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11-Hydroxythromboxane B_2 dehydrogenase is identical to cytosolic aldehyde dehydrogenase

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Abstract

11-Hydroxythromboxane B_2 dehydrogenase purified from porcine kidney has been identified as cytosolic aldehyde dehydrogenase (EC 1.2.1.3). This identification is based on protein characteristics, sequence analysis of one proteolytic digest, blocked N-terminus, subunit molecular mass of 55 kDa, and enzymatic activities. The sequence difference with the human enzyme is 7.5% in the fragments analyzed (29 exchanges of 388 positions, corresponding to the expected species variability for cytosolic aldehyde dehydrogenase). The substrate thromboxane B_2 contains a hemiacetal in its ring structure, but the reaction most likely proceeds via the aldehyde form of the substrate. This finding is in agreement with the proposed metabolism of 4-hydroxycyclophosphamide and highlights the possibility that molecules containing a hemiacetal structure can function as substrates for aldehyde dehydrogenase.

Key words: Thromboxane; Arachidonic acid; Cyclophosphamide; Aldehyde dehydrogenase; Amino acid sequence

1. Introduction

Eicosanoids encompass several groups of compounds: prostaglandins, thromboxanes, leukotrienes and lipoxins. Several of these substances are metabolized via dehydrogenase catalyzed reactions [1–3]. The most studied enzyme is the 15-hydroxyprostaglandin dehydrogenase from human placenta which is responsible for the inactivation of prostaglandins [2,3]. This enzyme has been found to belong to the short-chain alcohol dehydrogenase family [4,5].

The metabolic fate of thromboxane A_2, a potent platelet aggregator and vasoconstrictor, is nonenzymatic decomposition to the biologically inactive thromboxane B_2 (TXB_2) (Fig. 1). TXB_2 is further metabolized by two major pathways, one is β-oxidation from the carboxyl end, and the other reduction of carbon atom 11 through a dehydrogenase catalyzed reaction [3,6]. These two pathways lead to the major metabolites, 11-dehydro-TXB_2, 2,3-dinor-11-dehydro-TXB_2 and 2,3-dinor-TXB_2 (Fig. 1), which are excreted in human urine [6–10]. The major metabolite in the human circulation is 11-dehydro-TXB_2 [7,11]. The metabolites are used to quantify thromboxane production during different physiological and pathological events, such as heart attack, and one of the major treatment strategies in diseases with thromboembolic pathology is to inhibit platelet thromboxane synthesis with aspirin [12–15].

We have recently purified the 11-hydroxythromboxane B_2 dehydrogenase from porcine kidney [16]. The enzyme was found to have a subunit with a molecular mass of 55 kDa. The catalytic activity was dependent on NAD^+, showed irreversibility, and prostaglandins were not substrates for the enzyme. In the present work we have characterized the 11-hydroxy-thromboxane B_2 dehydrogenase, and identify it as cytosolic aldehyde dehydrogenase.

2. Materials and methods

2.1. 11-Hydroxythromboxane B_2 dehydrogenase

The enzyme was purified from porcine kidney as described [16] using ammonium sulphate precipitation of the proteins in the $100{,}000 \times g$ supernatant, ion exchange chromatography (DE-52, Whatman), affinity chromatography (5′-AMP-Sepharose, Pharmacia) and gel permeation chromatography (Protein Pak 125, Waters). The purity was controlled by SDS/polyacrylamide gel electrophoresis, using the Phast (Pharmacia) or Mini Protean II (Bio-Rad) systems. The purity was better than 95%.

The enzyme was tested for catalytic activity with 1 mM NAD^+ (Sigma) and 1 mM of [1-^{14}C]TXB_2 in 250 mM Tris-HCl, pH 8.0, 37°C. The labelled TXB_2 was obtained by incubation of [1-^{14}C]arachidonic acid (Amersham) with human washed platelets, and was diluted to the proper specific activity with synthetic TXB_2 (Cayman Chemical) [16]. Analysis of the enzymatic activity was performed by TLC in combination with autoradiography and scintillation counting [16].

