to observe two major activity peaks in both rat brain and heart. This apparent discrepancy could be explained on the basis of the different chromatographic techniques employed. The interaction of AMP deaminase with the phosphocellulose resin occurs through the phosphate moiety and is most probably an affinity type (Spychala, 1987). Therefore, inorganic phosphate might be more selective in eluting AMP deaminase forms from phosphocellulose than KCl or NaCl alone. The less probable explanation for the reduced number of peaks in rat brain may be related to the addition of PMSF, an inhibitor of serine proteases, since our preliminary studies without the inhibitor were almost identical to these presented here. Our two molecular forms of AMP deaminase may be classified as a low-K_m (form I) and high-K_m (form II).

Our studies are in agreement with the presence of two genes coding for AMP deaminase in rat and human tissues (Sabina *et al.*, 1990). RNase protection analysis revealed that the amp^1 gene is expressed at high level in skeletal muscle and heart and that the amp^2 gene is expressed at low level in skeletal muscle but at high level in liver, brain and heart (Morisaki *et al.*, 1990). The only unexplained discrepancy is the presence of AMP deaminase I in rat brain which was reported to express low level of amp^1 gene. However, the presence of an additional transcript from the amp^2 gene cannot be ruled out.

The kinetic properties of enzymes purified in essentially the same way were studied under conditions which approximate those found *in vivo*. Although the predominant forms of ATP and GTP in the cell are $MgATP^{-2}$ and $MgGTP^{-2}$, magnesium ions were rarely included in the incubation mixture in other studies. Studies with platelet and rat small intestine AMP deaminases have shown that magnesium ions strongly potentiate the allosteric effects exerted by ATP and GTP (Ashby and Holmsen, 1983; Spychala *et al.*, 1986).

The major kinetic difference between forms I and II relates to $S_{0.5}$ values in the absence of effectors and to the degree of activation by MgATP. Form II has a 6–28-fold higher substrate half saturation constant

Table 1. Kinetic properties of two forms of AMP deaminase from rat liver, brain, heart and skeletal muscle

Enzyme and condition	AMP deaminase I		AMP deaminase II	
	$S_{0.5}$ (mM)	h	$S_{0.5}$ (mM)	h
Liver				
Control	—	—	5.7 ± 0.4	2.5 ± 0.2
1 mM ATP	—	—	0.3 ± 0.1	1.0 ± 0.1
4 mM P_i	—	—	7.2 ± 0.5	2.8 ± 0.3
0.2 mM GTP	—	—	8.3 ± 0.5	3.5 ± 0.4
ATP, GTP, P_i	—	—	1.0 ± 0.2	1.2 ± 0.1
Brain				
Control	0.6 ± 0.1	1.0 ± 0.1	4.2 ± 0.2	2.3 ± 0.2
1 mM ATP	0.2 ± 0.02	0.8 ± 0.2	0.3 ± 0.2	1.3 ± 0.3
4 mM P_i	0.9 ± 0.1	1.9 ± 0.2	6.3 ± 0.2	2.9 ± 0.3
0.2 mM GTP	0.9 ± 0.1	2.1 ± 0.2	7.3 ± 0.2	2.8 ± 0.1
ATP, GTP, P_i	0.2 ± 0.02	1.0 ± 0.1	1.2 ± 0.1	1.1 ± 0.1
Heart				
Control	0.2 ± 0.1	1.4 ± 0.1	3.2 ± 0.1	2.4 ± 0.2
1 mM ATP	0.1 ± 0.01	1.0 ± 0.3	0.1 ± 0.02	1.0 ± 0.2
4 mM P_i	0.5 ± 0.1	1.4 ± 0.2	5.6 ± 0.1	2.7 ± 0.2
0.2 mM GTP	0.6 ± 0.1	2.4 ± 0.1	6.2 ± 0.1	3.8 ± 0.2
ATP, GTP, P_i	0.2 ± 0.03	2.2 ± 0.6	0.7 ± 0.1	1.2 ± 0.3
Muscle				
Control	0.7 ± 0.1	1.9 ± 0.1	—	—
1 mM ATP	0.4 ± 0.1	1.1 ± 0.1	—	—
4 mM P_i	0.7 ± 0.1	1.9 ± 0.1	—	—
0.2 mM GTP	1.1 ± 0.1	2.6 ± 0.2	—	—
ATP, GTP, P_i	0.9 ± 0.1	2.6 ± 0.2	—	—

Assays were performed as described in Materials and Methods. Data are means, +S.D. from three experiments. $S_{0.5}$ is defined as a substrate concentration at half maximal velocity and was calculated as described in Materials and Methods. h Represents Hill coefficient.

and is also more activated by MgATP. MgATP lowers substrate affinity of form II from 13 to 32 times, whereas the same parameter of form I is lowered by only a factor of 3. Interestingly, both AMP deaminase I and II are kinetically alike in the presence of MgATP. In general, form I is less susceptible to the regulation by MgATP, MgGTP and inorganic phosphate, whereas the activity of form II is significantly dependent on the presence of effectors, especially inhibitors. Although 4 mM P_i and 0.2 mM MgGTP alone affect the $S_{0.5}$ value of form I significantly, the fully activated enzyme (in the presence of MgATP) is not dramatically affected by these inhibitors. In contrast, AMP deaminase II from all sources and under these condition increase their $S_{0.5}$ value 3–7 times, from fully activated (MgATP only) to that in the presence of all effectors. Therefore, under presumably physiological conditions, AMP deaminase I would be much more active. Surprisingly, AMP deaminase from skeletal muscle does not confirm entirely to the properties described for form I from heart and brain and seems to share some properties with form II as well.

