Biochimica et Biophysica Acta, 570 (1979) 213—214

BBA Report

BBA 61356

DIHYDROPYRIMIDINE AMIDOHYDROLASE IS A ZINC METALLOENZYME

KATHLEEN P. BROOKS, BYUNG DONG KIM and EUGENE G. SANDER*

Department of Biochemistry, School of Medicine, West Virginia University, Morgantown, WV 26506 (U.S.A.)

(Received April 19th, 1979)

Key words: Dihydropyrimidine amidohydrolase; Zinc metalloenzyme

Summary

Bovine liver dihydropyrimidine amidohydrolase (EC 3.5.2.2) has been subjected to atomic absorption analysis. Three different preparations of homogeneous enzyme indicated that the enzyme contains 4.3 ± 0.3 g atoms of Zn^{2+} per mol of enzyme or 1.1 g atoms of Zn^{2+} per subunit. No Co^{2+}, Mn^{2+}, Mg^{2+} or Cd^{2+} was detected. Exhaustive dialysis against either *o*-phenanthroline or EDTA did not reduce enzyme activity; however, prolonged incubation with dipicolinic acid resulted in inactivation which can be reversed by either Zn^{2+} or Co^{2+} but not Mg^{2+}.

In addition to its role in the pathway for pyrimidine ring catabolism, dihydropyrimidine amidohydrolase (dihydropyrimidinase, EC 3.5.2.2) hydrolyzes the 5-halodihydropyrimidines [1] as well as certain hydantoin and succinimide drugs [2—4]. Recently, using methods to be published elsewhere, we have purified the enzyme to homogeneity (unpublished) and have confirmed the molecular weight and subunit composition reported for the partially purified enzyme by Maguire and Dudley [5]. Dihydroorotase from *Zymobacterium oroticum*, another enzyme responsible for reversible 5,6-dihydropyrimidine ring hydrolysis, is known to be a zinc metalloenzyme [6,7]. Hence, homogeneous dihydropyrimidinase has been subjected to metal analysis by atomic absorption spectrometry.

Three separate dihydropyrimidinase preparations, previously shown to be homogeneous with respect to contaminating proteins by SDS and native polyacrylamide gel electrophoresis were subjected to atomic absorption anal-

*To whom correspondence should be addressed.

ysis for Zn^{2+} Single preparations were analyzed for Co^{2+}, Mn^{2+}, Mg^{2+} and Cd^{2+}. The results (Table I) indicate that dihydropyrimidinase has no detectable Co^{2+}, Mg^{2+}, Mn^{2+} or Cd^{2+}. It does however contain approximately 4 mol

TABLE I

METAL ANALYSIS OF BOVINE LIVER DIHYDROPYRIMIDINE AMIDOHYDROLASE (DHPase)

Atomic absorption analysis was performed with a Jarrel-Ash Model 810 double beam spectrometer. Glassware was washed with 10% HCl prior to use. Buffers were analytically shown to be metal-free and the instrument was calibrated against Zn^{2+}, Co^{2+}, Mn^{2+}, Mg^{2+} and Cd^{2+} standards certified for atomic absorption (Fisher Scientific Co.). Samples of dihydropyrimidine amidohydrolase were dialyzed at 4°C against three changes of metal-free 0.001 M Tris-HCl buffer, pH 7.5 followed by centrifugation to remove a small precipitate. Dihydropyrimidine amidohydrolase concentration was measured by the Lowry protein method [8] and, when required, dilutions were made with the dialysis buffer. Gram atoms of metal per mol of enzyme and per mol of subunit were calculated from the analytical data, the protein concentration and molecular weight values of 226 000 and 56 500 for the enzyme and the subunit, respectively [5]. Values expressed as limits represent the detection limits of the instrument.

Metal	Metal concn. (nmol/ml)	DHPase concn. (nmol/ml)	nmol metal per nmol DHPase
Zinc	11.9	2.6	4.6
	11.6	2.8	4.1
	6.9	1.6	4.3
Cobalt	<0.8	1.0	<0.80
Manganese	<0.2	2.8	<0.07
Magnesium	<0.4	1.6	<0.25
Cadmium	<0.09	2.8	<0.03

of Zn^{2+} per mol of enzyme, or one Zn^{2+} per subunit.

Zinc appears to be very tightly chelated to native dihydropyrimidinase as exhaustive dialysis at 4°C against either 2.0 mM *o*-phenanthroline or 1.0 mM EDTA, each in 0.05 M Tris-HCl buffer, pH 7.0, failed to diminish enzyme activity when compared to controls dialyzed against chelator-free buffer. Incubation of dihydropyrimidinase with 0.50 mM dipicolonic acid in 0.05 M Tris-HCl buffer, pH 7.0, did, however, result in a time-dependent inhibition which required about 48 h to give 80% inhibition. In addition, enzyme reduced to half its original activity by treatment with dipicolinic acid could be completely reactivated, again in a time-dependent process, by incubation with either 0.005 mM Zn^{2+} or 0.100 mM Co^{2+}. Mg^{2+} (0.100 mM) did not reactivate dihydropyrimidinase under these conditions. Higher Zn^{2+} concentrations were not used for these experiments because preliminary data indicated that excessive Zn^{2+} is actually inhibitory to the fully active enzyme.

The authors wish to thank Dr. Donald Horvath for use of the atomic absorption spectrophotometer. This work was supported by grant CA21167 from the U.S. Public Health Service.

References

1 Kim, B.D., Keenan, S., Bodnar, J.K. and Sander, E.G. (1976) J. Biol. Chem. 251, 6909—6914
2 Dudley, K.H., Butler, T.C. and Bius, D.L. (1974) Drug Metab. Dispos. 2, 103—112
3 Dudley, K.H. and Roberts, S.B. (1978) Drug Metab. Dispos. 6, 133—139
4 Maguire, J.H. and Dudley, K.H. (1978) Drug Metab. Dispos. 6, 140—145
5 Maguire, J.H. and Dudley, K.H. (1978) Drug Metab. Dispos. 6, 601—605
6 Sander, E.G., Wright, L.D. and McCormick, D.B. (1965) J. Biol. Chem. 240, 3628—3630
7 Taylor, H., Taylor, M.L., Balch, W.E. and Gilchrist, P.S. (1976) J. Bacteriol. 127, 863—872
8 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265—275