Mechanism of reaction of allylamine with the quinoprotein methylamine dehydrogenase

Victor L. DAVIDSON,* M. Elizabeth GRAICHEN and Limei Hsu JONES

Department of Biochemistry, The University of Mississippi Medical Center, 2500 N. State St., Jackson, MS 39216-4505, U.S.A.

Allylamine did not serve as an efficient substrate for methylamine dehydrogenase (EC 1.4.99.3) in a steady-state assay of activity and appeared to act as a competitive inhibitor of methylamine oxidation by methylamine dehydrogenase. Transient kinetic studies, however, revealed that allylamine rapidly reduced the tryptophan tryptophylquinone (TTQ) cofactor of methylamine dehydrogenase. The rate of TTQ reduction by allylamine was 322 s^{-1} , slightly faster than the rate of reduction by methylamine. These data were explained by a kinetic mechanism in which allylamine and methylamine are alternative substrates for

methylamine dehydrogenase. The apparent competitive inhibition by allylamine is due to a very slow rate of release of the aldehyde product, 0.28 s^{-1} , relative to a rate of 18.6 s^{-1} for the release of the aldehyde product of methylamine oxidation. A reaction mechanism is proposed for the oxidative deamination of allylamine by methylamine dehydrogenase. This mechanism is discussed in relation to the reaction mechanisms of topa-bearing quinoprotein amine oxidases, the flavoprotein monoamine oxidase and the mammalian semicarbazide-sensitive amine oxidase.

INTRODUCTION

Methylamine dehydrogenase (EC 1.4.99.3) is a soluble bacterial enzyme which catalyses the oxidation of methylamine to formaldehyde plus ammonia [1]. It is similar to flavoprotein and quinoprotein amine oxidases in that it catalyses the oxidative deamination of primary amines, but different in that it possesses tryptophan tryptophylquinone (TTQ) (Figure 1) as a prosthetic group [2,3]. This prosthetic group is derived from a post-translational modification of two gene-encoded tryptophan residues [4]. Many of the physical, kinetic and redox properties of methylamine dehydrogenase have been characterized previously in this laboratory (reviewed in [1]). The reactions of methylamine dehydrogenase with a variety of substrates and inhibitors have been described. Methylamine dehydrogenase has a preference for short-chain aliphatic amines [5]. It is also reduced by benzylamines, but the enzyme has a very low affinity for these and they

do not react at detectable rates in a steady-state assay [6]. Methylamine dehydrogenase is also irreversibly inactivated by carbonyl agents, such as semicarbazide, as well as by other hydrazines [7], which form covalent adducts at the C-6 carbon of TTQ [8].

We report here that allylamine appears to act as a reversible competitive inhibitor of methylamine dehydrogenase, but is actually an alternative substrate which undergoes very slow release of the aldehyde product relative to the reaction with the preferred substrate. The kinetic parameters for the oxidative deamination of allylamine by methylamine dehydrogenase are completely characterized. This information is discussed in terms of the reaction mechanism by which methylamine dehydrogenase oxidizes amines and how it compares with those of quinoprotein and flavoprotein amine oxidases.

EXPERIMENTAL

The purification of methylamine dehydrogenase from *Paracoccus denitrificans* (ATCC 13543) was as described previously [9]. Protein concentrations were calculated from previously determined molar absorption coefficients [10]. Reagents were obtained from Aldrich and Sigma Chemical Co.

Absorbance spectra were recorded with a Milton Roy 3000 spectrophotometer. When necessary, excess reagents and non-covalently bound species were separated from the enzyme by passage over a small Ultrogel AcA 202 (IBF Biotechnics) desalting column. The steady-state kinetic activity of methylamine dehydrogenase was measured spectrophotometrically with a Kontron Uvicon 810 spectrophotometer using a dye-linked assay in which the oxidation of methylamine was coupled to the reduction of phenazine ethosulphate (PES) and a redox-sensitive dye, 2,6-dichloroindophenol (DCIP) [9].

Stopped-flow experiments were performed using an On-Line Instrument Systems (OLIS, Bogart, GA, U.S.A.) stopped-flow sample handling unit coupled to Durrum optics. A 486 class computer controlled by OLIS software was used to collect data.

Figure 1 Structure of the TTQ prosthetic group of methylamine dehydrogenase

Abbreviations used: DCIP, 2,6-dichloroindophenol; PES, phenazine ethosulphate; SSAO, semicarbazide-sensitive amine oxidase; TTQ, tryptophan tryptophylquinone.