2.2. Structural analysis

The protein was reduced with dithiothreitol and carboxymethylated with ^{14}C-labeled iodoacetate (Amersham) [17]. The radiolabeled enzyme was treated with a Lys-specific protease from Achromobactor (Vaco) for 4 h in 0.1 M ammonium bicarbonate, pH 8.0, 37°C. The digest was separated by reverse phase HPLC (Waters), applying a linear gradient of 0–60% acetonitrile in 0.1% aqueous trifluoroacetic acid for 90 min, using a C_4 column (5 μm, 250 × 4 mm, Vydac). The flow rate was 1 ml·min^{-1} and UV absorption was recorded at 214 and 280 nm.

Sequence degradations were carried out with AB1 470A and 447A sequencers (Applied Biosystems). Total compositions of the enzyme were determined with a Pharmacia Alpha Plus analyzer after hydrolysis in evacuated tubes with 6 M HCl, 0.5% phenol for 24 h at 110°C. Tryptophan was determined after hydrolysis with 4 M methane sulphonic acid for 20 h at 110°C.

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3. Results

11-Hydroxythromboxane B_2 dehydrogenase was purified from porcine kidney [16]. The enzyme obtained was pure as revealed by SDS/polyacrylamide gel electrophoresis and corresponded to a subunit molecular mass of 55 kDa. Determination of the molecular mass of the native enzyme was complicated by the fact that the enzyme lost activity in the affinity chromatography on 5'-AMP-Sepharose; the final purification step, gel permeation, has previously been interpreted to suggest that the enzyme may dissociate under loss of activity (cf. [16]).

The pure enzyme obtained was ^{14}C-carboxymethylated and submitted to structural analysis. Attempts at direct sequence analysis did not produce any results, showing that the N-terminus is blocked. Cleavage with a Lys-specific protease and subsequent peptide separations by HPLC produced peptides suitable for analysis and 35 peptide sequences were determined in 24 different fractions (Fig. 2) accounting for a total of 388 residues. These results identify the 11-hydroxythromboxane B_2 dehydrogenase as identical to cytosolic aldehyde dehydrogenase, with a residue identity of 92.5% between the porcine protein and human cytosolic aldehyde dehydrogenase [17]. Furthermore, total compositions are similar (Table 1), the present N-terminus is blocked like that of cytosolic aldehyde dehydrogenase (acetylated, cf. [17]), and subunit molecular masses are identical, around 55 kDa by SDS/polyacrylamide gel electrophoresis for the present enzyme vs. 54.8 kDa for the human enzyme [18]. Hence, although a few segments were not determined, compatible with the fact that a single digest was analyzed (Fig. 2), it can be safely concluded that porcine 11-hydroxythromboxane B_2 dehydrogenase corresponds to full-length cytosolic aldehyde dehydrogenase with a species variability of about 7.5% vs. the corresponding human enzyme. This value is smaller than the horse/human variability (9% cf. [19]) for this enzyme, thus further strengthening the argument of 11-hydroxythromboxane B_2 dehydrogenase and cytosolic aldehyde dehydrogenase as identical.

The cysteine at position 302, believed to be the catalytic centre, is conserved as expected. The amino acid substitutions compared to the human enzyme are evenly distributed (Table 2) except within the region 160–280 which showed relatively few positional differences. Only one exchange of those established (Table 2) requires a 2-base difference (Met/Asn at position 120).