The significance of the coexistence of forms I and II in several tissues is unclear. It is possible that the presence of a low-K_m form (either as an additional form in brain and heart of predominant form in skeletal muscle) may be associated with generally more intensive purine nucleotide metabolism; in chickens due to uricotelism, in hepatomas and small intestine epithelium due to the rapid growth, and in brain, skeletal muscle and heart due to intensive energy metabolism. Paradoxically, the comparison of rates of AMP deamination in isolated rat and chicken hepatocytes show that despite the presence of the form with low-K_m and less strictly controlled by effectors and therefore more active under physiological conditions in the chicken, the catabolism of AMP is much slower (Van den Berghe *et al.*, 1980; Spychala and Van den Berghe, 1987). Thus, it is speculated that the appearance of the additional form of AMP deaminase in rat hepatoma (Jackson *et al.*, 1977) or the regulation of the balance between form I and II in chicken liver on high protein feeding (Spychala and Marszalek, 1986) might not reflect the actual rate of AMP deamination but rather reflect the presence of an adaptive mechanism which regulates the ratio between the two forms of AMP deaminase. Whether it is the regulation of expression of amp^1 and amp^2 genes or involves the existence of other cellular factors, remains to be established.

REFERENCES

Ashby B. and Holmsen H. (1983) Platelet AMP deaminase. Regulation of MgATP and inorganic phosphate and inhibition by the transition state analog coformycin. *J. biol. Chem.* **258,** 3668–3672.

Bradford M. M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, *Analyt. Biochem.* **72,** 248–254.

Chaney A. L. and Marbach E. P. (1962) Modified reagents for determination of urea and ammonia. *Clin. Chem.* **8,** 130–132.

Chapman A. G. and Atkinson D. E. (1973) Stabilization of adenylate energy charge by the adenylate deaminase reaction. *J. biol. Chem.* **248,** 8309–8312.

Chapman A. G., Miller A. L. and Atkinson D. E. (1976) Role of the adenylate deaminase reaction in regulation of adenine nucleotide metabolism in Ehrlich ascites tumor cells. *Cancer Res.* **36,** 1144–1150.

Itoh R., Oka, J. and Ozasa H. (1986) Regulation of rat heart cytosol 5′-nucleotidase by adenylate energy charge. *Biochem. J.* **235,** 847–851.

Jackson R. C., Morris H. P. and Weber G. (1977) Enzymes of the purine ribonucleotide cycle in rat hepatomas and kidney tumors. *Cancer Res.* **37,** 3057–3065.

Marquardt D. W. (1963) An algorithm for least squares estimation of non-linear parameters. *J. Soc. ind. appl. Math.* **11,** 431–441.

Marszalek J., Kostrowicki J. and Spychala J. (1989) LEHM: a convenient non-linear regression microcomputer program for fitting Michaelis–Menten and Hill models to enzyme kinetic data. *CABIOS* **5,** 239–340.

Morisaki T., Sabina R. L. and Holmes E. W. (1990) Adenylate deaminase. A multigene family in humans and rats. *J. biol. chem.* **265,** 11482–11486.

Ogasawara N., Yoshino M. and Kawamura Y. (1972) Multiple forms of AMP deaminase in rat brain. *Biochim. biophys. Acta* **258,** 680–684.

Ogasawara N., Goto H., Yamada Y. and Watanabe T. (1978) Distribution of AMP-deaminase isozymes in rat tissues, *Eur. J. Biochem.* **87,** 297–304.

Ogasawara N., Goto H., Yamada Y., Watanabe T. and Asano T. (1982) AMP deaminase isozymes in human tissues. *Biochim. biophys. Acta* **714,** 298–306.

Purzycka J. (1982) AMP and adenosine aminohydrolases in rat tissues. *Acta biochim. pol.* **9,** 83–93.

Sabina R. L., Morisaki T., Clarke P. Eddy R., Shows T. B., Morton C. C. and Holmes E. W. (1990) Characterization of the Human and rat myoadenylate deaminase genes. *J. biol. Chem.* **265,** 9423–9433.

Setlow B., Burger R. and Lowenstein J. M. (1966) Adenylate deaminase. I. The effects of adenosine and

guanosine triphosphates on activity and the organ distribution of the regulated enzyme. *J. biol. Chem.* **242,** 1244–1245.

Skladanowski A. C. and Newby A. C. (1990) Partial purification and properties of an AMP-specific 5′-nucleotidase from pigeon heart *Biochem. J.* **268,** 117–122.

Spychala J. (1987) The interaction of polyphosphoinositols with AMP deaminase. *Biochem. biophys. Res. Commun.* **148,** 106–111.

Spychala J. and Makarewicz W. (1983) Two forms of AMP deaminase from chicken liver. *Biochem biophys. Res. Commun.* **114,** 1011–1016.

Spychala J. and Marszalek J. (1986) The effect of high protein diet on the regulatory properties of AMP deaminase from chicken liver. *Acta biochim. pol.* **33,** 187–195.

Spychala J. and Van den Berghe G. (1987) Adenine nucleotide metabolism in isolated chicken hepatocytes. *Biochem. J.* **242,** 551–558.

Spychala J., Marszalek J. and Kucharczyk E. (1986) AMP deaminases of rat small intestine. *Biochim. biophys. Acta* **880,** 123–130.

Van den Berghe G., Bontemps F. and Hers H.-G. (1980) Purine catabolism in isolated rat hepatocytes. Influence of coformycin. *Biochem. J.* **188,** 913–920.

Van den Berghe G., Bronfman M., Vanneste R. and Hers H.-G. (1977) The mechanism of adenosine triphosphate depletion in the liver after a load of fructose. A kinetic study of liver AMP deaminase. *Biochem. J.* **162,** 601–609.