Cell. Mol. Life Sci. 63 (2006) 1205–1213
1420-682X/06/101205-9
DOI 10.1007/s00018-006-6013-y

Cellular and Molecular Life Sciences

Research Article

Hep27, a member of the short-chain dehydrogenase/reductase family, is an NADPH-dependent dicarbonyl reductase expressed in vascular endothelial tissue

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Received 16 January 2006; received after revision 28 February 2006; accepted 31 March 2006
Online First 10 May 2006

Abstract. Human Hep27 was originally isolated from growth-arrested HepG2 cells and identified as a member of the superfamily of short-chain dehydrogenases/reductases (SDR). Its substrate specificity has not been determined, but a cross-species comparison suggests that it occurs in widely divergent species, such as human, *Cenorhabditis elegans*, *Drosophila* and *Arabidopsis thaliana.* In this study, Hep27 was expressed as a His_6 fusion protein, and subjected to a substrate screen, using a compound library of SDR substrates, comprising steroids, retinoids, sugars and carbonyl compounds. Whereas no steroid dehydrogenase or retinoid activity was detected, it was found that Hep27 catalyzed the NADPH-dependent reduction of dicarbonyl compounds, like 3,4-hexanedione and 1-phenyl-1,2-propanedione with similar turnover numbers as DCXR (a mitochondrial dicarbonyl reductase/xylulose reductase). In contrast, Hep27 does not convert sugar substrates like xylulose or threose. Based on its substrate specificity and expression in endothelial tissues, it is suggested that Hep27 functions as a dicarbonyl reductase in enzymatic inactivation of reactive carbonyls, involved in covalent modification of cellular components.

Keywords. Hep27, carbonyl reductase, short-chain dehydrogenase/reductase, dicarbonyl compounds

Introduction

Hep27 (gene symbol DHRS2) was originally identified as a protein specifically regulated in the G1 phase of the cell cycle [1]. It was isolated from the nuclear fraction of the hepatoblastoma cell line HepG2, cloned, and found to be a member of the superfamily of short-chain dehydrogenases/reductases (SDR) [2]. Members of this family are mainly oligomeric enzymes with about 250–350-residue subunits. SDR members share a few distinct sequence motifs, comprising conserved nucleotide cofactor and active site residues [3–5], with a typical overall residue identity of 15–30%. Despite a low level of conservation (often 15–30%), enzymes of this family show a conserved α/β sandwich folding pattern [3–5]. Accordingly, SDR enzymes are mostly NAD(P)(H)-dependent dehydrogenases/reductases, acting on a heterogeneous set of substrates, including steroids, retinoids, prostaglandins,

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sugars and xenobiotics. However, the enzyme activities are not restricted to oxidoreductions, since several members also display isomerase or lyase activities [3]. At present, well over 3000 SDR primary structures from all life forms are deposited in sequence databases, and over 60 SDR members were identified within the human genome [6].

In a previous investigation, we identified Hep27 as a prototype of one of eight conserved clusters found in a genome-wide cross-species comparison of SDR enzymes between human, *Drosophila*, *Cenorhabditis elegans* and *Arabidopsis thaliana* [6]. Within this cluster, three distinct human proteins are found (Fig. 1): the mitochondrial dicarbonyl reductase/xylulose reductase DCXR (GI:7705925), the peroxisomal enzyme SRL (GI:12804321), and Hep27. Out of these, only the substrate specificity of DCXR has been determined thus far [7], displaying activity towards several dicarbonyl compounds and sugars.

The nuclear localization of Hep27, its chromosomal localization at 14q11.2, a region characterized by loss of heterozygosity in several human tumors, and high levels of expressed Hep27 in some tumor types suggested a possible function in tumorigenesis [8, 9]. Accordingly, it was postulated that the underlying mechanism and involvement of Hep27 implied activation of nuclear hormone receptor ligands (steroids, retinoids), resulting in the malignant phenotype. These suggestions make it important to clarify the substrate profile and expression pattern of Hep27.

