

Research article

Open Access

Modification of carbonic anhydrase II with acetaldehyde, the first metabolite of ethanol, leads to decreased enzyme activity

Fatemeh Botorabi^{1,2}, Janne Jänis³, Jarkko Valjakka¹, Sari Isoniemi³, Pirjo Vainiotalo³, Daniela Vullo⁴, Claudiu T Supuran⁴, Abdul Waheed⁵, William S Sly⁵, Onni Niemelä⁶ and Seppo Parkkila^{*1,2}

Address: ¹Institute of Medical Technology, Tampere University Hospital, 33520 Tampere, Finland, ²School of Medicine, University of Tampere and Tampere University Hospital, 33520 Tampere, Finland, ³Department of Chemistry, University of Joensuu, 80101 Joensuu, Finland, ⁴Università degli studi di Firenze, Laboratorio di Chimica Bioinorganica, I-50019 Sesto Fiorentino (Firenze), Italy, ⁵Edward A. Doisy Department of Biochemistry and Molecular Biology, Saint Louis University School of Medicine, St. Louis, 63104 Missouri, USA and ⁶Department of Laboratory Medicine and Medical Research Unit, Seinäjoki Central Hospital and University of Tampere, 60220 Seinäjoki, Finland

Email: Fatemeh Botorabi - fatemeh.ahmad@uta.fi; Janne Jänis - janne.janis@joensuu.fi; Jarkko Valjakka - jarkko.valjakka@uta.fi; Sari Isoniemi - sari.isoniemi@joensuu.fi; Pirjo Vainiotalo - pirjo.vainiotalo@joensuu.fi; Daniela Vullo - daniela.vullo@unifi.it; Claudiu T Supuran - claudiu.supuran@unifi.it; Abdul Waheed - waheeda@slu.edu; William S Sly - slyws@slu.edu; Onni Niemelä - onni.niemela@epshp.fi; Seppo Parkkila* - seppo.parkkila@uta.fi

* Corresponding author

Published: 27 November 2008

Received: 3 September 2008

BMC Biochemistry 2008, 9:32 doi:10.1186/1471-2091-9-32

Accepted: 27 November 2008

This article is available from: <http://www.biomedcentral.com/1471-2091/9/32>

© 2008 Botorabi et al; licensee BioMed Central Ltd.

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/2.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Abstract

Background: Acetaldehyde, the first metabolite of ethanol, can generate covalent modifications of proteins and cellular constituents. However, functional consequences of such modification remain poorly defined. In the present study, we examined acetaldehyde reaction with human carbonic anhydrase (CA) isozyme II, which has several features that make it a suitable target protein: It is widely expressed, its enzymatic activity can be monitored, its structural and catalytic properties are known, and it contains 24 lysine residues, which are accessible sites for aldehyde reaction.

Results: Acetaldehyde treatment in the absence and presence of a reducing agent (NaBH₃CN) caused shifts in the pI values of CA II. SDS-PAGE indicated a shift toward a slightly higher molecular mass. High-resolution mass spectra of CA II, measured with and without NaBH₃CN, indicated the presence of an unmodified protein, as expected. Mass spectra of CA II treated with acetaldehyde revealed a modified protein form (+26 Da), consistent with a "Schiff base" formation between acetaldehyde and one of the primary NH₂ groups (e.g., in lysine side chain) in the protein structure. This reaction was highly specific, given the relative abundance of over 90% of the modified protein. In reducing conditions, each CA II molecule had reacted with 9–19 (14 on average) acetaldehyde molecules (+28 Da), consistent with further reduction of the "Schiff bases" to substituted amines (N-ethyllysine residues). The acetaldehyde-modified protein showed decreased CA enzymatic activity.

Conclusion: The acetaldehyde-derived modifications in CA II molecule may have physiological consequences in alcoholic patients.

Background

Acetaldehyde, a product of ethanol metabolism, has been suggested to play a pivotal role in the toxicity of ethanol to human tissues [1-3]. It can form covalent, stable or unstable adducts with amino acids and nucleophilic biomolecules [4-10]. The adduct formation may result in changes in the physicochemical properties of proteins, nucleic acids, and lipids, disturb normal cellular functions, and create adverse immunological responses [8,11-15].

Aldehyde incorporation can lead to the formation of several different types of protein modifications, which have been previously identified in *in vitro* studies and in studies on alcohol abusers *in vivo* [6,8,14,16,17]. Acetaldehyde reacts primarily with reactive lysine residues of preferred target proteins [18-25]. It appears that under appropriate reducing conditions, proteins with abundant amounts of reactive lysine residues are modified at acetaldehyde concentrations that may be present in tissues after alcohol intake [8,25-28]. Figure 1 depicts the common reaction mechanism of the side chain amino groups (ϵ -NH₂) of lysine residues with acetaldehyde, in the absence (A) and presence (B) of a reducing agent NaBH₃(CN). In the former case, an unstable adduct (a "Schiff base") is formed, while in the latter case a "Schiff base" is further reduced to form a stable adduct (an N-ethyllysine residue). In the absence of reducing agents, stable cyclic imidazolidinone structures are also formed in a reaction between acetaldehyde and the free α -amino group of the aminoterminal of haemoglobin [16,20,29,30]. Previous studies have identified adducts with erythrocyte membrane proteins [31], haemoglobin [5,20,32-34], albumin, transferrin and lipoproteins [14,35,36], tubulin [21], ethanol-metabolizing cytochrome P450IIE1 enzyme [37], collagens [38], and ketosteroid reductase [39].

