Mycothiol, 1-*O*-(2′-[*N*-acetyl-L-cysteinyl]amido-2′-deoxy-α-D-glucopyranosyl)-D-*myo*-inositol, is the factor of NAD/factor-dependent formaldehyde dehydrogenase

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Received 17 March 1997; revised version received 21 April 1997

Abstract Two different NAD/coenzyme-dependent formaldehyde dehydrogenases exist, the well-known NAD/GSH-dependent (EC 1.2.1.1) and the more recently discovered NAD/Factor-dependent enzyme. The GSH-dependent one has been found in eukaryotes and Gram-negative bacteria, the Factor-dependent one in two different Gram-positive bacteria. Previous work also showed that Factor and GSH are not interchangeable in the enzymatic reactions. Here it is revealed that the Factor is identical to mycothiol (MySH), 1-*O*-(2′-[*N*-acetyl-L-cysteinyl]-amido-2′-deoxy-α-D-glucopyranosyl)-D-*myo*-inositol, a thiol compound which has recently been detected in Actinomycetes. Thus, MySH is GSH's companion as it is the coenzyme for the enzyme which henceforth can be indicated as NAD/MySH-dependent formaldehyde dehydrogenase.

Key words: Mycothiol; Formaldehyde dehydrogenase; Glutathione; Actinomycetes; Alcohol dehydrogenase class III

1. Introduction

The ability to remove toxic formaldehyde is widespread among living organisms. In vitro formaldehyde oxidation is catalyzed by a surprisingly large number of oxidoreductases differing in the type of coenzyme/cofactor used (see the introduction section of [1]). However, whether each of these enzymes has a physiological role in formaldehyde removal is questionable. For instance, it has been shown [2] that the Gram-negative bacterium *Paracoccus denitrificans* is unable to dissimilate formaldehyde-yielding substrates when NAD/GSH-dependent formaldehyde dehydrogenase (GD-FAlDH, EC 1.2.1.1) is lacking. Thus, for this organism, GD-FAlDH is crucial in removal of formaldehyde and other formaldehyde oxidoreductases do not take over its role. The distribution of GD-FAlDH (that is (NAD-dependent, zinc-containing) class III alcohol dehydrogenase [3]) is from microbes [4] to vertebrates [5], and the enzyme has been deduced to be an ancestral form of importance at all levels of evolution. However, when grown on formaldehyde-yielding substrates, the Gram-positive bacteria *Amycolatopsis methanolica* and *Rhodococcus erythropolis* do not produce GD-FAlDH but a so-called NAD/Factor-dependent formaldehyde dehydrogenase (FD-FAlDH) [6,7]. Comparison of the properties of GD-FAlDH and FD-FAlDH showed similarities and dissimilarities, the most striking difference being the inequality of GSH and Factor with respect to biological and physicochemical properties [7].

After all, the occurrance of a non-GSH-dependent formaldehyde dehydrogenase in the Gram-positives mentioned above is not so surprising. Although GSH is generally regarded as a compound with universal biological significance, several organisms are presently known which contain other low-molecular-weight thiols instead of GSH [8]. One of these is mycothiol (MySH), for which the structure, 1-*O*-(2′-[*N*-acetyl-L-cysteinyl]amido-2′-deoxy-α-D-glucopyranosyl)-D-*myo*-inositol (Fig. 1), was recently elucidated [9–11]. Since in our previous work [7], the Factor could not be replaced by known coenzymes or (co)factors but it behaved as a sugar-like compound in chromatography, and MySH has been detected in a number of Actinomycetes [8] (taxonomically, *A. methanolica* and *R. erythropolis* belong to this group), this prompted us to investigate the possibility that MySH and Factor are identical.

2. Materials and methods

2.1. Materials

MySSyM, the disulfide form of MySH, was isolated from *Streptomyces* sp. AJ 9463 [9], and Factor solution and Factor-free FD-FAlDH from 3,4-dimethoxybenzoate-grown *R. erythropolis* [6] and from methanol-grown *A. methanolica* [7]. Dithiothreithol (DTT) was included in the enzyme assay [7] to convert MySSyM into MySH (or oxidized, inactive Factor into active Factor).

2.2. HPLC of MySSyM and Factor solution

An Asahipak ODP-50 column was equilibrated with water acified with phosphoric acid to pH 2.9. Samples of MySSyM or Factor-so-

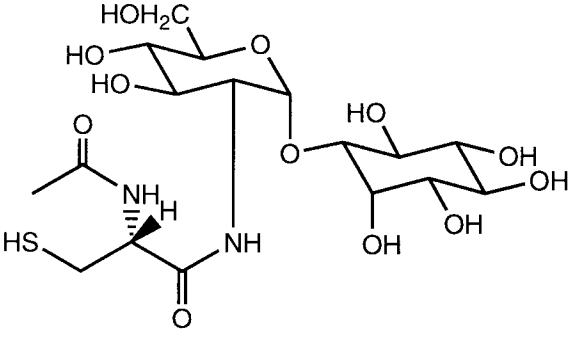

Fig. 1. Structure of mycothiol (MySH).