Material and methods

Cloning, protein expression and purification of human Hep27. Expression constructs were obtained by cloning human Hep27 [2] into the pET15b vector (Novagen) by PCR with gene-specific primers using a cDNA library prepared from the hepatoblastoma cell line HepG2. The expression plasmid codes for an N-terminal His_6-tagged protein [10] containing an internal thrombin cleavage site. The entire construct sequence was verified by analysis on an ABI 377 system. The expression plasmid was transformed into *E. coli* strain BL21(DE3) pLysS, grown at 37 °C, and recombinant proteins were expressed by isopropylthiogalactoside induction at an OD of 0.6 for 2 h. Cells were harvested, lysed by sonication, and recombinant proteins were purified by immobilized metal-ion-affinity chromatography (IMAC) on His-bind resin (Novagen). Thrombin protease (Amersham Pharmacia Biotech) was used to cleave the His-tag, and protein was further purified by anion exchange chromatography. Purity was confirmed by SDS-PAGE, and protein concentrations were determined spectrophotometrically or by compositional analysis on a Biochrome amino acid analyzer after hydrolysis of samples in 6 M HCl, 0.1% phenol. Assessment of protein conformation was achieved using circular dichroism spectroscopy by recording the ellipticity as a function of wavelength between 260 and 195 nm using an AVIV Model 62 DS spectropolarimeter.

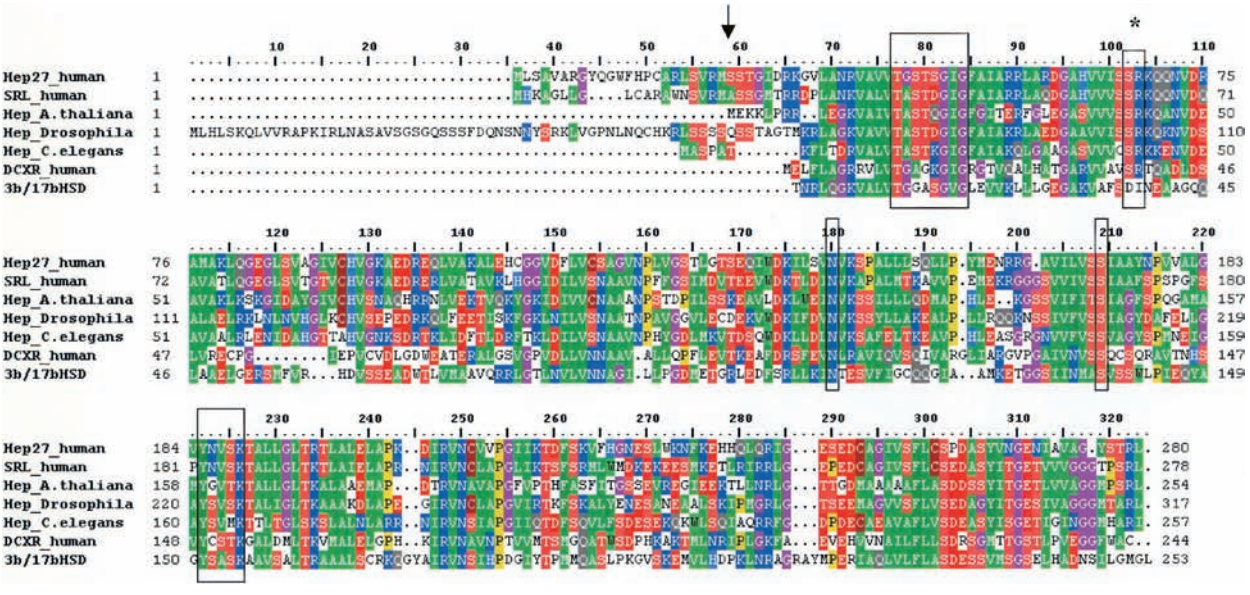

Figure 1. Sequence alignment of human Hep27 and related SDR enzymes. Sequences shown are human Hep27 (GI:5031737), peroxisomal SRL (GI:12804321), human DCXR (GI:7705925), forming a conserved cluster with Hep_*Drosophila* (GI:21355319), Hep_C.elegans (GI:17560676) and Hep_A.thaliana (GI:18412959). For comparison, bacterial 3β/17β-hydroxysteroid dehydrogenase (1hxh) is included. Boxing indicates sequence motifs and conserved residues found in SDR enzymes. Arrow points to the mature N terminus as observed from sequence analysis of isolated Hep27 [1, 2], also used for recombinant expression in *E. coli*. The asterisk indicates the residues important for cofactor specificity. NAD(H) specificity is determined by an acidic residue (D at position 37 in 3β/17β-HSD), whereas NAD(P)(H)-dependent enzymes have no acidic residue at this position, and a basic residue at +1 (R at position 39 in human DCXR).