In the present study, we used carbonic anhydrase (CA) isozyme II as a model to investigate the effects of acetaldehyde reaction with the protein. CA II is a well characterized enzyme expressed in several organs, including the

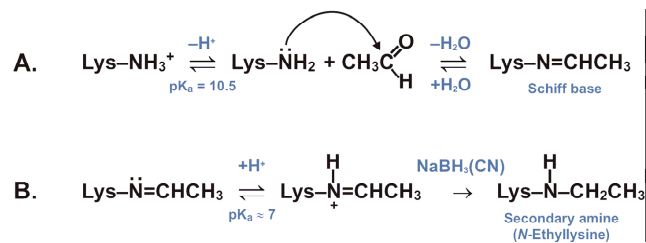


Figure 1
The reaction mechanism of lysine residues with acetaldehyde, in the absence (A) and presence (B) of a reducing agent NaBH₃(CN).

brain, stomach, gut, kidney, and reproductive organs, and it is highly abundant in erythrocytes [40]. It is one of the most efficient enzymes known in the animal kingdom, catalyzing the reversible hydration of carbon dioxide at a rate of 1.4×10^6 molecules per second [41]. CA II was our choice of model protein, because its structural and catalytic properties are well known and it contains 24 lysine residues (out of 260 amino acids), each a potential site for acetaldehyde reaction. Currently, there are also valid methods to monitor CA II catalytic activity to assess the functional consequences of acetaldehyde reaction. Importantly, CA II catalytic activity is essential for several physiological processes such as gastric acid formation, alkalization of pancreatic juice and bile, renal proton secretion, bone resorption, and cerebrospinal fluid secretion [40]. Although proteomic *in vivo* evidence of this modification is still lacking, one would expect a wide variety of adverse effects in these physiological processes, if CA II activity was disturbed due to acetaldehyde reaction in alcoholic patients.

Methods

Production of recombinant human CA II

The recombinant human CA II enzyme was produced in *E. coli* [42] and purified to homogeneity using CA inhibitor affinity chromatography as described in [43].

Labelling of CA II with acetaldehyde

Human blood samples and recombinant human CA II enzyme were treated with various concentrations of acetaldehyde either in the presence or absence of a reducing agent, NaBH₃(CN). All reagents were maintained and pipeting was performed at +4°C to minimize acetaldehyde evaporation. The sample tubes containing 1/10 diluted (in H₂O) blood or CA II enzyme with or without acetaldehyde in H₂O were tightly sealed and incubated at 37°C for 2 hr. Then 10 mM NaBH₃(CN) or equal volume of H₂O was added to each sample tube, and the incubation at +37°C was continued for 22 hr. After the incubation the samples were quickly cooled down to +4°C.

Isoelectric focusing and SDS-PAGE

IEF was carried out using Novex Pre-Cast vertical IEF gels (pH 3–10) (Invitrogen, Carlsbad, CA) containing 2% ampholytes. One μg samples of recombinant CA II protein untreated or treated with 10 mM NaBH₃(CN) and various acetaldehyde concentrations were applied to each lane. The electrophoreses were performed in an Xcell SureLock™ Mini-Cell unit (Invitrogen) at a constant power of 2 W per gel for 2 hr with a voltage limit of 500 V. The polypeptides were visualized using Colloidal Blue staining kit (Invitrogen).

All the reagents for SDS-PAGE were from Invitrogen except for the protein markers that were obtained from

Bio-Rad Laboratories (Richmond, CA). The electrophoreses were performed in an Xcell SureLock™ Mini-Cell unit (Invitrogen) under reducing conditions, using NuPAGE™ 10% Bis-Tris gels. The polypeptide bands were stained using Colloidal Blue staining kit (Invitrogen).

CA activity measurements

CA catalytic activity was first determined using a slightly modified end-point titration method of Maren et al. [44,45]. Briefly the steps included: 150 ng of human CA II or 1 µl of 1/10 diluted blood sample was added to 500 µl of ice-cold assay buffer (20 mM imidazole, 5 mM Tris (BASE), 0.2 mM *p*-nitrophenol). The cuvette containing the sample and assay buffer was placed in a Lambda 35 UV/VIS (Perkin Elmer Instruments, Waltham, MA) spectrophotometer and 500 µl of ice-cold CO₂-saturated H₂O was added into the cuvette. The exact time for the yellow color disappearance was counted. In the control experiments without the CA II enzyme, the color disappeared in 50 sec. Statistical significance of the enzyme activity results was assessed using Student's *t* test and was denoted as *p* values.

The enzymatic activities of native recombinant human CA II and the enzyme treated with 100 mM acetaldehyde under reducing conditions were also assayed using an Applied Photophysics (Leatherhead, UK) stopped-flow instrument. Reaction was measured using 0.2 mM phenol red as an indicator, in 10 mM Hepes, 0.1 M Na₂SO₄, pH 7.5, for a period of 10–100 sec. To determine the kinetic parameters, CO₂ concentration ranged from 1.7 to 17 mM. Kinetic parameters were obtained from Lineweaver-Burk plots, as reported earlier, and represent the mean from at least three different determinations.

Sample preparation for mass spectrometry

Due to a low tolerance of ESI for high salt concentrations, protein samples were first desalted using a PD-10 column (Amersham-Biosciences, Billingham, UK) equilibrated in advance with 10 mM ammonium acetate (pH 6.8) buffer and ten 1-mL fractions were collected. Fractions 3–5 were combined and concentrated to ~500 µL using an Ultra-free-0.5 (10-kDa cut-off) centrifugal filter device (Millipore, Billerica, MA, USA). Protein concentrations were determined by absorbance at 280 nm using a calculated extinction coefficient of $\epsilon_{280} = 50070 \text{ M}^{-1} \text{ cm}^{-1}$. For mass spectrometry, samples were further diluted with acetonitrile/water/acetic acid (49.5:49.5:1.0, v/v) solution to an approximate concentration of 5 µM.

Mass spectrometry

All experiments were performed on a 4.7-T Bruker Bio-APEX-Qe Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer (Bruker Daltonics, Billerica, MA, USA), interfaced to an external Apollo-II™ electrospray

ionization (ESI) source. Protein samples were directly infused at a flow rate of 1.5 µL min⁻¹. ESI-generated ions were externally accumulated in an RF-hexapole ion trap for 400 ms and transmitted through a high-voltage optics region to an Infinity ICR cell for "sidekick" trapping, conventional "RF-chirp" excitation and broadband detection. For each spectrum, a total of 256 co-added (512-kWord) time-domain transients were zero-filled once prior to fast Fourier transform and magnitude calculation. Frequency-to-*m/z* calibration was performed externally with respect to the ions of an ES Tuning Mix (Agilent Technologies, Santa Clara, CA, USA) calibration mixture. All data were processed using Bruker XMASS 7.0.8 software.