* To whom correspondence should be addressed.

All experiments were performed at 30 °C. The reaction between oxidized methylamine dehydrogenase and allylamine was monitored at 440 nm. The reaction between reduced methylamine dehydrogenase and PES was monitored at 483 nm, an isosbestic wavelength for the oxidized and reduced forms of PES. The data were fitted using OLIS software to the equation for a single exponential:

$$A = Ce^{-kt} + b \quad (1)$$

where C is a constant related to the initial absorbance and b represents an offset value to account for a non-zero baseline. It was assumed that the observed reactions obeyed the scheme shown in eqn. (2):

To determine these rate constants, k_{obs} was fitted to eqn. (3) [11]:

$$k_{\text{obs}} = 0.5\{k_1[S] + k_{-1} + k_2 + k_{-2} - [(k_1[S] + k_{-1} + k_2 + k_{-2})^2 - 4(k_1k_2[S] + k_1k_{-2}[S] + k_{-1}k_{-2})]^{0.5}\} \quad (3)$$

In all experiments the concentration of substrate was sufficiently greater than the enzyme concentration so that k_{obs} was independent of enzyme concentration as predicted by eqn. (3). It should be noted that it was not possible to directly determine K_d from these experimental data because the concentration-dependence of k_{obs} could not be described by a simple hyperbola [11,12]. This necessitated the use of eqn. (3), which considers k_1 and k_{-1} as independent unknown variables. Non-linear curve fitting of these data was performed with Sigma Plot v.5.01 (Jandel Scientific, San Raphael, CA, U.S.A.).

RESULTS

Effect of allylamine on the absorption spectrum of methylamine dehydrogenase

The reaction of methylamine dehydrogenase with allylamine was examined spectroscopically. Addition of allylamine to oxidized methylamine dehydrogenase caused changes in its visible absorption spectrum (Figure 2) which indicated that the protein-bound TTQ was being reduced [10]. Methylamine dehydrogenase

Figure 2 Spectral changes caused by addition of allylamine to methylamine dehydrogenase

Oxidized methylamine dehydrogenase (12.7 μM) was present in 50 mM potassium phosphate, pH 7.5. Spectra were recorded before (—) and after (---) addition of 2.0 molar equivalents of allylamine.

Figure 3 Secondary plots of the inhibition by allylamine of methylamine oxidation by methylamine dehydrogenase

(a) Initial rates of methylamine oxidation were measured while varying the methylamine concentration at a fixed concentration of 4.8 mM PES in the presence of different fixed concentrations of allylamine. (b) Initial rates of methylamine oxidation were measured while varying the PES concentration at a fixed concentration of 100 μM methylamine in the presence of different fixed concentrations of allylamine. The units of k_{cat} are s^{-1} . ●, No allylamine; △, 0.5 μM allylamine; ○, 1.0 μM allylamine; ▲, 1.5 μM allylamine.

has an $\alpha_2\beta_2$ structure, with one TTQ on each β subunit. Complete reduction of the enzyme was achieved by addition of a 2:1 molar ratio of allylamine to enzyme (1:1 per TTQ).

Inhibition by allylamine of methylamine oxidation by methylamine dehydrogenase

Allylamine was not an efficient substrate for methylamine dehydrogenase in the steady-state kinetic assay using PES as an electron acceptor. Addition of up to 1 mM allylamine caused only barely detectable enzyme-mediated reduction of PES and DCIP. It was not possible to determine a k_{cat} and K_m because the rate of allylamine-dependent DCIP reduction was not sufficiently greater than background levels. Addition of allylamine did, however, inhibit the reaction of methylamine dehydrogenase with methylamine. Maximum inhibition was observed when allylamine was added simultaneously with substrate, and the extent of inhibition decreased with time when allylamine was preincubated with the enzyme prior to the addition of methylamine. At a 2:1 molar ratio of allylamine to enzyme, the inhibitory effect was lost after as little as 15 s of preincubation. As the concentration of allylamine preincubated with enzyme

Figure 4 Reduction of methylamine dehydrogenase by allylamine

(a) Allylamine (17.2 μM) was mixed with oxidized methylamine dehydrogenase (2.0 μM) in 0.1 M potassium phosphate, pH 7.5, at 30 °C. Absorbance was monitored at 440 nm. (b) The k_{obs} was plotted against allylamine concentration for several reactions such as that shown in (a). The values of k_{obs} were calculated by fitting the decrease in A_{440} to eqn. (1). The line drawn through these points is the fit of these data to eqn. (3) with k_{-2} set at zero.

was increased, longer times were required for full recovery of enzyme activity.