The identification of 11-hydroxythromboxane B_2 dehydrogenase as cytosolic aldehyde dehydrogenase is a novel finding, revealing molecular properties of the little-studied 11-hydroxythromboxane B_2 dehydrogenase and adding a further functional role to the more well-studied aldehyde dehydrogenase. The identity is also proven by

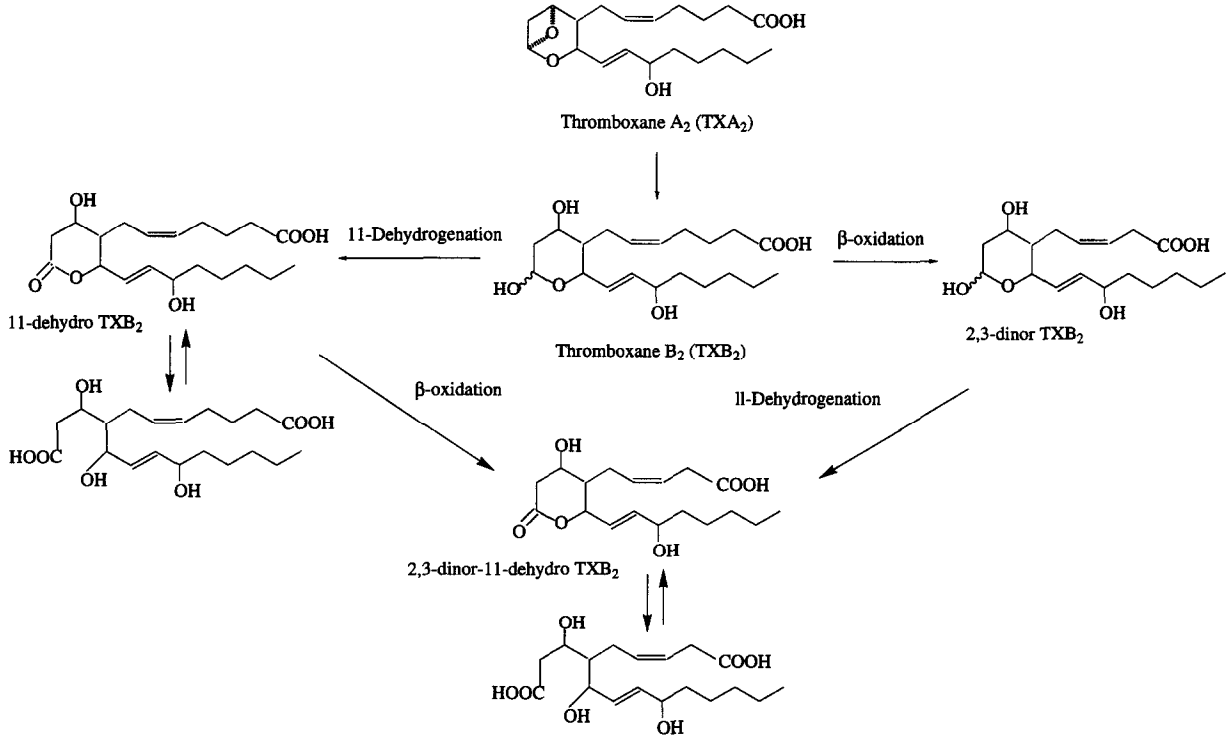

Fig. 1. Major metabolic pathways for thromboxanes. Thromboxane A_2 (TXA_2) decomposes to thromboxane B_2 (TXB_2) which is further metabolized via β-oxidation and/or dehydrogenation. Whether the metabolite 2,3-dinor-11-dehydro-TXB_2 is formed via the two possible pathways or only one of them is not known.

enzymatic properties. Thus, the 11-hydroxythromboxane B_2 dehydrogenase preparation had an enzymatic activity for acetaldehyde and propanal typical of cytosolic aldehyde dehydrogenase. Furthermore, TXB_2 is really a hemiacetal, and upon ring opening gives rise to an aldehyde (Fig. 3) which is likely to constitute the true substrate for the enzyme (cf. section 4).

4. Discussion

This study identifies the 11-hydroxythromboxane B_2 dehydrogenase as identical to cytosolic aldehyde dehydrogenase, clarifying the mechanism of the 11-hydroxythromboxane B_2 dehydrogenase and proving additional important functions for the aldehyde dehydrogenase. Three points are of special interest and constitute important facts for further investigations.