Results

Isoelectric focusing and SDS PAGE

Recombinant human CA II treated with various concentrations of acetaldehyde was subjected to isoelectric focusing (Fig. 2, upper panel). The pI value of untreated enzyme was about 7.9. Acetaldehyde treatment in the presence or absence of a reducing agent NaBH₃(CN) (sodium cyanoborohydride) caused shifts in the pI values. In the presence of the reducing agent, the isoelectric point became slightly more basic after acetaldehyde treatment. The results shown in Fig. 2 (lower panel) showed a concentration dependent shift to lower pI with increased aldehyde concentrations. Even 100 µM acetaldehyde caused a visible change in the charge of the CA II molecule. Bands with pI values of 6.8, 7.1 and 7.3 were identified with increasing acetaldehyde concentrations. Without NaBH₃(CN), all changes in isoelectric points were toward more acidic values. Acetaldehyde concentrations of 100 µM–10 mM produced two extra bands of pI values at 7.3 and 7.4. At 10 mM concentration, the 7.3 pI value became most prominent. Surprisingly, the pattern was completely different when acetaldehyde reached 100 mM concentration. The protein appeared as a smear with three faint bands of 6.4, 6.7 and 6.9 pI values. It is notable that NaBH₃(CN) alone had no effect on the isoelectric point of CA II (Fig. 2, upper panel).

SDS PAGE was performed on acetaldehyde-treated CA II samples. The results in Figure 3 demonstrate that 100 mM acetaldehyde in the presence of NaBH₃(CN) produced a shift toward a slightly higher molecular mass. In contrast, the same acetaldehyde concentration without the reducing agent produced a faint smear, which could indicate a partial degradation of the protein or it may be a consequence of unwanted interactions between the ampholytes and acetaldehyde in a medium.

Kinetic analyses

CA activity measurements were performed using a previously described assay method of Maren [44,45] combined to a spectrophotometric color determination. First, we

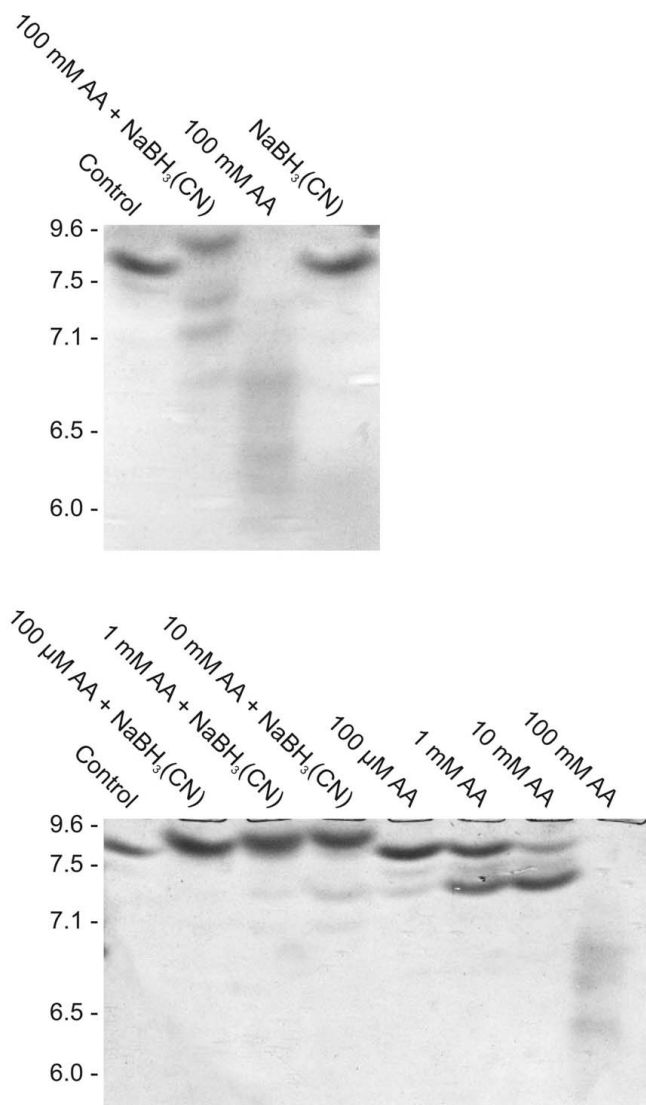


Figure 2
Isoelectric focusing of human recombinant CA II treated with various concentrations of acetaldehyde (AA) in the presence or absence of a reducing agent, 10 mM NaBH₃(CN).

determined the CA activities of blood samples treated with acetaldehyde. Figure 4 demonstrates that 100 mM acetaldehyde produced over 40% reduction in CA activity both in the presence ($p = 0.016$) and absence ($p = 0.012$) of NaBH₃(CN). A 10 mM concentration of acetaldehyde also reduced the CA activity in the presence of NaBH₃(CN), but this change did not reach statistical significance ($p = 0.052$). In the second set of experiments, we measured enzymatic activities of CA II samples in analogous conditions as described above. Acetaldehyde (100 mM) in the presence of NaBH₃(CN) caused 23.7% reduction in CA II activity ($p = 0.001$) (Fig. 5). In the absence of NaBH₃(CN), the activity was only slightly reduced ($p =$

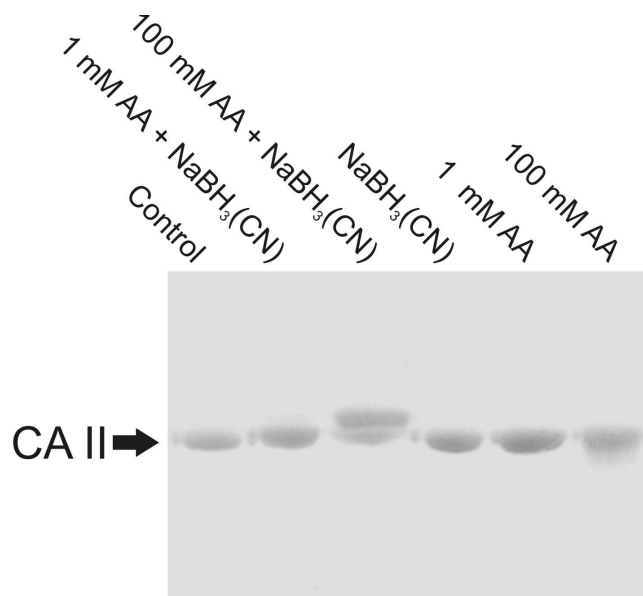


Figure 3
SDS-PAGE of human recombinant CA II treated with acetaldehyde (AA). The acetaldehyde concentrations are shown in the figure. Some of the samples were subjected to reducing conditions using NaBH₃(CN).