Steady-state kinetic experiments were performed to determine the mechanism of inhibition. Initial rates of methylamine oxidation were measured while varying the methylamine concentration at different fixed concentrations of allylamine. In these experiments allylamine was added to the reaction mixture immediately before the addition of substrate. Reciprocal plots of $1/k_{\text{cat}}$ against $1/[S]$ produced a family of lines which intersected approximately on the $1/k_{\text{cat}}$ axis (Figure 3a). Initial rates of methylamine oxidation were also measured while varying the PES concentration at different fixed concentrations of allylamine. Reciprocal plots of $1/k_{\text{cat}}$ against $1/[S]$ produced a family of lines which appeared to be parallel (Figure 3b). The reaction of methylamine dehydrogenase with methylamine and PES is known to proceed by a Ping Pong reaction mechanism [5,13,14]. The inhibition patterns observed in Figure 3 are consistent with allylamine acting as a reversible dead-end inhibitor which is competitive with respect to methylamine [15].

Stopped-flow kinetic studies of the reduction of methylamine dehydrogenase by allylamine

As allylamine was observed to reduce the enzyme-bound TTQ, this reaction was examined by stopped-flow spectroscopy. The

Figure 5 Oxidation of methylamine dehydrogenase by PES

(a) PES (765 μM) was mixed with reduced methylamine dehydrogenase (2.0 μM) in 0.1 M potassium phosphate, pH 7.5, at 30 °C. Absorbance was monitored at 483 nm. (b) The k_{obs} was plotted against PES concentration for several reactions such as that shown in (a). The values of k_{obs} were calculated by fitting the increase in A_{483} to the equation for an exponential rise. The line drawn through these points is the fit of these data to eqn. (3).

reduction of oxidized methylamine dehydrogenase by allylamine was monitored by following the decrease in absorbance at 440 nm on addition of various concentrations of allylamine to the enzyme. Using allylamine concentrations of 11.5 μM or greater, the absorbance changes with time could be fitted to the equation for a single exponential, as shown in Figure 4(a). Since complete reduction of the enzyme was observed upon the addition of a stoichiometric amount of allylamine (Figure 2), k_{-2} was assumed to be zero to minimize error in the fitted parameters. The fit of the data to eqn. (3) (Figure 4b) yielded values of $k_1 = 1.5 \pm 0.2 \mu\text{M}^{-1} \cdot \text{s}^{-1}$, $k_{-1} = 102 \pm 47 \text{ s}^{-1}$ and $k_2 = 322 \pm 10 \text{ s}^{-1}$. It is interesting to note that the value for k_2 for the reaction with allylamine is actually greater than that of 280 s^{-1} which was observed previously for the reaction with methylamine under these buffer conditions [16]. The K_d (k_{-1}/k_1) of 67 μM is similar to that observed for methylamine under these conditions.

Stopped-flow kinetic studies of the oxidation of methylamine dehydrogenase by PES

In order to understand the apparent inhibition of methylamine dehydrogenase by allylamine in the steady-state kinetic assay, it was also necessary to characterize the kinetics of the re-oxidation of reduced methylamine dehydrogenase by PES. This reaction

Scheme 1 Kinetic model for the reaction of methylamine dehydrogenase with both allylamine and methylamine

A is methylamine; A' is allylamine; B is oxidized PES; P is formaldehyde; P' is acrolein; Q is reduced PESH₂; E is oxidized methylamine dehydrogenase; F is reduced methylamine dehydrogenase.

Table 1 Rate constants for the reaction of methylamine, allylamine and PES with methylamine dehydrogenase

Rate constants refer to the mechanism which is shown in Scheme 1. The values for k_1 , k_{-1} , k_2 and k_{-2} were taken from [16] and H. B. Brooks and V. L. Davidson, unpublished work. All other constants were calculated from stopped-flow and steady-state kinetic experiments as described in the text. All reactions were performed in 0.1 M potassium phosphate, pH 7.5, at 30 °C.