Substrate screening and determination of kinetic constants for Hep27. Enzyme activities were measured as NAD(P)(H)-dependent conversions using a set of substrates comprising about 60 different steroids, retinol derivatives, and aliphatic carbonyl and sugar compounds (Table 1). Reactions were measured at 100 µM substrate concentrations, and were performed in 1.0 ml at 25 °C. Activities were recorded by determination of the change of absorbance at 340 nm, using a molar extinction coefficient for NADH of 6.22 mM^{-1} cm^{-1}. Recordings were carried out with a Cary 300Bio instrument. Retinoid activities were analyzed by HPLC using an isocratic eluent system (85% acetonitrile) on C18 columns (Waters) at 373 nm [10]. Product formation of steroid activities determined by UV spectroscopy (above) was verified by gas chromatography-mass spectrometry (GC-MS) [11]. Experiments were carried out at varied substrate concentrations with saturating cofactor concentrations. The pH profiles were obtained using a set of 100 mM phosphate buffers ranging from pH 5 to 10, and under the conditions employed no change in pH was observed. Kinetic constants were calculated from initial velocity data by direct curve fitting using non-linear or linear regression analysis (GraphPad).

Expression analysis of Hep27. The expression pattern of Hep27 in human tissues was analyzed by Northern blot hybridization and reverse-transcriptase PCR (RT-PCR) using mRNA isolated from different tissues. For Northern blot analysis, an array of human tissue preparations (multiple tissue blots, normal and cancer tissues, Clontech) were used. Endothelial expression analysis was performed by Western and Northern blot, using homogenates for SDS-PAGE and RNA, isolated from primary human cells (saphena magna vein, umbilical vein) and the microvascular endothelial cell line HMEC [12, 13]. Antibodies against Hep27 were obtained by immunizing a rabbit with full-length, SDS-denatured human Hep27. RT-PCR was carried out using M-MLV reverse transcriptase and a cDNA library primed with a set of random $pd(N)_6$ oligonucleotides (Gibco Life Sciences).

Cell culture. COS-7 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) fetal calf serum (FCS), 100 U/ml penicillin, 100 mg/l streptomycin and 2 mM L-glutamine. HepG2 cells and HMEC were grown in minimum essential medium and MCDB131 cell medium, respectively, as described [2]. Cells were grown in the presence of antibiotics (ampicillin or gentamicin) at 37 °C in a humidified 5% CO_2 atmosphere. HepG2 cells were obtained from American Tissue Culture Collection, primary human endothelial cells (saphena magna and umbilical veins) were obtained after vascular surgery or delivery, and HMEC were cultivated as described [12].

Transfection and expression of Hep27 in COS-7 cells. COS-7 cells were seeded into 100-mm petri dishes (3×10^7 cells/dish) and transfected with 10 µg Hep27 plasmid DNA subcloned into the mammalian expression vector pcDNA3.1 (Invitrogen) using the FuGENE 6 transfection reagent (Roche Molecular Biochemicals). Control cells were transfected with equal amount of the parental vector alone. Cells were lysed 24 h post-transfection and harvested with a rubber scraper. Later cells were collected by a low speed centrifugation and then resuspended in hypotonic buffer (10 mM Tris-HCl, pH 7.5, containing 1 mM phenylmethylsulfonyl fluoride), put on ice for 20 min, and finally homogenized using a Dounce homogenizer. The remaining cell debris was pelleted at 14 000 rpm for 15 min. The supernatant was collected and the remaining pellet was dissolved in 10 mM Tris-HCl.

HPLC analysis of retinoids in cells overexpressing Hep27. To determine the enzymatic activity of Hep27 with retinoids, 5–7 µg of either cell lysate, supernatant or cell pellet from the infected COS-7 cells in a 10 mM Tris–HCl, pH 7.4 buffer were incubated with 50 µM all-trans retinol, all-trans retinal, 9-*cis* retinol and 9-cis retinal. In order to not be restricted by the cofactor, an excess of cofactors was used (500 µM). NAD and NADP were used for 9-*cis* retinol and all-trans retinol, and NADH and NADPH for 9-cis retinal and all-trans retinal, respectively. After a 30-min incubation at 37 °C, samples ware extracted with hexane twice. All previous steps were done in the dark with minimal red light illumination. The organic phase was carefully removed and analyzed using a Waters Alliance 2690 HPLC system connected to a C18 reversed-phase column (Supelco; 4.6×250 mm). The mobile phase was acetonitrile-water (85:15, vol/vol). Elution was at 1.0 ml/min, and the effluent was monitored with a spectrum between 280 and 400 nm. Retinol was quantified at 320 nm and retinal at 370 nm.