0.045). All other changes observed were non-significant ($p > 0.05$).

The enzyme activity data obtained from the stopped-flow analysis are shown in Table 1. This method indicated that the acetaldehyde-modified enzyme is about one-third as active as the native CA II (considering the specificity constant k_{cat}/K_m). On the other hand, the inhibition constant of acetazolamide is about three times higher for the modified protein compared to the native one.

Mass spectrometry

Figure 6A presents an ESI FT-ICR mass spectrum measured for native CA II. A protein ion charge state distribution from 13+ to 32+ representing apo-protein (i.e., protein without a bound Zn²⁺ cation) was observed. The most abundant isotopic mass ($m_{m.a.}$) of the protein was determined to be 29098.07 ± 0.07 Da which agrees well with the calculated value ($m_{m.a.} = 29097.93$ Da for CAH2_HUMAN (Swiss-Prot entry [P00918](#)), with the initial methionine removed and the second residue changed from serine to alanine due to the used cloning strategy).

Figure 6B presents an expanded view on the charge state 24+ of the mass spectra of native CA II (Figure 6A) as well as CA II incubated in the presence of NaBH₃(CN), acetaldehyde (AA), and both. In the presence of NaBH₃(CN), the mass spectrum was identical to that observed for native CA II. In contrast, when reacted with 100 mM

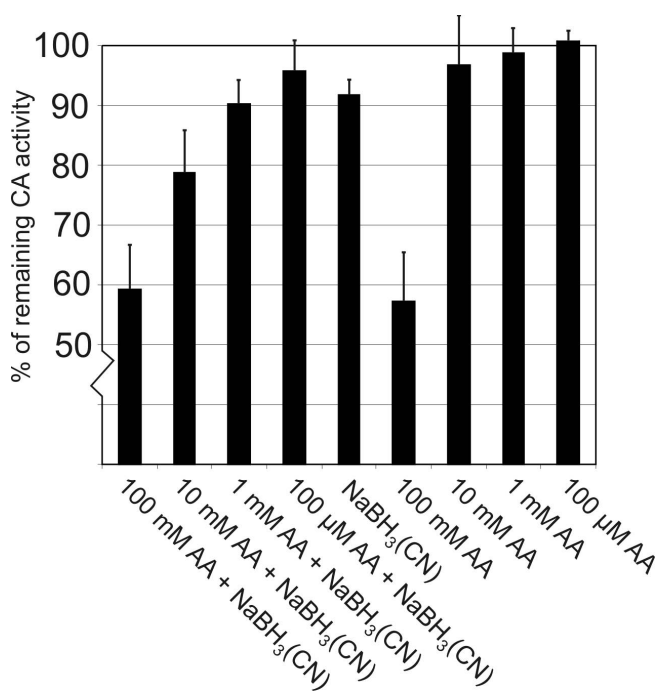


Figure 4
Carbonic anhydrase activity of human blood samples treated with various concentrations of acetaldehyde (AA) in the presence or absence of a reducing agent, 10 mM NaBH₃(CN). The activity assay was performed using a previously described assay method of Maren [44,45]. The obtained values from three assays are indicated as mean \pm standard deviation.

acetaldehyde in the absence of NaBH₃(CN), the mass of CA II increased by ~ 26 Da ($m_{m.a.} = 29124.09 \pm 0.09$ Da), consistent with a "Schiff base" formation between acetaldehyde and a primary amino group, e.g., ϵ -NH₂ of lysine ($\Delta m_{theor} = +26.02$ Da). This reaction was highly specific given that the modified protein form was present for as much as 90% as compared to the unmodified form. In the presence of NaBH₃(CN), a more drastic change was observed. Evenly distributed new signals appeared in the mass spectrum, with a mass increase of $\sim n \times 28$ Da, n ranging from 9 to 19 (for the three most abundant forms, $m_{m.a.} = 29462.51 \pm 0.06$ Da ($n = 13$), 29490.54 ± 0.05 Da ($n = 14$), and 29518.59 ± 0.05 Da ($n = 15$)). This is consistent with the formation of multiple stable covalent acetaldehyde adducts with CA II, most probably *N*-ethyllysine residues ($\Delta m_{theor} = +28.03$ Da).

Discussion

Acetaldehyde has been demonstrated to be able to bind to several different proteins, both *in vitro* and *in vivo*. The structural modifications due to acetaldehyde binding may affect the conformation, acid-base properties, and/or hydrogen-bonding patterns of amino acids on the surface

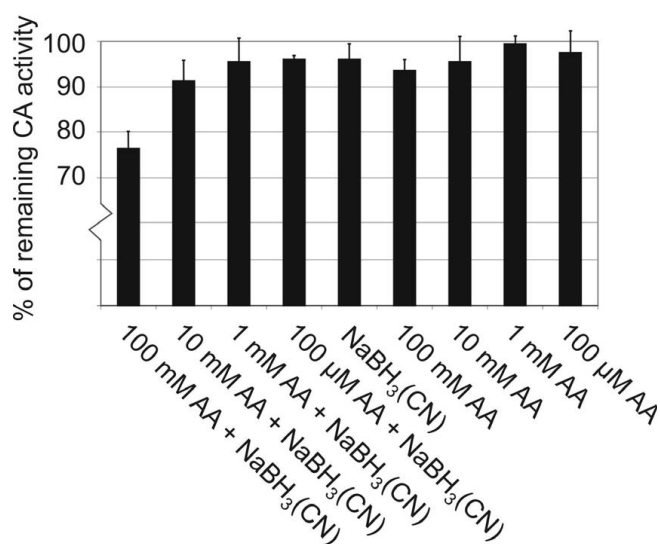


Figure 5
Carbonic anhydrase activity of human recombinant CA II treated with various concentrations of acetaldehyde (AA) in the presence or absence of a reducing agent, 10 mM NaBH₃(CN). The activity assay was performed using a previously described assay method of Maren [44,45]. The obtained values from three assays are indicated as mean \pm standard deviation.