Constant	Rate
k_1	$2.4 \pm 0.2 \mu\text{M}^{-1} \cdot \text{s}^{-1}$
k_{-1}	$246 \pm 42 \text{ s}^{-1}$
k_2	$278 \pm 2 \text{ s}^{-1}$
k_{-2}	0
k_3	$18.6 \pm 0.3 \text{ s}^{-1}$
k_4	$0.14 \pm 0.04 \mu\text{M}^{-1} \cdot \text{s}^{-1}$
k_{-4}	$62 \pm 37 \text{ s}^{-1}$
k_5	$102 \pm 7 \text{ s}^{-1}$
k_{-5}	0
k_7	$1.5 \pm 0.2 \mu\text{M}^{-1} \cdot \text{s}^{-1}$
k_{-7}	$102 \pm 47 \text{ s}^{-1}$
k_8	$322 \pm 10 \text{ s}^{-1}$
k_{-8}	0
k_9	$0.28 \pm 0.01 \text{ s}^{-1}$

was monitored by following the increase in absorbance at 483 nm on addition of various concentrations of oxidized PES to the reduced enzyme. The oxidized and reduced forms of PES were isosbestic at this wavelength and the absorbance of oxidized methylamine dehydrogenase was 70% of that at its absorption maximum. In all cases, the absorbance changes with time could be fitted to the equation for a single exponential, as shown in Figure 5(a). A fit of these data to eqn. (3) (Figure 5b) yielded values of $k_1 = 0.14 \pm 0.04 \mu\text{M}^{-1} \cdot \text{s}^{-1}$, $k_{-1} = 62 \pm 37 \text{ s}^{-1}$ and $k_2 = 102 \pm 7 \text{ s}^{-1}$. A fitted value of $k_{-2} = 0 \pm 1 \text{ s}^{-1}$ was also obtained.

Calculation of rate constants for aldehyde product release from steady-state and transient kinetic data

It is evident from the rapid kinetic studies that allylamine is a competent substrate for methylamine dehydrogenase to the extent that it binds with a reasonable affinity and reduces the enzyme even more rapidly than the preferred substrate. The

Figure 6 Correlation of steady-state kinetic data with microscopic rate constants

Data were obtained from steady-state kinetic experiments. The solid lines represent the predicted relationship, using eqn. (4) and the values listed in Table 1, between k_{cat} and methylamine (a) or PES (b) at different fixed concentrations of allylamine. (a) Initial rates of methylamine oxidation were measured while varying the methylamine concentration at a fixed concentration of 4.8 mM PES in the presence of different fixed concentrations of allylamine. (b) Initial rates of methylamine oxidation were measured while varying the PES concentration at a fixed concentration of 100 μM methylamine in the presence of different fixed concentrations of allylamine. The concentrations of allylamine were: ●, none present; △, 0.5 μM ; ○, 1.0 μM ; ▲, 1.5 μM .

preincubation studies (discussed above) demonstrated that allylamine does not irreversibly inactivate the enzyme. These observations suggested that allylamine is a substrate for methylamine dehydrogenase but that the release of the aldehyde product must be very slow relative to that of the formaldehyde product of the preferred substrate. That being the case it would be appropriate to treat allylamine as an alternative substrate rather than a reversible inhibitor when it is present with methylamine.

Methylamine dehydrogenase is known to obey a Ping Pong reaction mechanism [5,13,14]. The kinetic model for the reaction of methylamine dehydrogenase with both allylamine and methylamine simultaneously is given in Scheme 1. A rate equation for this mechanism was derived using the method of King and Altman [17]. Certain terms could be eliminated from this equation based on the results of the stopped-flow kinetic studies. The rate constants for the reverse of the catalytic steps with methylamine and allylamine, k_{-2} and k_{-8} respectively, were set at

zero. When this was done, k_6 appeared in all remaining terms and consequently dropped out of the equation. This simplified rate equation is given as eqn. (4):

$$k_{\text{cat}} = \frac{[A][B](k_1k_2k_3k_4k_5k_9)(k_{-7}+k_8) + [B][A'](k_3k_4k_5k_7k_8k_9)(k_{-1}+k_2)}{\{[A](k_1k_2k_3k_9)(k_{-7}+k_8)(k_{-4}+k_5) + [B](k_3k_4k_5k_9)(k_{-1}+k_2)(k_{-7}+k_8) + [A'](k_3k_7k_8k_9)(k_{-1}+k_2)(k_{-4}+k_5) + [A][B](k_1k_4k_9)(k_{-7}+k_8)(k_2k_3+k_2k_5+k_3k_5) + [A'][B](k_3k_4k_7)(k_{-1}+k_2)(k_5k_8+k_5k_9+k_8k_9)\}}$$
(4)

