A novel NADPH/NADH-dependent aldehyde reduction enzyme isolated from the tapeworm *Moniezia expansa*

Peter M. Brophy*, Paul Crowley and John Barrett

Department of Biological Science, University College of Wales, Aberystwyth, Dyfed SY23 3DA, Wales, UK

Received 7 February 1990

An aldehyde reduction enzyme has been purified from the cytosol of the tapeworm, *Moniezia expansa*, by chromatofocusing and Reactive-Red chromatography. The enzyme is monomeric (subunit 34 kDa) and can utilise NADH and NADPH as co-factors. Substrates of the enzyme include alkanals, alka-2,4-dienals and alk-2-enals, established secondary products of lipid peroxidation. The enzyme reduced methylglyoxal, another possible natural substrate (*M. expansa* lacks glyoxalase I activity). The parasite enzyme may help form a final line of defence against cytotoxic aldehydes arising from host immune initiated lipid peroxidation.

Lipid peroxidation; Aldehyde reductase; Alcohol dehydrogenase; (*Moniezia expansa*)

1. INTRODUCTION

Cytotoxic aldehydes can be formed in vivo by lipid peroxidation [1]. The intracellular enzymatic defences against aldehydes have not been fully established. There are 3 candidate routes: via reduction by alcohol dehydrogenase/aldehyde reductase, via oxidation by aldehyde dehydrogenase and via glutathione conjugation by glutathione transferases [2–6]. Cytotoxic aldehydes in parasitic worms may arise from lipid peroxidation produced by the release of free-radicals from host-immune effector cells [7,8]. Glutathione transferase has previously been implicated as an aldehyde defence enzyme in parasites including *Moniezia expansa* [6].

Methylglyoxal is another important cytotoxic endogenous aldehyde [9]. Glyoxalase I is usually associated with the metabolism of methylglyoxal but this enzymatic activity is apparently absent in *Moniezia expansa* and many other related parasites [9]. This report describes the purification of a major aldehyde reduction enzyme from the tapeworm, *Moniezia expansa*.

2. MATERIALS AND METHODS

Moniezia expansa was obtained from the intestines of freshly slaughtered sheep at a local abattoir. The NADPH-dependent aldehyde reduction activity was measured using *trans*-2-nonenal as the standard aldehyde, at a final concentration of 0.1 mM, at 37°C in 100 mM sodium phosphate buffer, pH 7.0. Protein was determined using a dye binding method [10]. Microsomal and cytosolic fractions were prepared as previously described [6].

The major NADPH-linked *trans*-2-nonenal reduction activity was purified by chromatofocusing at pH 7–4 (Pharmacia, Uppsala, Sweden) followed by Reactive-Red agarose chromatography (Pharmacia). SDS/PAGE was carried out as previously described [6] in horizontal thin-layer gels (Pharmacia). The native molecular mass was determined by Sephadex G-100 chromatography (2.6 × 70 cm matrix) using 20 mM sodium phosphate buffer, pH 7.4, containing 50 mM potassium chloride as the running solution.

3. RESULTS

3.1. *Purification*

Over 95% of the NADPH-linked *trans*-2-nonenal reduction activity in *M. expansa* was detected in the cytosol compared to the microsomes. A cytosolic fraction prepared from 20 g of tissue, previously concentrated by poly(ethylene)glycol, could be resolved into three NADPH/*trans*-2-nonenal reduction forms by chromatofocusing at pH 7–4 (Fig.1). Active fractions of the major form were applied to a Reactive-Red affinity matrix and NADPH/*trans*-2-nonenal reduction activity was eluted as a single symmetrical peak by a pulse of 0.1 mM NADPH and one protein band was detected on SDS-PAGE at 34 kDa (Fig.2). On gel filtration the NADPH/*trans*-2-nonenal reduction activity had a molecular mass of 30 kDa. The activity was purified approximately 40-fold (Table I) but loss of activity occurred during the affinity chromatography step. The enzyme failed to bind an NADP-agarose (Sigma) and Blue-Dextran affinity (Sigma) matrices while significant loss of activity was observed during elution from a Reactive-Orange affinity (Amicon) matrix.