or within the active site of an enzyme, thereby disrupting the normal protein function. Although several lines of previous investigations during the last two decades have focused on the generation of acetaldehyde adducts, it has turned out to be a great challenge to explore the functional consequences of these acetaldehyde-induced modifications without proper functional assays. Acetaldehyde is a very reactive compound, and thus, it has been particularly difficult to determine the presence of unstable adducts formed under non-reductive conditions.

The present results showed that human CA II is a good target protein for acetaldehyde modification. It has a high number of lysine residues (24 in total) in its primary sequence. The highly sensitive mass spectrometric method indicated that, under reducing conditions, each CA II enzyme molecule had reacted with up to 19 (14 on average) acetaldehyde molecules to form stable covalent adducts (*N*-ethyllysine residues). Although such a high number of acetaldehyde was bound to the enzyme, the modified protein still retained much of the enzymatic activity. Using the modified Maren's method combined with a spectrophotometric analysis, we found that the enzyme activity decreased 23.7% under supraphysiological (100 mM) acetaldehyde concentrations in reducing conditions. The stopped-flow CA activity assay showed a significantly greater decrease in the enzyme activity when compared to the spectrophotometric assay. Even though

Table 1: Kinetic and inhibitory properties of CA II measured by the stopped-flow method

Enzyme	k_{cat} (s^{-1})	K_M (mM)	k_{cat}/K_M ($M^{-1}s^{-1}$)	K_i (acetazolamide) (nM)
Native CA II	$1.40 \cdot 10^6$	9.3	$1.5 \cdot 10^8$	12
CA II + AA + NaBH ₃ (CN)	$0.77 \cdot 10^6$	9.3	$0.5 \cdot 10^8$	35

these assay methods produced different rates for the acetaldehyde-induced inhibition, both techniques clearly indicated that acetaldehyde binding reduces the catalytic activity.

Interestingly, only a single acetaldehyde molecule had reacted with CA II under non-reductive conditions, forming the unstable covalent adduct ("Schiff base"). Due to high mass resolution inherent for the FT-ICR technique, unequivocal differentiation between the "Schiff base" (+26.02 Da) and the substituted amine (+28.03 Da), i.e. ~2-Da mass difference at 29 kDa, was possible (Figure 1). According to the mass spectrometry, over 90% of the enzyme molecules were modified with acetaldehyde. However, repeated measurements with the acetaldehyde-treated sample, after being stored two weeks at 4 °C, indicated the presence of an unmodified CA II only, consistent with unstable character of the formed "Schiff base". To further localize the modification site, both native and

acetaldehyde-treated CA II samples were subjected to on-line pepsin digestion prior to mass spectrometry. Although the resulting peptide maps had 100% sequence coverage, the observed peptides were markedly larger (up to 10 kDa) in the presence of acetaldehyde, possibly due to decreased protease activity, making spectral assignments occasionally ambiguous. However, two of the observed 6.3-kDa peptic peptides had a mass difference of 26.01 Da, suggesting the modification site within the first 56 residues. Owing to the experimental difficulties in obtaining good tandem mass spectra for these large peptic peptides, more experiments with different proteases are warranted for the further identification of the modification site in CA II.

Both SDS-PAGE and isoelectric focusing suggested that 100 mM acetaldehyde might induce degradation of CA II protein under non-reductive conditions. However, mass spectrometry did not show any evidence for the accelerated degradation of CA II (i.e., no change in the absolute intensity of the intact protein or evidence of the peptide signals), suggesting that the smear observed in isoelectric focusing may have resulted from chemical interactions between ampholytes and acetaldehyde under non-reductive conditions.

More detailed structural studies are needed to characterize the acetaldehyde-induced modifications of CA II. This enzyme, although only weakly expressed in liver [46,47], represents an excellent model for both structural and functional studies of acetaldehyde modification. Its crystal structure and kinetic properties have been reported by several groups [48-53]. These studies can be expanded to other CA isozymes that are more highly expressed in the liver, including CA III, VA, and CA XIV [54-56]. These enzymes may also be susceptible to modification because acetaldehyde can reach over 100 μ M concentration in the liver of alcoholic patients [57], even though concentrations are lower, though still in a micromolar range, in the blood [58,59].

Accurate acetaldehyde determination from biological samples has been extremely challenging. It is widely accepted that single doses of ethanol do not significantly increase blood free acetaldehyde concentrations [60,61]. Nonetheless, such doses may elevate acetaldehyde levels within intracellular compartments or cell membranes. Baraona et al. [62] tested the blood of 5 healthy individu-

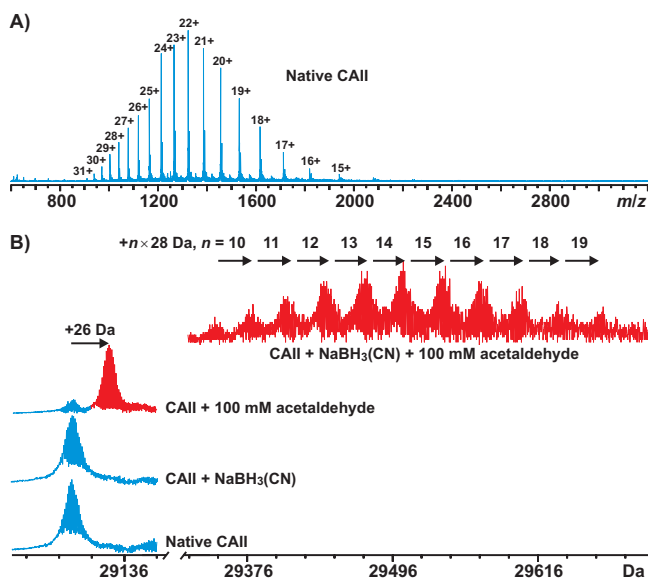


Figure 6
ESI FT-ICR mass spectra of human recombinant CA II. (A) A broadband mass spectrum of native CA II (different protein ion charge states have been assigned as $z + = [CA II + zH]^{z+}$) and (B) an expanded view on the charge state 24+ for native CA II, and CA II samples treated with 100 mM acetaldehyde in the absence and presence of NaBH₃(CN). The determined masses have been indicated. In panel B, the mass scale in Da is set for the charge state 24+.