Initial rates were determined at different concentrations of methylamine, allylamine and PES in the steady-state kinetic experiments which were described earlier. All microscopic rate constants in eqn. (4) were determined from the stopped-flow experiments described above, except for k_3 and k_9 . They are the rate constants that describe the product release steps for the aldehyde products which are derived from methylamine and allylamine respectively.

The steady-state kinetic data which are shown in Figure 3 were fitted to eqn. (4) while assigning all rate constants except k_3 and k_9 (see Table 1). A very good fit to the data was obtained, which supported the validity of the kinetic model, and yielded values for the unknown rate constants of $18.6 \pm 0.3 \text{ s}^{-1}$ for k_3 and $0.28 \pm 0.01 \text{ s}^{-1}$ for k_9 . The errors which are given in Table 1 are the standard errors of the fit. The correlation between the steady-state data and the curves predicted by eqn. (4) using the values in Table 1 are shown in Figure 6. Although some small deviation was observed for the steady state data in the absence of allylamine, given the complexity of eqn. (4), this represents a very good correlation with the kinetic model. The very slow rate for the product release of acrolein is consistent with the barely detectable reaction of methylamine dehydrogenase with allylamine alone in the steady-state assay. A rate this slow would be at the lower limits of our levels of detection in the steady-state assay.

DISCUSSION

A likely mechanism for the reactions of methylamine dehydrogenase with methylamine and allylamine is shown in Figure 7. The reaction is initiated by a nucleophilic attack by the amine nitrogen on one of the quinone carbonyls. This most probably

results in formation of a carbinolamine intermediate which loses water to form the imine intermediate (I) shown. These two steps

leading to imine formation will be reflected in the kinetically determined K_i . The k_2 reflects the rate of proton abstraction of the imine intermediate by an active-site nucleophile and concomitant reduction of TTQ [16]. The comparable values of k_2 exhibited by methylamine and allylamine suggest that the allyl function does not appreciably influence this process. The reaction of amines and PES with methylamine dehydrogenase is known to proceed via a Ping Pong mechanism in which the aldehyde product is released prior to reaction with PES. Thus hydrolysis of the imine intermediate (II) must occur before re-oxidation. Comparison of the steady-state and transient kinetic parameters for the reaction catalysed by methylamine dehydrogenase (Table 1) indicates that the release of the aldehyde product is likely to be the rate-determining step in the overall oxidation–reduction reaction with methylamine and PES. The value of k_3 of 18.6 s^{-1} is approximately the value of k_{cat} of 17 s^{-1} per TTQ for the steady-state reaction of methylamine and PES with methylamine dehydrogenase [5]. The hydrolysis of the enzyme–product imine intermediate (II) in the reaction with allylamine is evidently much slower than the hydrolysis of this intermediate in the reaction with methylamine. This suggests either that the enzyme–product complex formed on reaction with allylamine is significantly more stable than the complex formed by reaction with methylamine, or that the transition state for the hydrolysis of the allylamine-derived intermediate is much less stable than that of the methylamine-derived intermediate, or both. This is probably a consequence of the extended conjugation which is provided by the allylic group relative to the methyl group.

The reaction of methylamine dehydrogenase with allylamine distinguishes it from flavoprotein amine oxidases which also catalyse the oxidative deamination of primary amines. Allylamine is a true inhibitor of the flavoprotein monoamine oxidase. This enzyme also catalyses the oxidative deamination of primary amines. With monoamine oxidase, allylamine is a mechanism-based inactivator which forms a covalent adduct with an active-site amino acid residue [18]. The different ways in which allylamine affects the activities of monoamine oxidase and methylamine dehydrogenase reflect the different mechanisms by which these two enzymes perform similar catalytic reactions. In contrast to the mechanism shown in Figure 7, the mechanism of amine oxidation by monoamine oxidase proceeds through one-electron transfers with radical intermediates [19].