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Abbreviations: MySH, mycothiol (1-*O*-(2′-[*N*-acetyl-L-cysteinyl]amido-2′-deoxy-α-D-glucopyranosyl)-D-*myo*-inositol); MySSyM, the disulfide form of MySH; GD-FAlDH, NAD/glutathione-dependent formaldehyde dehydrogenase; MD-(formerly FD-)-dependent FAlDH, NAD/mycothiol-dependent formaldehyde dehydrogenase; DTT, dithiothreitol

PII S0014-5793(97)00510-3

lution were applied and elution occurred with the acidified water. The eluate was monitored with a photodiode-array detector at 220 nm. Fractions were collected to test them in the enzyme assay.

3. Results and discussion

No activity was found in the absence of MySH or Factor or in the presence of MySSyM when DTT was omitted. Substantial activity (up to 0.23 U/mg protein in the presence of 0.2 mM MySH) was observed when Factor solution or MySSyM was added. L-Cysteine, *N*-acetyl-L-cysteine or *N*-acetyl-L-cysteamine were not active. HPLC of Factor solution and MySSyM (Fig. 2) and assay of the collected fractions showed that both elute at fractions around 4.8 min. The other fractions were not active and the total amount of activity found in the fractions around 4.8 min. was similar to that applied to the column. Thus, both Factor and MySH are active in the assay and they have similar chromatographic properties (in their oxidized form). Since no other compounds active with FD-FAlDH occur in the Factor solution and GSH or cysteine and its derivatives could not replace MySH, it is concluded that Factor and MySH are identical and that the enzyme has a high specificity for MySH. It is proposed, therefore, to rename the enzyme as NAD/MySH-dependent formaldehyde dehydrogenase (MD-FAlDH). Although evidence has been provided for a role of MySH as protector against oxidative attack on the cell [8,11], this is the first example in which its role is shown as coenzyme in a dissimilation pathway step. Whether other GSH-dependent enzymes have also an MySH-dependent counterpart in Actinomycetes remains to be investigated. Since MySH but not GSH occurs [8–10] in pathogens like *Mycobacteria* and in economically important antibiotics producers like *Streptomycetes*, further studies on the coenzyme function of MSH could be rewarding with respect to drug development and production of certain antibiotics.

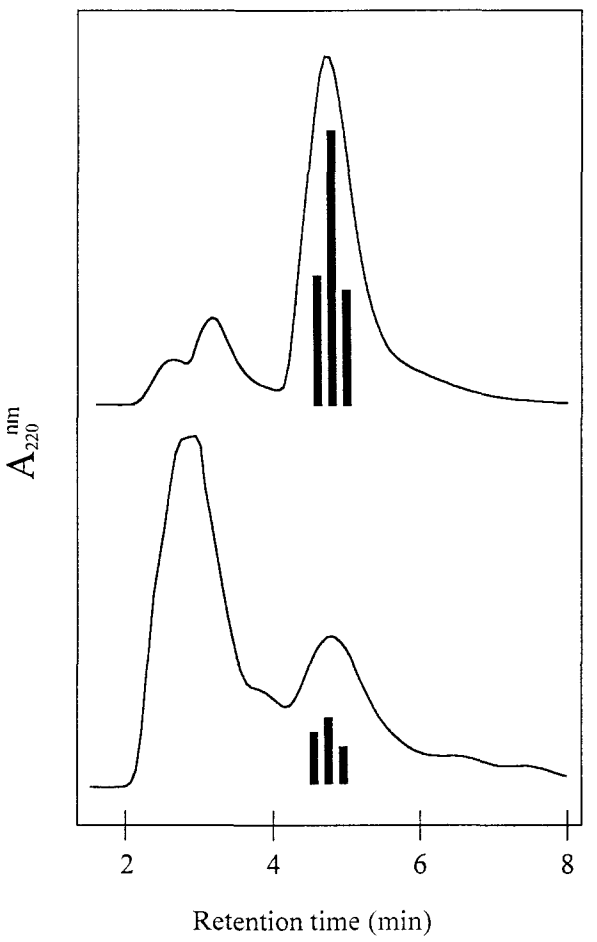

Fig. 2. HPLC chromatograms for a sample of MySSyM (curve A) and Factor solution (curve B) obtained by injecting the samples on an Asahipak ODP-50 column and eluting under isocratic conditions (water acidified with phosphoric acid to pH 2.9).

Amino acid sequence comparisons suggested that alcohol dehydrogenase class III is the ancestor of all other classes of (NAD-dependent) alcohol dehydrogenase [12]. From the amino acid sequence and the structure obtained by molecular modelling, it appears now that MD-FAlDH is distantly related to medium-chain alcohol dehydrogenases and related enzymes, linking the cluster of vertebrate alcohol dehydrogenases and that of yeast ethanol dehydrogenase and other tetrameric enzymes [13]. Thus, in addition to GD-FAlDH for removal of formaldehyde, evolutionary history generated MD-FAlDH for that purpose (and perhaps also a special esterase [7] for hydrolysis of the product, analogous to *S*-formylglutathione esterase (EC 3.1.2.12) [14], although an oxidative conversion of the product could also be possible [15]). Since ovothiols (4-mercaptohistidines) and several unknown low-molecular-weight thiols have been found in organisms lacking MySH and GSH [8,10], it is not unlikely that still other GSH coenzyme counterparts will be found in the future.

Acknowledgements: We thank Barend Groen for technical assistance, Harlof Roelen for help in the preparation of Fig. 1, and Sander Piersma for proviving *A. methanolica* MD-FAlDH.

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