4.1. Catalytic mechanism

The catalytic mechanism for the conversion of TXB_2 to 11-dehydro-TXB_2 by aldehyde dehydrogenase is probably explained by the hemiacetal structure formed between carbon atom 11 and 12 in the ring system of TXB_2 (Fig. 3). TXB_2 can exist in three forms, two anomers with the 11-hydroxyl group in α or β-position, and the aldo form with an open ring structure. The aldo form of TXB_2 is the most likely substrate candidate. There are only a few studies dealing with the conformation of TXB_2 in different solutions. However, it is dependent on the solvent. Kotovych and Aarts [20] reported 76% 11α-OH TXB_2 and 24% 11β-OH TXB_2 in $CDCl_3$. However, in CD_3OD the predominant anomer was 11β-OH (80%). The same authors did not report any aldehyde form of the molecule. However, it is a continuous equilibrium between the two forms [21]. The relatively high K_m and low V_{max} values for TXB_2 (cf. [16]), most likely depends on the fact that only a small portion of the substrate exists in the aldo form.

4-Hydroxycyclophosphamide, the metabolite of cyclophosphamide, has also been shown to be a substrate for aldehyde dehydrogenase; for review see [22]. The structure of 4-hydroxycyclophosphamide resembles that of TXB_2, with three possible conformations. In this case the aldo form, aldophosphamide, is regarded as the substrate for aldehyde dehydrogenase [22], further supporting the aldo form of TXB_2 as the suitable substrate for aldehyde dehydrogenase.

Another similarity exists between the metabolism of 4-hydroxycyclophosphamide and TXB_2. After dehydrogenation the product can exist in two interconvertable conformations, an acyclic form and a δ-lactone or δ-lactam. This has clearly been demonstrated for 11-dehydro-TXB_2 with a preference for the δ-lactone at acidic pH and the open ring form at basic pH (Fig. 1) [23]. A similar interconversion between carboxyphosphamide and 4-ketocyclophosphamide is not believed to take place, although this has not been studied in detail [22].

Our findings together with the metabolic data earlier reported for 4-hydroxycyclophosphamide highlight a novel group of substrates for aldehyde dehydrogenase, although not previously recognized from the conformation of the substrate, hemiacetal, or of the product, δ-lactone (Figs. 1 and 3).

4.2. Metabolic interaction

The identification of 11-hydroxythromboxane B_2 dehydrogenase as cytosolic aldehyde dehydrogenase further stresses the importance of measurements of metabolites from the two pathways (Fig. 1) when estimating thromboxane biosynthesis in vivo, since several endogenous and exogenous (cf. above) compounds might interfere with the dehydrogenase-catalyzed reaction and thus influence the recorded urinary and blood levels of both 11-dehydro-TXB_2 and 2,3-dinor-TXB_2. Relying on only one parameter might lead to a false interpretation of the data. Thus, measurements of an increase or decrease of one metabolite might reflect altered metabolism and not a change in the biosynthesis of the biologically important compound, TXA_2 (Fig. 1).

In in vitro studies the organ distribution and propor-

Table 1
Total composition of 11-hydroxythromboxane B_2 dehydrogenase after acid hydrolysis of the carboxymethylated porcine enzyme

Amino acid	11-Hydroxythromboxane B_2 dehydrogenase (porcine) (mol/mol)	Cytosolic aldehyde dehydrogenase (human) (mol/mol)
Cys (Cm)	9.4	11
Asx	45.9	46
Thr	25.9	28
Ser	30.8	29
Glx	51.9	50
Pro	27.2	24
Gly	49.1	48
Ala	40.3	38
Val	34.3	38
Met	11.4	8
Ile	32.1	35
Leu	39.8	37
Tyr	14.2	16
Phe	23.4	22
Trp	5.4	7
Lys	38.6	38
His	8.8	8
Arg	16.2	17
Sum		500

Values are from acid hydrolysis with 6 M HCl/0.5% phenol for 24 h at 110°C, and for tryptophan, hydrolysis with 4 M methane sulphonic acid for 20 h at 110°C; all values without corrections for destruction, incomplete release or impurities. For comparison, the total composition of human liver cytosolic aldehyde dehydrogenase is also given (from sum of amino acid sequence [17]).