In mammals, allylamine is apparently a substrate for the copper-containing semicarbazide-sensitive amine oxidase (SSAO) [20,21]. The SSAO of vascular tissues has been implicated in the conversion of allylamine [20,22], and perhaps aliphatic amines [23], to their corresponding toxic aldehydes. This enzyme also possesses an organic cofactor of unknown identity; however, it now appears that all copper-containing amine oxidases are probably quinoproteins [24], which possess 6-hydroxydopa-quinone [25]. The possible relevance of these studies with methylamine dehydrogenase to the question of allylamine metabolism by mammals must await further characterization of SSAO. These results do, however, provide additional insight into

Figure 7 Proposed mechanisms for the reductive half-reaction of methylamine dehydrogenase with (a) methylamine and (b) allylamine

the alternative cofactors and mechanisms which are present in nature for the metabolism of primary amines.

We thank Harold B. Brooks for providing a computer program to facilitate the derivation of rate equations using the King–Altman method. This work was supported by National Institutes of Health grant GM-41574.

REFERENCES

- Davidson, V. L. (1993) in *Principles and Applications of Quinoproteins* (Davidson, V. L., ed.), pp. 73–95, Marcel Dekker, New York
- McIntire, W. S., Wemmer, D. E., Christoserdov, A. Y. and Lidstrom, M. E. (1991) *Science* **252**, 817–824
- Chen, L., Mathews, F. S., Davidson, V. L., Huizinga, E., Vellieux, F. M. D., Duine, J. A. and Hol, W. G. J. (1991) *FEBS Lett.* **287**, 163–166
- Christoserdov, A. Y., Tsygankov, Y. D. and Lidstrom, M. E. (1990) *Biochem. Biophys. Res. Commun.* **172**, 211–216
- Davidson, V. L. (1989) *Biochem. J.* **261**, 107–111
- Davidson, V. L., Jones, L. H. and Graichen, M. E. (1992) *Biochemistry* **31**, 3385–3390
- Davidson, V. L. and Jones, L. H. (1992) *Biochim. Biophys. Acta* **1121**, 104–110
- Huizinga, E. G., van Zanten, B. A. M., Duine, J. A., Jongejan, J. A., Huitema, F., Wilson, K. S. and Hol, W. G. J. (1992) *Biochemistry* **31**, 9789–9795
- Davidson, V. L. (1990) *Methods Enzymol.* **188**, 241–246
- Husain, M., Davidson, V. L., Gray, K. A. and Knaff, D. B. (1987) *Biochemistry* **26**, 4139–4143
- Hirami, K. (1979) *Kinetics of Fast Reactions*, Halsted Press, New York
- Strickland, S., Palmer, G. and Massey, V. (1975) *J. Biol. Chem.* **250**, 4048–4052
- Eady, R. R. and Large, P. J. (1968) *Biochem. J.* **106**, 245–255
- Eady, R. R. and Large, P. J. (1971) *Biochem. J.* **123**, 757–771
- Segel, I. H. (1975) *Enzyme Kinetics*, John Wiley & Sons, New York
- Brooks, H. B., Jones, L. H. and Davidson, V. L. (1993) *Biochemistry* **32**, 2725–2729
- King, E. L. and Altman, C. (1956) *J. Phys. Chem.* **60**, 1375–1378
- Silverman, R. B., Hiebert, C. K. and Vazquez, M. L. (1985) *J. Biol. Chem.* **260**, 14648–14652
- Silverman, R. B., Hoffman, S. J. and Catus, W. B., III (1980) *J. Am. Chem. Soc.* **102**, 7126–7128
- Boor, P. J., Sanduja, R., Nelson, T. J. and Ansari, G. A. S. (1987) *Biochem. Pharmacol.* **36**, 4347–4353
- Boor, P. J. and Nelson, T. J. (1980) *Toxicology* **18**, 87–102
- Boor, P. J. and Hysmith, R. M. (1987) *Toxicology* **44**, 129–144
- Precious, E., Gunn, C. E. and Lyles, G. A. (1988) *Biochem. Pharmacol.* **37**, 707–713
- Klinman, J. P. and Mu, D. (1994) *Annu. Rev. Biochem.* **63**, 299–344
- Janes, S. M., Mu, D., Wemmer, D., Smith, A. J., Kaur, S., Mautry, D., Burlingame, A. L. and Klinman, J. P. (1990) *Science* **248**, 981–987

Received 6 October 1994/3 January 1995; accepted 10 January 1995