GC-MS. For GC-MS analysis, steroids were converted into volatile methyl ester trimethylsilyl (TMS) ether derivatives, as described previously [14]. Compounds were separated isothermically at 280 °C on a fused-silica capillary column coated with 100% cross-linked methyl silicone (HP-1; Hewlett–Packard, Wiesbaden, Germany). Steroids were identified by comparisons with authentic compounds and retention indices. For GC-MS, derivatives were automatically injected into 1 µl hexane at 180 °C in splitless mode. The temperature was taken to 220 °C at 20 °C/min and then to 315 °C at 4 °C/min. GC and GC-MS were performed on Hewlett–Packard HP 6890 ChemStation instruments.

Table 1. Substrate screening of human Hep27 activity with different retinoids, steroids, bile acids, carbonyl compounds and sugars. For retinoids, assays of Hep27 expressed in *E. coli* or COS-7 cells were employed, and for steroids, bile acids, sugars, carbonyl compounds, *E. coli* expressed recombinant Hep27 was used. Cofactors used to measure dehydrogenase and reductase activities were NAD^+/NADP and NADH/NADPH, respectively. Recordings were carried out at cofactor concentrations ranging from 100 µM to 1.0 mM and substrate from 1 µM to 100 µM.

Substrate	Activity	Hep27
Retinoids:		
All-trans retinol	All-trans retinol-DH	na
All-trans retinal	All-trans retinal-Red	na
9-Cis retinol	9-Cis retinol-DH	na
9-Cis retinal	9-Cis retinal-Red	na
Steroids:		
Androsterone	3α-DH	na
Testosterone	17β-DH	na
Testosterone	3oxo-Red	na
20α-Hydroxyprogesterone	20α-DH	na
5α-Pregnan-3β,20α-diol	3β/20α-DH	na
5α-Pregnan-20β-ol-3-one	20β-DH	na
5β-Pregnan-3α,20β-diol	3α/20β-DH	na
5α-Androstan-3β,17β-diol	3β/17β-DH	na
Cortisol	11β-DH	na
Dehydroepiandrosterone	3β-DH	na
7α-Hydroxy DHEA	7α-DH	na
7β-Hydroxy DHEA	7β-DH	na
5-Androsten-3β,7β,17β-triol	3β/7β/17β-DH	na
Androstenedione	3/17oxo-Red	na
5α-Androstanedione	3/17oxo-Red	na
Estradiol	17β-DH	na
Ursodeoxycholic acid (150 µM)	7β-DH	55 nmol/min.mg
Isoursodeoxycholic acid (150 µM)	7β-DH	76 nmol/min.mg
Deoxycholic acid	3α/12α-DH	na
Glycodeoxycholic acid	3α/12α-DH	na
7α- Hydroxycholesterol	3β/7α-DH	na
7β-Hydroxycholesterol	3β/7β-DH	na
Miscellaneous Carbonyls:		
Acetone	CR	na
Pyridine-3-aldehyde	CR	na
Pyridine-4-aldehyde	CR	na
4-Nitroacetophenone	CR	na
Menadione	CR	na
2-Propanol	DH	na
Ethanol	DH	na
Methanol	DH	na
Cyclohex-2-en-1-ol (CHX)	DH	na
(S)-Indan-1-ol	DH	na
(R)-Indan-1-ol	DH	na
3,4-Hexanedione (1.5mM)	CR	353 nmol/min.mg
2,3-Heptanedione (2mM)	CR	637 nmol/min.mg
1-Phenyl-1,2-propanedione (1.5mM)	CR	516 nmol/min.mg
Methylglyoxal	CR	na
DL-Glyceraldehyde	CR	na
DL-Glyceraldehyde	CR	na
D-Threose	DH	na
D-Sorbitol	DH	na
D-Fructose	DH	na
D-Maltose	DH	na
L-Arabinose	DH	na
Sucrose	DH	na
Ribitol	DH	na
Xylitol	DH	na

na: no activity; CR: carbonyl reductase; DH: dehydrogenase; Red: reductase.