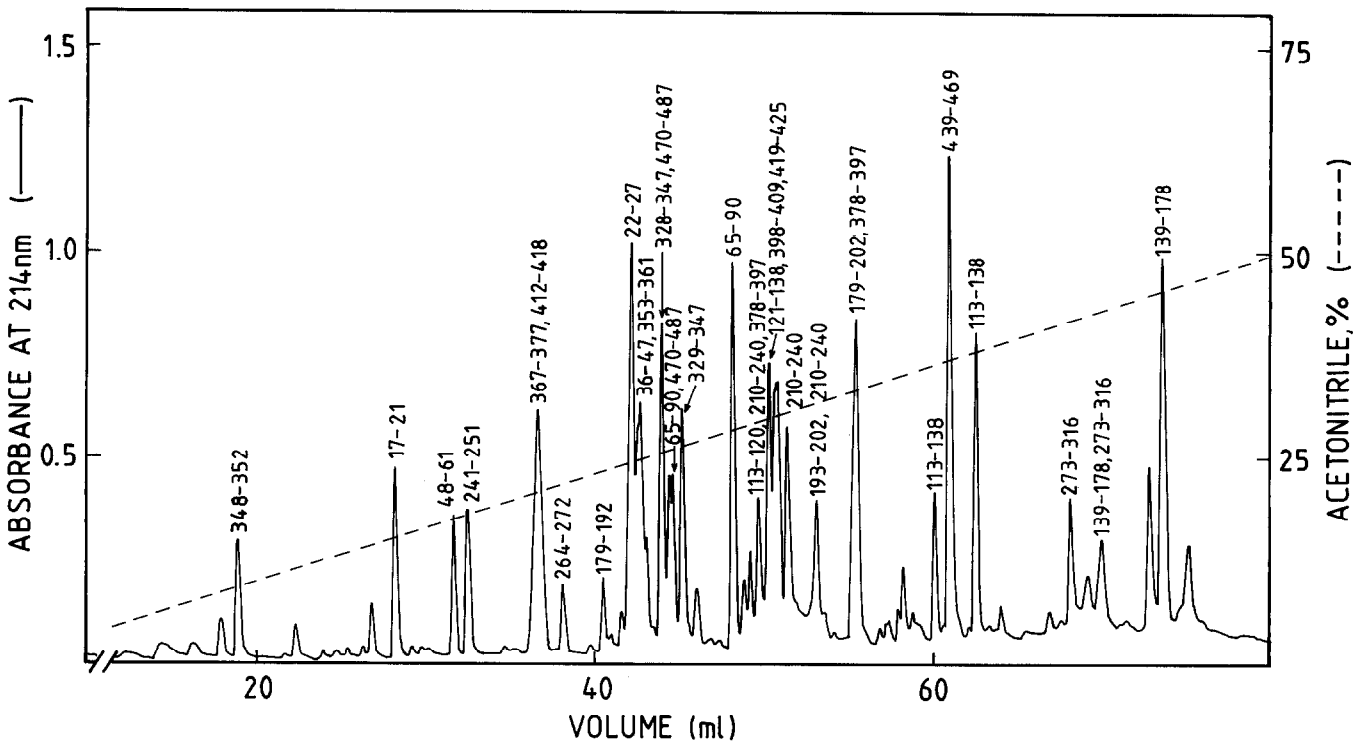

Fig. 2. HPLC chromatogram from the purification of the peptides obtained after digestion of 11-hydroxy-thromboxane B_2 dehydrogenase with a Lys-specific protease. The start and stop positions in the aldehyde dehydrogenase structure [17] of all peptides analyzed from each fraction are indicated by the numbers.

