The International Journal of Biochemistry & Cell Biology 41 (2009) 1079–1093

Contents lists available at ScienceDirect

The International Journal of Biochemistry & Cell Biology

journal homepage: www.elsevier.com/locate/biocel

Murine serum deoxyribonuclease 1 (Dnase1) activity partly originates from the liver

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ARTICLE INFO

Article history:
Received 12 June 2008
Received in revised form
29 September 2008
Accepted 30 September 2008
Available online 14 October 2008

Keywords:
Deoxyribonuclease 1
Liver
Serum
Hepatectomy
Gene expression

ABSTRACT

Reduction of serum DNASE1 (DNase I) activity is supposed to aggravate anti-nuclear autoimmunity, i.e. Systemic Lupus Erythematosus (SLE) in man and mice. To evaluate the etiology of this reduction, more information is needed about the source(s) and regulation of serum DNASE1. In this work we used male C57BL/6 wild-type (WT) mice to verify that serum Dnase1 activity partly depends on hepatic *Dnase1* gene expression. Thus serum and liver Dnase1 activity showed a parallel oscillatory course during 24 h, which was accompanied by a phase-shifted fluctuation of the hepatic *Dnase1* mRNA content. Performing native PAGE zymography (NPZ) we detected a presumably premature non-sialylated and a mature sialylated hepatic Dnase1 isoform, which both show a parallel circadian fluctuation, indicating continuous secretion of Dnase1. The sialylated form was also detectable in serum. By immunostaining the hepatocytes were identified as the source of hepatic *Dnase1* gene expression. After 70% hepatectomy, the serum Dnase1 activity increased markedly due to the occurrence of ischemic hepatocellular necrosis in the vicinity of the surgical suture. Similarly, hepatocellular necrosis induced by injection of streptolysin-O (SLO) into the liver led to a rapid parallel increase of Dnase1 and of aspartate- and alanine aminotransferase (AST/ALT) in serum. Subsequent to hepatectomy, *Dnase1* gene expression was up-regulated in the regenerating liver most likely leading to an enhanced serum Dnase1 level until complete regeneration. These data demonstrate that serum Dnase1 at least partly originates from the liver and hint to the possibility that natural as well as pathological hepatic conditions influence its activity.

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1. Introduction

Mammalian DNASE1 is a Ca^{2+} and Mg^{2+}/Mn^{2+}-dependent secretory endonuclease with a pH-optimum at about pH 7.0 (Laskowski, 1971). It belongs to a family of four nucleases (DNASE1 and DNASE1like1–3), which share a high sequence homology but differ in their substrate specificities and expression pattern (Shiokawa and Tanuma, 2001). DNASE1 preferentially cleaves double-stranded DNA into 3′-OH/5′-phospho-tri- and/or -tetra-oligonucleotides and is specifically inhibited by monomeric actin. However, the strength of this inhibition is species-specific due to a few amino-acid substitutions in DNASE1 (Mannherz et al., 1982). Phylogenetic analyses revealed the presence of DNASE1 in all vertebrate families (Yasuda et al., 2004). Since its discovery in bovine pancreas (Sachs, 1905), it has been regarded to be mainly a digestive enzyme. In lower vertebrates *DNASE1* gene expression is restricted to the pancreas and to a minor extend the small intestine (Mogi et al., 2003; Takeshita et al., 2001), whereas in mammals it is expressed in a number of further organs lining the gastrointestinal tract like the parotid gland, stomach and liver (Napirei et al., 2004a). According to the expression pattern within the gut, mammals can be grouped into three types: pancreas type (pig and human), parotid type (rat and mouse) and mixed pancreas-parotid type (bovine and rabbit), most probably reflecting differences in the eating habits of these species (Takeshita et al., 2000). The function of DNASE1 within the alimentary tract remains elusive,

Abbreviations: Ab, antibody; ACTB, β-actin; ALB1, albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; AP, alkaline phosphatase; DNASE1, deoxyribonuclease 1; DPZ, denaturing SDS-PAGE zymography; Endo H, endoglycosidase H; EST, expressed sequence tag; KO, knockout; mAb, monoclonal Ab; NPZ, native PAGE zymography; rhDNASE1, recombinant human DNASE1; ORF, open reading frame; PBS, phosphate-buffered saline; rER, rough endoplasmic reticulum; RT, room temperature; RT-PCR, reverse transcription with subsequent polymerase chain reaction; SDS, sodium dodecylsulfate; SLE, systemic Lupus erythematosus; SLO, streptolysin-O; TBS, Tris-buffered saline; TSS, transcription start site; WT, wild-type.