Results

Features of human Hep27. Large conservation of a Hep27 and related proteins in *Homo sapiens*, *Drosophila melanogaster*, *C. elegans*, and *A. thaliana* ([6], Fig. 1) indicates an essential role of all these proteins within the organisms. Thus far, we have identified about 70 SDR genes in humans, and 3 of these cluster closely together, namely Hep27, mitochondrial DCXR and peroxisomal SRL ([6], Fig. 1). From the SDR alignment shown in Figure 1, the typical features of enzymes of this family, active site motifs and conserved amino acid residues follow the classical pattern [3, 15, 16], and are present in Hep27. Specifically, human Hep27 is predicted to have a cofactor specificity towards NADP(H) and falls into the cP2 classification of SDR enzymes as identified through the highly conserved residue Arg68 (Fig. 1) [15, 16]. Based on this alignment both SRL and DCXR are also classified as NADPH-dependent reductases, highlighted through the absence of an acidic residue at positions 64 [SRL] and 38 [DCXR] [fingerprint for NAD(H) specificity], and the presence of Arg at positions 65 (SRL) and 39 (DCXR) (Fig. 1).

Determination of coenzyme specificity, substrate profile and kinetic analysis of human Hep27. A construct encoding human Hep27 with a start site at Ser59 (Fig. 1) was used for expression in *E. coli*. IMAC purification from induced cultures resulted in an apparently homogeneous enzyme preparation with a molecular mass of 28 kDa, as judged from SDS-PAGE (Fig. 2a), in accordance with the theoretical mass of 27.4 kDa. The material obtained was subjected to N- and C-terminal sequence analysis, demonstrating the expected sequences at the N and C termini. Additionally, the correct structure was supported by compositional analysis, which was also used for determination of protein concentrations. Circular dichroism spectroscopy revealed characteristics of a folded protein, documented by the absence of a minimum at 190 nm and the presence of minima between 220 and 230 nm (Fig. 2b). Having established purification of an apparently homogeneous and folded enzyme preparation, a substrate screen of Hep27 was performed. A small library (containing about 100 compounds) of possible SDR substrates was used, comprising four different classes of compounds, *i.e.* steroids, retinoids, sugars and miscellaneous xenobiotic carbonyl compounds (Table 1). The substrate screens were performed by recording the change of absorbance at 340 nm at a potential substrate concentration of 100 μM, using NAD(H) and NADP(H) as cofactors in the reactions.

Using this method, it was found that Hep27 converts dicarbonyl compounds like 3,4-hexanedione, 2,3-heptanedione and 1-phenyl-1,2-propanedione in an NADPH-dependent manner (Table 1). No other activity towards xenobiotic or sugar compounds was found, and NAD(H) was not found to promote electron transfer under any conditions. Likewise, no activity towards retinoids was detected, and among the steroids tested only the bile acids ursodeoxycholic acid (UDCA) and isoursodeoxycholic acid (isoUDCA) were converted at position 7β with a minor activity (10% of 2,3-heptanedione activity). No activity towards 7-oxygenated steroids, oxysterols, 7α-OH DHEA, 7β-OH DHEA or other vertebrate-type steroids at positions 3, 11, 17, 20 or 21 was detectable (Table 1). , this screen establishes Hep27 as a novel NADPH-dependent dicarbonyl reductase.

Determination of kinetic constants for Hep27. Using 2,3-heptanedione as substrate, the pH optimum of Hep27-catalyzed reduction was found to be pH 6.5 (Fig. 3a), in line with the chemical mechanism of the conserved Tyr185 functioning as an acid-base catalyst [5]. Kinetic constants for Hep27-mediated carbonyl reduction were

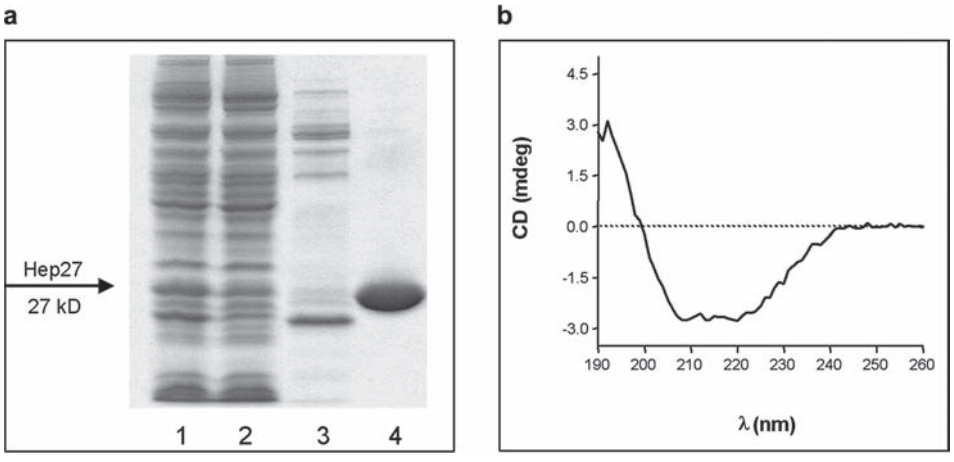

Figure 2. Characterization of recombinant human Hep27. (*a*) SDS/PAGE of purification steps of recombinant Hep27. Lane 1: total lysate, lane 2: flow through of IMAC column, lane 3: wash fraction, lane 4: elution fraction. (*b*) Circular dichroism spectrum of purified Hep27.