tion of the different metabolizing systems, β-oxidation and 11-hydroxy-dehydrogenation, are of importance for the recorded levels of the metabolites. The organ distribution of 11-hydroxythromboxane B_2 dehydrogenase has only been reported for one species [24]. In the rabbit, the lung showed the highest activity followed by the kidney, stomach, liver, aorta, spleen and heart, whereas the brain showed no detectable activity. Although the distribution of cytosolic aldehyde dehydrogenase often is regarded as predominating in liver, it also has an overall similar distribution. Noticeably, the organ distribution of alcohol dehydrogenase class I [25], which in gen-

Table 2
Sum of established positional differences between 11-hydroxythromboxane B_2 dehydrogenase now analyzed and cytosolic aldehyde dehydrogenase previously known, corresponding to species variants between porcine and human forms of 11-hydroxytromboxane B_2 dehydrogenase/cytosolic aldehyde dehydrogenase

Position	Porcine	Human	Position	Porcine	Human
17	Phe	Ile	285	Ala	Asn
47	Lys	Glu	294	Leu	Val
50	Glu	Gln	308	Leu	Ile
86	Gln	Arg	316	Asn	Asp
89	Asn	Tyr	330	Val	Ile
114	Phe	Tyr	340	Asn	Thr
120	Met	Asn	351	Glu	Asp
123	Gly	Ala	381	Ile	Val
127	Arg	Lys	424	Tyr	Phe
140	His	Gln	442	Val	Ile
146	Met	Ile	448	Ser	Ala
156	Ser	His	459	Leu	Val
238	Val	Ile	462	His	Gln
248	Gln	Glu	482	Leu	Phe
278	Phe	Leu			

Differences shown are those established after analysis of 388 positions, covering the following positions of the human enzyme : 17–27, 36–61, 65–90, 113–202, 210–251 (235 and 239 incompletely confirmed), 264–316, 328–361, 367–409, 412–425 and 439–487.

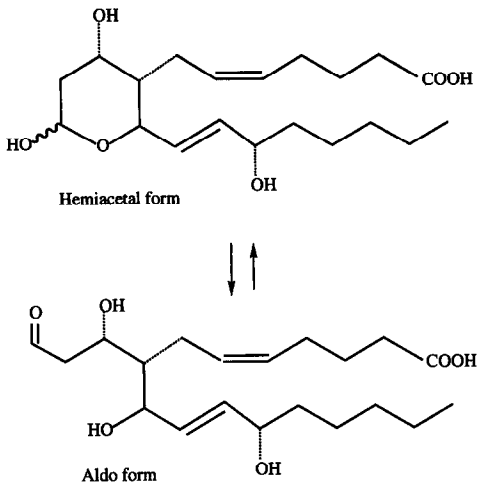

Fig. 3. Conformations of thromboxane B_2. Because of the hemiacetal structure within the ring of TXB_2 the molecule can exist in three different forms, the 11-α and 11-β anomers, and the aldo form. It is a continuous equilibrium between the different forms and the proportions of the isomers are dependent on the solvent [20,21].

eral is believed to follow the distribution of cytosolic aldehyde dehydrogenase, shows a pattern similar to that of 11-hydroxy-thromboxane B_2 dehydrogenase.

4.3. Enzyme structure

The cytosolic aldehyde dehydrogenase from liver of different species are all tetramers and an N-terminal segment has been suggested to play a role in subunit interaction [26].

The enzyme described in the present paper was previously regarded as a monomer, based on behaviour upon gel permeation chromatography. However, the chromatography was also dependent on ionic interactions which influenced the results [16]. The present identification therefore supports the notion that native 11-hydroxy-thromboxane B_2 dehydrogenase may be tetrameric, like aldehyde dehydrogenase, and that the quaternary structure is influenced by environmental interactions [16].

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