Correspondence address: J. Barrett, Department of Biological Science, University College of Wales, Aberystwyth, Dyfed SY23 3DA, Wales, UK

* *Present address:* Leicester Biocentre, University of Leicester, Leicester LE1 7RH, UK

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/90/$3.50

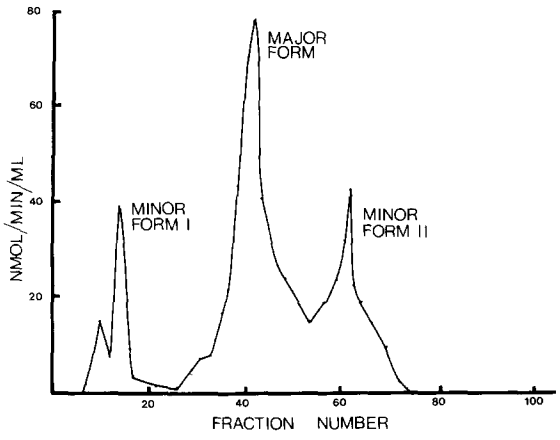

Fig.1. Chromatofocusing at pH 7–4 of NADPH-dependent *trans*-2-nonenal reduction activity in *M. expansa* cytosol. Cytosol (70 ml) was concentrated to 8 ml by poly(ethylene)glycol and dialysed against 100 vols of chromatofocusing start buffer (25 mM imidazole-HCl, pH 7.4, containing 5 mM mercaptoethanol). A 0.9 × 30 cm PBE 9-4 polybuffer exchange matrix was used at 4°C using a flow rate of 12 ml/h and the pH gradient was established by a 1:8 dilution of polybuffer, pH 4.0, containing 5 mM mercaptoethanol. The activity was eluted into fractions (2.75 ml) containing 20% (v/v) glycerol and 200 mM potassium phosphate buffer, pH 7.8. No activity was further eluted by the addition of 1 M NaCl.

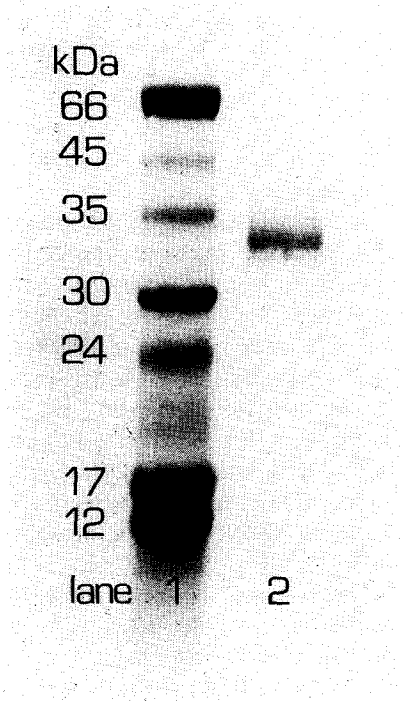

Fig.2. SDS/polyacrylamide electrophoresis of the aldehyde reduction enzyme of *M. expansa* cytosol after purification by chromatofocusing and Reactive-Red affinity chromatography. 2 μg of *M. expansa* protein was added to the slot. (Lane 1) Protein standards; (lane 2) aldehyde reduction enzyme. Protein was stained by Coomassie blue R-250.

Table I

Purification of the major NADPH-linked *trans*-2-nonenal reduction enzyme in *M. expansa* cytosol

Fraction	Total activity (nmol/min)	Total protein (mg)	Specific activity (nmol/min per mg)
Cytosol	3220	296	10.8
Chromatofocusing			
Unresolved	20	-	
Minor I	125	-	
Minor II	520	10.8	48.1
Major	1685	19.9	84.7
Reactive Red			
Major	354	0.9	393.3

- = not determined

Table II

Substrate specificity of the *M. expansa* aldehyde reduction enzyme with cytotoxic aldehydes using NADPH as the co-factor

Substrate	Activity (nmol/min per mg)
Trans-2-nonenal	382 ± 17 (5)
Trans-2-octenal	335 ± 14 (3)
Trans-trans-2,4-decadienal	592 ± 65 (3)
Hexanal	248 ± 24 (3)
Methylglyoxal	683 ± 64 (5)