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doi:10.1016/j.biocel.2008.09.030

since *Dnase1* knockout (KO) mice do not exhibit malnutrition (own observation).

Beside saliva and intestinal juice DNASE1 is secreted into a variety of further body fluids like urine (kidney), seminal fluid (prostate), lachrymal fluid (lachrymal gland) and serum implying that DNASE1 fulfils further physiological functions (Nadano et al., 1993). Indeed, lack or reduction of serum DNASE1 activity is supposed to be involved in the induction of anti-nuclear autoimmunity (Lachmann, 2003; Napirei et al., 2006b; Tsukumo and Yasutomo, 2004). Thus, *Dnase1* KO mice exhibit an enhanced occurrence of anti-nucleosome autoantibodies and Lupus-nephritis when of the genetically SLE-predisposed mixed 129 × C57BL/6 genetic background (Napirei et al., 2000). Reduced serum DNASE1 activity was found also in many SLE-patients and in the NZB/W F1 SLE-mouse model (Napirei et al., 2006b). However, the reasons for its reduction under these instances are still unclear (Lachmann, 2003; Napirei et al., 2006b). The primary immunogen during SLE is the nucleosome (Berden et al., 2002). Therefore degradation of chromatin released during cell death by nucleases seems to be protective towards anti-nuclear autoimmunity. Indeed, it was shown that serum DNASE1 in cooperation with the plasminogen system degrades chromatin derived from dead cells (Napirei et al., 2004b). Since a reduction of serum DNASE1 often coincides with SLE, its elevation will be of therapeutic value. However, application of recombinant human DNASE1 (rhDNASE1) to SLE-patients and NZB/W F1 mice led to only minor success (Lachmann, 2003; Napirei et al., 2006b). Therefore, information about the source, regulation and inhibitory factors of serum DNASE1 activity are of interest.

So far only sparse information about the source of serum DNASE1 exists. For humans it was supposed that serum DNASE1 originates from the pancreas (Love and Hewitt, 1979). However, DNASE1 is produced by the exocrine and not the endocrine part of the pancreas (Kraehenbuhl et al., 1977), suggesting that the pancreas is not necessarily a source of serum DNASE1. The elevation of serum DNASE1 activity, which was detected in patients suffering from acute pancreatitis (Miyauchi et al., 1986), is most likely caused by damage of the exocrine glandular cells. In addition, serum DNASE1 activity can be found in all mammalian species, i.e., also in species belonging to the parotid-type (rat and mouse), which do not display *Dnase1* gene expression in the pancreas (Takeshita et al., 2000).

For rats it was described that somatostatin administration led to a reduced serum Dnase1 activity in parallel to a reduced *Dnase1* gene expression in the lower gastrointestinal tract and the pituitary gland, whereas liver *Dnase1* gene expression was not altered (Yasuda et al., 2001). These data imply that the pituitary gland and organs of the lower gastrointestinal tract (corpus of the stomach, duodenum, small and large intestine) are further sources of serum DNASE1 activity. However, for humans it was described that the age-dependent gradual decline in *DNASE1* gene expression in the pituitary gland as observed in males and in menopausal females is not accompanied by a reduced serum DNASE1 activity (Yasuda et al., 2002). These data indicate that in humans the pituitary gland cannot be a major source of serum DNASE1 activity. It therefore appears that the organs of the lower gastrointestinal tract may contribute to the overall serum DNASE1 activity.