als and 6 alcoholic patients and showed that most of the blood acetaldehyde was present in the erythrocytes after alcohol consumption. *In vivo*, acetaldehyde concentration within the erythrocytes is about 3–10 times higher than in the plasma [62,63]. Although acetaldehyde concentrations are probably highest in the liver, the site of ethanol metabolism, acetaldehyde modified protein epitopes have been also located to other organs and cell types. Positive immunohistochemical staining for acetaldehyde adducts has been demonstrated in the brain, heart, skeletal muscle, and erythrocytes [14,64-66]. All of these tissues and cells contain several CA isozymes that may become functionally impaired because of adduct formation. Therefore, these modifications deserve further study to determine the exact submolecular defects caused by acetaldehyde in each isozyme.

Conclusion

In the present study, we showed that acetaldehyde, the first metabolite of ethanol, can modify the ubiquitous enzyme, carbonic anhydrase. Mass spectrometric analysis indicated that one of the primary NH₂ groups (e.g., in lysine side chain) in the CA isozyme II had reacted with acetaldehyde under non-reducing condition, consistent with a "Schiff base" formation. In reducing conditions, each CA II molecule had reacted with 9–19 (14 on average) acetaldehyde molecules, consistent with further reduction of the "Schiff bases" to substituted amines (N-ethyllysine residues). The latter structural change led to decreased enzyme activity, which may have important physiological consequences in alcohol abusers.

Authors' contributions

ON, JV, JJ and SP designed the study. AW and WSS provided the recombinant *E. coli* bacteria for CA II production. FA purified the recombinant CA II protein. FA, DV, CTS, and SP carried out the enzyme activity assays. JJ, SI, JV and PV performed the mass spectrometry. FA, ON, JV, JJ, CTS and SP drafted the manuscript. All authors read and approved the final manuscript.

Acknowledgements

This work was supported by grants from the EU projects Euroxy (CTS) and DeZnIT (CTS, SP), the Finnish Foundation for Alcohol Studies (ON), National Institutes of Health, Grant DK-40163 (WSS), and the Academy of Finland, Grant I08533 (JJ). The authors thank Jukka Lehtonen, Aulikki Lehmus and Ritva Romppanen for skilful technical assistance.