Assays were carried out at 37°C using 100 μM aldehyde. Activity was expressed as ± SD, with the number of replicates in parentheses

3.2. *Biochemical characteristics*

The enzyme reduced a range of known aldehyde products of lipid peroxidation (Table II) and methylglyoxal. Using 0.4 mM NADPH, the aldehyde reduction enzyme had low apparent K_m values for *trans*-2-nonenal 6.6 μM) and methylglyoxal (10.7 μM), respectively. The enzyme was only weakly inhibited by phenobarbital (30% inhibition by 500 μM) and pyrazole (35% inhibition by 500 μM), characteristic inhibitors of aldehyde reductase and alcohol dehydrogenase, respectively [11]. The helminth enzyme was able to use NADH as a co-factor at approximately 50% of the rate of NADPH-linked reduction and also catalysed NAD and NADP-linked oxidation of ethanol and propanol at approximately 5% of the rate of NADPH-linked reductions. No activity was detected using D-fructose as a substrate of the aldehyde reduction enzyme.

4. DISCUSSION

The aldehyde reduction enzyme of *M. expansa* cytosol may have an important role in the detoxification of naturally occurring aldehydes such as alk-2-enals, alkanals and alka-2,4-dienals and 2-oxoaldehydes. It is not as yet possible to clarify the relationship of the helminth enzyme to aldehyde reduc-

tase. The monomeric structure and subunit size indicates homology to aldehyde reductase but the helminth enzyme can utilise both NADH and NADPH and is insensitive to phenobarbital inhibition.

The relative in vivo contribution of parasitic helminth aldehyde reduction enzyme and glutathione transferase towards defence against host immune initiated lipid peroxidation is still to be determined. Both enzymes appear to detoxify alk-2-enals and alka-2,4-enals and the aldehyde reduction enzyme can also apparently, unlike helminth glutathione transferase (Brophy and Barrett, unpublished results), detoxify alkanals.

Methylglyoxal was also indicated as a possible endogenous natural substrate of the *M. expansa* aldehyde reduction enzyme but the helminth enzyme does not appear related to a yeast methylglyoxal reductase which is apparently 2-oxoaldehyde/NADPH-specific and irreversible in the aldehyde reduction direction [12].

Acknowledgement: P.M.B. and J.B. acknowledge the support of the Medical Research Council (UK).

REFERENCES

[1] Esterbauer, H., Cheeseman, K.H., Diazani, M.U., Poli, G. and Slater, T.F. (1982) Biochem. J. 208, 129–140.
[2] Antonenkov, V.D., Pirozhkov, S.V. and Panchenko, L.F. (1987) FEBS Lett. 224, 357–360.
[3] Esterbauer, H., Zollner, H. and Lang, J. (1985) Biochem. J. 228, 363–374.
[4] Schauenstein, E., Esterbauer, H. and Zolner, H. (1977) in: Aldehydes in Biological Systems, Pion, London.
[5] Alin, P., Danielson, U. and Mannervik, B. (1985) FEBS Lett. 179, 267–270.
[6] Brophy, P.M., Southan, C. and Barrett, J. (1989) Biochem. J. 262, 939–946.
[7] Callahan, H.L., Crouch, R.K. and James, E.R. (1988) Parasitol. Today 4, 218–225.
[8] Brophy, P.M. and Barrett, J. (1990) Parasitology, in press.
[9] Brophy, P.M., Crowley, P. and Barrett, J. (1990) Int. J. Parasitol., in press.
[10] Sedmak, J.J. and Grossberg, S.E. (1977) Anal. Biochem. 79, 544–552.
[11] Von Wartburg, J.P. and Wermuth, B. (1980) in: Enzymatic Basis of Detoxification, vol. 1 (Jakoby W.B. ed.) pp. 249–260, Academic Press, New York.
[12] Murata, K., Fukuda, Y., Simosaka, M., Watanabe, K., Saikusa, T. and Kimura, A. (1985) Eur. J. Biochem. 151, 631–636.