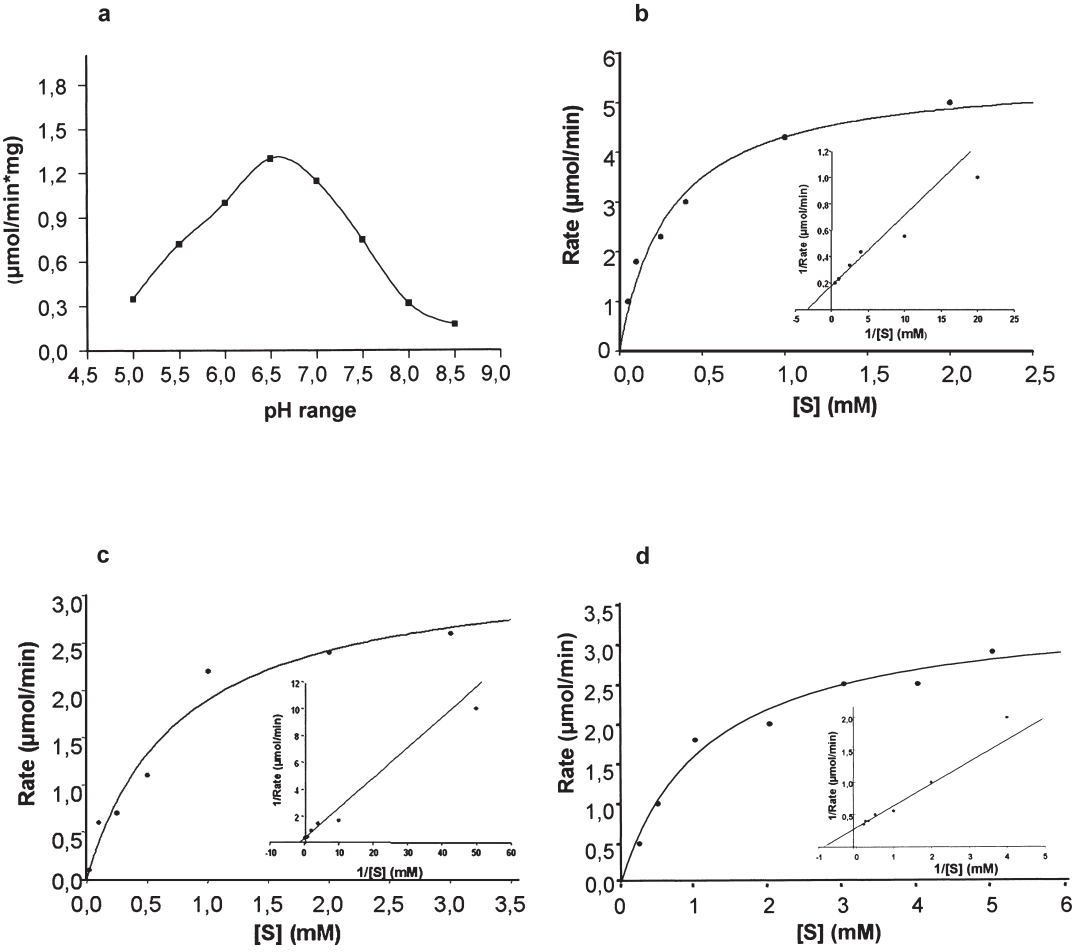

Figure 3. Kinetic characterization of recombinant human Hep27. (*a*) Determination of pH optima using a range of phosphate buffer at different pH. (*b–d*) Non-linear and linear regression analysis of Hep27 activity with 1-phenyl-1,2-propanedione (*b*), 3,4-hexanedione (*c*), and 2,3-heptanedione (*d*).

obtained. Non-linear regression analysis revealed Michaelis-Menten kinetics (Fig. 3b–d), with K_M values ranging from 0.3 to 1.1 mM for 1-phenyl-1,2-propanedione and 2,3-heptanedione, respectively, and K_{cat} values ranging from 15.2 min^{-1} (1-phenyl-1,2-propanedione) to 40 min^{-1} (2,3-heptanedione) (Table 2). Comparison of the corresponding catalytic efficiencies revealed similar K_{cat}/K_M values for 1-phenyl-1,2-propanedione and for the aliphatic dicarbonyl compounds (Table 2). Kinetic measurements at concentrations up to 200 µM were carried out with UDCA and isoUDCA, using initially the photometric method. The observed absorbance change, was verified by product analysis using GC-MS of TMS ethers (data not shown). As no saturation behavior was observed up to 200 µM concentration of UDCA or isoUDCA, no kinetic parameters could be unambiguously determined.