In addition to the organs discussed above, we have demonstrated *Dnase1* gene expression within the thyroid gland and liver of mice, two organs potentially able to secrete DNASE1 into serum (Napirei et al., 2004a). The liver is a digestive gland fulfilling both exocrine and endocrine functions and is known to be the source of many serum proteins. So far, only minor information exists about hepatic *DNASE1* gene expression. For lower vertebrates the data are controversial. One group detected DNASE1 activity by DPZ in the liver of frogs (*Rana catesbeiana* and *Rana esculenta*) and fish (*Cyprinus carpio*) (Malicka-Blaszkiewicz, 1986). Other groups did not detect *DNASE1* gene expression on protein (measured by the single radial enzyme diffusion assay) and mRNA level in amphibian livers (*Rana catesbeiana*, *Xenopus laevis*, *Bufo vulgaris*, *Cynops phyrrogaster*) (Takeshita et al., 2001). Immunostaining revealed that *DNASE1* gene expression was restricted to the pancreatic islands of the carp hepatopancreas (Mogi et al., 2003). In mammals, hepatic *DNASE1* gene expression was demonstrated for mice (Napirei et al., 2004a; Takeshita et al., 1997), rats (Yasuda et al., 2001) and rabbits (Yasuda et al., 1997). The observation that infection of mice by hepatitis virus leads to an increase of hepatic and serum Dnase1 activity supports the assumption of an interdependence of serum Dnase1 activity on hepatic *Dnase1* gene expression (Giusti and Cacciatore, 1965). Furthermore, an increase of serum Dnase1 activity which occurred after acetaminophen-induced hepatocellular necrosis in WT mice supports the assumption of Dnase1 synthesis by hepatocytes (Napirei et al., 2006a).

Since the cellular source of serum DNASE1 was never investigated in detail, we assessed the interdependence of murine hepatic *Dnase1* gene expression and the level of serum Dnase1 activity. We found a parallel oscillation of hepatic and serum Dnase1 activity, and a phase-shifted oscillation of *Dnase1* mRNA transcription in the liver. Hepatectomy experiments as well as injection of the bacterial pore-forming toxin SLO into the liver revealed the passive release of Dnase1 from necrotic hepatocytes resulting in an elevated increase of serum Dnase1 activity. Furthermore, regeneration of the liver after hepatectomy was accompanied by an up-regulation of *Dnase1* gene expression and a prolonged increase of serum Dnase1 activity until complete regeneration after approximately three weeks. Since Dnase1 was shown to be a secretory protein these experiments imply that under physiological conditions Dnase1 is secreted into serum by hepatocytes. These data demonstrate for the first time that the liver is a source of serum Dnase1. However, we cannot exclude that other organs display a similar circadian fluctuation of *Dnase1* gene expression like the liver and therefore may also contribute to the serum Dnase1 activity.

2. Materials and methods

2.1. Materials

Bovine pancreatic DNASE1: Worthington (Lakewood, NJ, USA); rhDNASE1: Hoffmann La Roche (Basel, Switzerland); calf thymus DNA, oligo-dT primers, protease inhibitor cocktail and rabbit anti-actin antibody A2066: Sigma–Aldrich (Deisenhofen, Germany); Omniscript RT-Kit and RNeasy Mini Kit: Qiagen (Hilden, Germany); PCR primers: MWG-Biotech (Ebersberg, Germany); RNAlater: Ambion (Austin, TX, USA); Taq Polymerase, PageRuler^{TM} Prestained Protein Ladder, Random Hexamer primers and ϕX174/BsuRI DNA-marker: MBI Fermentas (St. Leon-Rot, Germany); endoglycosidase H (Endo H) and acetyl-neuraminyl hydrolase (sialidase): New England Biolabs (Frankfurt a.M., Germany); polyclonal chicken anti-mouse IgG (sc-2966) and chicken anti-rabbit IgG (sc-2967) antibody (Ab) conjugated with alkaline phosphatase (AP): Santa Cruz Biotechnology (Heidelberg, Germany); mouse monoclonal anti-actin Ab (Ab-1): Oncogene (Schwalbach, Germany); polyclonal rabbit anti-albumin Ab (0220-1829): Biotrend (Köln, Germany); polyclonal goat anti-mouse Ig Ab conjugated with AP (D0486), Fuchsin Substrate-Chromogen System (K0624) and 3-amino-9-ethylcarbazole (AEC): DAKO Cytomation (Hamburg, Germany); TdT *in situ* kit: R&D Systems (Wiesbaden, Germany).

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