References

- Lieber CS: **Medical disorders of alcoholism.** *N Engl J Med* 1995, **333(16)**:1058-1065.
- Niemela O, Parkkila S, Yla-Herttuala S, Halsted C, Witztum JL, Lanca A, Israel Y: **Covalent protein adducts in the liver as a result of ethanol metabolism and lipid peroxidation.** *Lab Invest* 1994, **70(4)**:537-546.
- Freeman TL, Tuma DJ, Thiele GM, Klassen LW, Worrall S, Niemela O, Parkkila S, Emery PV, Preeedy VR: **Recent advances in alcohol-induced adduct formation.** *Alcohol Clin Exp Res* 2005, **29(7)**:1310-1316.
- Tuma DJ, Klassen LW: **Immune responses to acetaldehyde-protein adducts: role in alcoholic liver disease.** *Gastroenterology* 1992, **103(6)**:1969-1973.
- Stevens VJ, Fantl WJ, Newman CB, Sims RV, Cerami A, Peterson CM: **Acetaldehyde adducts with hemoglobin.** *J Clin Invest* 1981, **67(2)**:361-369.
- Niemela O: **Aldehyde-protein adducts in the liver as a result of ethanol-induced oxidative stress.** *Front Biosci* 1999, **4**:D506-513.
- Israel Y, Hurwitz E, Niemela O, Arnon R: **Monoclonal and polyclonal antibodies against acetaldehyde-containing epitopes in acetaldehyde-protein adducts.** *Proc Natl Acad Sci USA* 1986, **83(20)**:7923-7927.
- Sorrell MF, Tuma DJ: **The functional implications of acetaldehyde binding to cell constituents.** *Ann N Y Acad Sci* 1987, **492**:50-62.
- Worrall S, de Jersey J, Nicholls R, Wilce P: **Acetaldehyde/protein interactions: are they involved in the pathogenesis of alcoholic liver disease?** *Dig Dis* 1993, **11(4-5)**:265-277.
- Nicholls R, de Jersey J, Worrall S, Wilce P: **Modification of proteins and other biological molecules by acetaldehyde: adduct structure and functional significance.** *Int J Biochem* 1992, **24(12)**:1899-1906.
- Mauch TJ, Donohue TM Jr, Zetterman RK, Sorrell MF, Tuma DJ: **Covalent binding of acetaldehyde selectively inhibits the catalytic activity of lysine-dependent enzymes.** *Hepatology* 1986, **6(2)**:263-269.
- Mauch TJ, Tuma DJ, Sorrell MF: **The binding of acetaldehyde to the active site of ribonuclease: alterations in catalytic activity and effects of phosphate.** *Alcohol Alcohol* 1987, **22(2)**:103-112.
- Viitala K, Makkonen K, Israel Y, Lehtimäki T, Jaakkola O, Koivula T, Blake JE, Niemela O: **Autoimmune responses against oxidant stress and acetaldehyde-derived epitopes in human alcohol consumers.** *Alcohol Clin Exp Res* 2000, **24(7)**:1103-1109.
- Niemela O, Parkkila S: **Alcoholic macrocytosis – is there a role for acetaldehyde and adducts?** *Addict Biol* 2004, **9(1)**:3-10.
- Viitala K, Israel Y, Blake JE, Niemela O: **Serum IgA, IgG, and IgM antibodies directed against acetaldehyde-derived epitopes: relationship to liver disease severity and alcohol consumption.** *Hepatology* 1997, **25(6)**:1418-1424.
- Fowles LF, Beck E, Worrall S, Shanley BC, de Jersey J: **The formation and stability of imidazolidinone adducts from acetaldehyde and model peptides. A kinetic study with implications for protein modification in alcohol abuse.** *Biochem Pharmacol* 1996, **51(10)**:1259-1267.
- Niemela O: **Distribution of ethanol-induced protein adducts in vivo: relationship to tissue injury.** *Free Radic Biol Med* 2001, **31(12)**:1533-1538.
- Tuma DJ, Hoffman T, Sorrell MF: **The chemistry of acetaldehyde-protein adducts.** *Alcohol Alcohol Suppl* 1991, **1**:271-276.
- Donohue TM Jr, Tuma DJ, Sorrell MF: **Acetaldehyde adducts with proteins: binding of [¹⁴C]acetaldehyde to serum albumin.** *Arch Biochem Biophys* 1983, **220(1)**:239-246.
- San George RC, Hoberman HD: **Reaction of acetaldehyde with hemoglobin.** *J Biol Chem* 1986, **261(15)**:6811-6821.
- Tuma DJ, Jennett RB, Sorrell MF: **The interaction of acetaldehyde with tubulin.** *Ann N Y Acad Sci* 1987, **492**:277-286.
- Jennett RB, Sorrell MF, Johnson EL, Tuma DJ: **Covalent binding of acetaldehyde to tubulin: evidence for preferential binding to the alpha-chain.** *Arch Biochem Biophys* 1987, **256(1)**:10-18.
- Jennett RB, Saffari-Fard A, Sorrell MF, Smith SL, Tuma DJ: **Increased covalent binding of acetaldehyde to calmodulin in the presence of calcium.** *Life Sci* 1989, **45(16)**:1461-1466.
- Xu DS, Jennett RB, Smith SL, Sorrell MF, Tuma DJ: **Covalent interactions of acetaldehyde with the actin/microfilament system.** *Alcohol Alcohol* 1989, **24(4)**:281-289.
- Tuma DJ, Newman MR, Donohue TM Jr, Sorrell MF: **Covalent binding of acetaldehyde to proteins: participation of lysine residues.** *Alcohol Clin Exp Res* 1987, **11(6)**:579-584.
- Jennett RB, Sorrell MF, Saffari-Fard A, Ockner JL, Tuma DJ: **Preferential covalent binding of acetaldehyde to the alpha-chain of purified rat liver tubulin.** *Hepatology* 1989, **9(1)**:57-62.
- Klassen LW, Tuma DJ, Sorrell MF, McDonald TL, DeVasure JM, Thiele GM: **Detection of reduced acetaldehyde protein adducts using a unique monoclonal antibody.** *Alcohol Clin Exp Res* 1994, **18(1)**:164-171.