Table 2: Kinetic constants for human Hep27. K_M values are given in 10^{-3} M, K_{cat} values are given in min^{-1} and K_{cat}/K_M values are given in $M^{-1}min^{-1}$. Values shown are the average of three to five experiments and its corresponding standard deviation values. Measurements are performed at pH optimum of 6.5. The kinetic constants for the substrates were determined with 200 µM NADPH.

Substrate	K_M (mM)	K_{cat} min^{-1}	K_{cat}/K_M $M^{-1}min^{-1}$
1-Phenyl-1,2-propanedione	0.3 ± 0.04	15.2 ± 1.5	5.0×10^4
2,3-Heptanedione	1.1 ± 0.5	40.0 ± 8.9	3.6×10^4
3,4-Hexanedione	0.8 ± 0.3	11.7 ± 1.8	1.5×10^4

Endothelial expression of Hep27. To determine expression patterns of Hep27, Northern blot analysis of RNA isolated from multiple human tissues was carried out using a radiolabeled Hep27 probe. The expression pattern that was detected with a transcript size of about 4.4 kbp was restricted mainly to liver, to a lesser degree to kidney, and to several cell lines, like HEK, MOLT4 or HeLa (data not shown). These data largely agree with observations reported earlier [8, 9]. Surprisingly, we discovered expression of Hep27 in the microvascular human endothelial cell line HMEC by Northern blot and RT-PCR analysis (Fig. 4a, c). Western blot analysis with anti-human

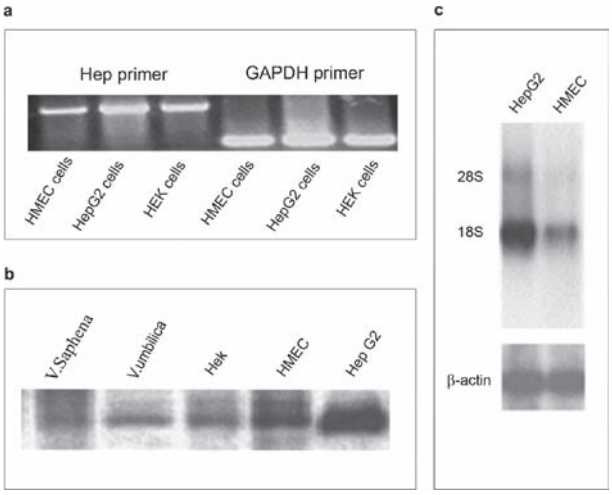

Figure 4. Expression analysis of Hep27 in endothelial tissue and different cell lines. (*a*) Reverse-transcriptase PCR and detection of Hep27 in HMEC, HepG2 and HEK cells, as control GAPDH primers were used. (*b*) Western blot of different cell homogenates probed with anti-humanHep27 antibodies. (*c*) Northern blot analysis and transcript comparison of HepG2 and HMEC cells. As loading control the blot was hybridized with a labeled probe detecting actin.

Hep27 antiserum revealed that Hep27 was also detectable as protein product, both in the endothelial cell line and in primary endothelial cells isolated under surgery or delivery (Fig. 4b).

Discussion

Hep27 is a functionally unannotated member of a conserved cluster of SDR enzymes comprising, besides Hep27, DCXR and SRL in humans, with 26% and 62% identities, respectively, towards Hep27. To define the substrate specificity of Hep27, we followed two lines. First, we used a steroid compound library, allowing for screens of known mammalian steroid dehydrogenase activities, by utilizing a large set of differentially substituted steroids of various hormone classes and bile acids. This approach was based on the assumption that the function of Hep27 is potentially similar to that of known hydroxysteroid dehydrogenases, like 3α-HSDs, 17β-HSDs or 11β-HSDs, converting nuclear hormone receptor ligands. Consequently, these steroid dehydrogenases function as a pre-receptor control mechanism and constitute important novel drug targets [17]. Despite intensive screening efforts using different assay formats (UV/VIS, HPLC, GC-MS), we only found minor 7β-OH dehydrogenase activity with UDCA and isoUDCA, which makes it, therefore, unlikely that the function of Hep27 is to control hormone levels in an analogous manner to the known steroid dehydrogenases. This distinguishes Hep27 from other related enzymes in the cluster, like mouse lung carbonyl reductase with its 3α-hydroxysteroid dehydrogenase activity.

Second, we reasoned that the other human members (SRL, DCXR) of the conserved Hep27 cluster have similar or overlapping activities with Hep27. SRL is annotated as peroxisomal retinol dehydrogenase, consequently we tested if Hep27 converts retinoids. However, no activity was found in our screens. To exclude artifactual loss of enzyme activity during IMAC purification, we expressed human Hep27 in COS7 cells and analyzed for retinoid conversion. No difference from mock-transformed cells was detected, indicating that Hep27 is not involved in retinoid metabolism. DCXR is a mitochondrial dicarbonyl/xylulose reductase with a large range of activities towards dicarbonyl and sugar compounds. These properties suggest that DCXR is involved in the uronate cycle of glucose metabolism, accounting for about 5% of the daily glucose catabolism in humans [7]. In our screen we found that Hep27 has overlapping substrate specificity with DCXR, including several reactive dicarbonyl compounds such as 3,4-hexanedione, 2,3-heptanedione and 1-phenyl-1,2-propanedione, displaying K_{cat} values of 108 min^{-1} for DCXR and 40 min^{-1} for Hep27 (substrate 2,3-heptanedione). However, in contrast to DCXR, no activity towards the aldehyde methylglyoxal was found. As opposed to DCXR, Hep27 does not accept sugar substrates like xylulose or threose. These properties make Hep27 a specific dicarbonyl reductase, in contrast to the wider substrate specificity observed with DCXR.

Reactive carbonyl compounds are generated during the course of metabolic processes, through oxidative stress in a variety of biological systems, or are present in dietary constituents [18]. According to the World Health Organization (WHO) and the International Program on Chemical Safety (IPCS), in Europe the estimated daily dietary intake of 1-phenyl-1,2 propanedione, 2,3-heptanedione and 3,4-hexanedione is 6, 2 and 33 µg/day respectively; however, no further concentration data for humans are available. When two carbonyl groups are adjacent on a carbon chain, the reactivity of each carbonyl group tends to be elevated, and those compounds with α-dicarbonyl groups are cytotoxic, *e.g.* causing mitotic chromosome loss [19], or are prone to conversion into advanced glycation end-products (AGEs) [7, 20–23]. The starting compounds are assumed to originate from carbohydrates such as glucose and fructose, or from lipids, either from dietary or endogenous sources, which then undergo a non-enzymatic Maillard reaction, ultimately leading to the AGEs through intermediates with reactive α-dicarbonyl formation. AGEs are a group of insoluble complex compounds that frequently accumulate in plasma proteins and tissues of diabetic patients [7, 20–23], and are thought to produce their intrinsic effects by stimulation of scavenger receptors, called RAGEs,

subsequently leading to an aberrant production of inflammatory cytokines. Furthermore, AGEs can cause sclerotic complications through their ability to modify extracellular matrix proteins such as collagens or laminins [21, 22, 24–26].

A detoxification mechanism against reactive α-dicarbonyls exists, comprising several members of the aldo-keto reductase (AKR) and SDR families, including aldose reductase, aldehyde reductase, dihydrodiol dehydrogenase, monomeric carbonyl reductase, DCXR and sepiapterin reductase [7]. The substrate analysis performed in the present study adds Hep27 as a novel member to this group of scavenging carbonyl reductases, involved in protection against reactive carbonyls. In this respect, the endothelial expression of Hep27 might be of particular importance. The role of Hep27 might be to protect against detrimental effects of dicarbonyl compounds, and we assume that Hep27 constitutes a first line of defense within the endothelium. Regarding the nuclear localization, further experiments are still required to unequivocally establish this subcellular localization and the mechanism of nuclear import. Our own data [9] suggest that nuclear localization is observed in a stage-specific manner, with a large fraction of transfected cells displaying cytosolic localization, which is consistent with a role as a detoxifying enzyme.

Acknowledgements. This study was supported by grants from Novo-Nordisk foundation (UO), Swedish Research Council (HJ), Karolinska Institutet (UO, HJ), and the Deutsche Forschungsgemeinschaft (grant no. Ei68/2-3 to GE).

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