28. Braun KP, Cody RB Jr, Jones DR, Peterson CM: **A structural assignment for a stable acetaldehyde-lysine adduct.** *J Biol Chem* 1995, **270(19)**:11263-11266.
29. Braun KP, Pavlovich JG, Jones DR, Peterson CM: **Stable acetaldehyde adducts: structural characterization of acetaldehyde adducts of human hemoglobin N-terminal beta-globin chain peptides.** *Alcohol Clin Exp Res* 1997, **21(1)**:40-43.
30. Lin RC, Shahidi S, Lumeng L: **Production of antibodies that recognize the heterogeneity of immunoreactive sites in human hemoglobin chemically modified by acetaldehyde.** *Alcohol Clin Exp Res* 1993, **17(4)**:882-886.
31. Gaines KC, Salhany JM, Tuma DJ, Sorrell MF: **Reaction of acetaldehyde with human erythrocyte membrane proteins.** *FEBS Lett* 1977, **75(1)**:115-119.
32. Peterson CM, Nguyen LB: **Clinical implications of acetaldehyde adducts with hemoglobin.** *Prog Clin Biol Res* 1985, **183**:19-30.
33. Niemela O, Israel Y: **Hemoglobin-acetaldehyde adducts in human alcohol abusers.** *Lab Invest* 1992, **67(2)**:246-252.
34. Niemela O, Israel Y, Mizoi Y, Fukunaga T, Eriksson CJ: **Hemoglobin-acetaldehyde adducts in human volunteers following acute ethanol ingestion.** *Alcohol Clin Exp Res* 1990, **14(6)**:838-841.
35. Savolainen MJ, Baraona E, Lieber CS: **Acetaldehyde binding increases the catabolism of rat serum low-density lipoproteins.** *Life Sci* 1987, **40(9)**:841-846.
36. Wickramasinghe SN, Gardner B, Barden G: **Cytotoxic protein molecules generated as a consequence of ethanol metabolism in vitro and in vivo.** *Lancet* 1986, **2(8511)**:823-826.
37. Behrens UJ, Hoerner M, Lasker JM, Lieber CS: **Formation of acetaldehyde adducts with ethanol-inducible P450IIIE1 in vivo.** *Biochem Biophys Res Commun* 1988, **154(2)**:584-590.
38. Jukkola A, Niemela O: **Covalent binding of acetaldehyde to type III collagen.** *Biochem Biophys Res Commun* 1989, **159(1)**:163-169.
39. Zhu Y, Fillenwarth MJ, Crabb D, Lumeng L, Lin RC: **Identification of the 37-kd rat liver protein that forms an acetaldehyde adduct in vivo as delta 4-3-ketosteroid 5 beta-reductase.** *Hepatology* 1996, **23(1)**:115-122.
40. Parkkila S: **An overview of the distribution and function of carbonic anhydrase in mammals.** *Exs* 2000:79-93.
41. Lehtonen J, Shen B, Vihinen M, Casini A, Scozzafava A, Supuran CT, Parkkila AK, Saarnio J, Kivela AJ, Waheed A, et al.: **Characterization of CA XIII, a novel member of the carbonic anhydrase isozyme family.** *J Biol Chem* 2004, **279(4)**:2719-2727.
42. Hu PY, Waheed A, Sly WS: **Partial rescue of human carbonic anhydrase II frameshift mutation by ribosomal frameshift.** *Proc Natl Acad Sci USA* 1995, **92(6)**:2136-2140.
43. Parkkila S, Kaunisto K, Rajaniemi L, Kumpulainen T, Jokinen K, Rajaniemi H: **Immunohistochemical localization of carbonic anhydrase isoenzymes VI, II, and I in human parotid and submandibular glands.** *J Histochem Cytochem* 1990, **38(7)**:941-947.
44. Maren TH: **A simplified micromethod for the determination of carbonic anhydrase and its inhibitors.** *J Pharmacol Exp Ther* 1960, **130**:26-29.
45. Sundaram V, Rumbolo P, Grubb J, Strisciuglio P, Sly WS: **Carbonic anhydrase II deficiency: diagnosis and carrier detection using differential enzyme inhibition and inactivation.** *Am J Hum Genet* 1986, **38(2)**:125-136.
46. Parkkila S, Parkkila AK, Juvenon T, Rajaniemi H: **Distribution of the carbonic anhydrase isoenzymes I, II, and VI in the human alimentary tract.** *Gut* 1994, **35(5)**:646-650.
47. Pan PW, Rodriguez A, Parkkila S: **A systematic quantification of carbonic anhydrase transcripts in the mouse digestive system.** *BMC Mol Biol* 2007, **8**:22.
48. Supuran CT: **Carbonic anhydrases: novel therapeutic applications for inhibitors and activators.** *Nat Rev Drug Discov* 2008, **7(2)**:168-181.
49. Liljas A, Kannan KK, Bergsten PC, Waara I, Fridborg K, Strandberg B, Carlbom U, Jarup L, Lovgren S, Petef M: **Crystal structure of human carbonic anhydrase C.** *Nat New Biol* 1972, **235(57)**:131-137.
50. Hakansson K, Carlsson M, Svensson LA, Liljas A: **Structure of native and apo carbonic anhydrase II and structure of some of its anion-ligand complexes.** *J Mol Biol* 1992, **227(4)**:1192-1204.
51. Duda D, Govindasamy L, Agbandje-McKenna M, Tu C, Silverman DN, McKenna R: **The refined atomic structure of carbonic anhydrase II at 1.05 Å resolution: implications of chemical rescue of proton transfer.** *Acta Crystallogr D Biol Crystallogr* 2003, **59(Pt 1)**:93-104.
52. Di Fiore A, Scozzafava A, Winun JY, Montero JL, Pedone C, Supuran CT, De Simone G: **Carbonic anhydrase inhibitors: binding of an antiglaucoma glycosyl-sulfanilamide derivative to human isoform II and its consequences for the drug design of enzyme inhibitors incorporating sugar moieties.** *Bioorg Med Chem Lett* 2007, **17(6)**:1726-1731.
53. Fisher SZ, Maupin CM, Budayova-Spano M, Govindasamy L, Tu C, Agbandje-McKenna M, Silverman DN, Voth GA, McKenna R: **Atomic crystal and molecular dynamics simulation structures of human carbonic anhydrase II: insights into the proton transfer mechanism.** *Biochemistry* 2007, **46(11)**:2930-2937.
54. Parkkila S, Halsted CH, Villanueva JA, Vaananen HK, Niemela O: **Expression of testosterone-dependent enzyme, carbonic anhydrase III, and oxidative stress in experimental alcoholic liver disease.** *Dig Dis Sci* 1999, **44(11)**:2205-2213.
55. Nagao Y, Platero JS, Waheed A, Sly WS: **Human mitochondrial carbonic anhydrase: cDNA cloning, expression, subcellular localization, and mapping to chromosome 16.** *Proc Natl Acad Sci USA* 1993, **90(16)**:7623-7627.
56. Parkkila S, Kivela AJ, Kaunisto K, Parkkila AK, Hakkola J, Rajaniemi H, Waheed A, Sly WS: **The plasma membrane carbonic anhydrase in murine hepatocytes identified as isozyme XIV.** *BMC Gastroenterol* 2002, **2**:13.
57. Eriksson CJ: **Determination of hepatic acetaldehyde and its biphasic relationship to the ethanol concentration in rats.** *Adv Exp Med Biol* 1980, **132**:459-467.
58. Matthewson K, Al Mardini H, Bartlett K, Record CO: **Impaired acetaldehyde metabolism in patients with non-alcoholic liver disorders.** *Gut* 1986, **27(7)**:756-764.
59. Lindros KO, Stowell A, Pikkariainen P, Salaspuro M: **Elevated blood acetaldehyde in alcoholics with accelerated ethanol elimination.** *Pharmacol Biochem Behav* 1980, **13(Suppl 1)**:119-124.
60. Eriksson CJ, Fukunaga T: **Human blood acetaldehyde (update 1992).** *Alcohol Alcohol Suppl* 1993, **2**:9-25.
61. Eriksson CJ: **Measurement of acetaldehyde: what levels occur naturally and in response to alcohol?** *Novartis Found Symp* 2007, **285**:247-255. discussion 256-260.
62. Baraona E, Di Padova C, Tabasco J, Lieber CS: **Red blood cells: a new major modality for acetaldehyde transport from liver to other tissues.** *Life Sci* 1987, **40(3)**:253-258.
63. Hernandez-Munoz R, Ma XL, Baraona E, Lieber CS: **Method of acetaldehyde measurement with minimal artifactual formation in red blood cells and plasma of actively drinking subjects with alcoholism.** *J Lab Clin Med* 1992, **120(1)**:35-41.
64. Niemela O, Parkkila S, Worrall S, Emery PV, Preedy VR: **Generation of aldehyde-derived protein modifications in ethanol-exposed heart.** *Alcohol Clin Exp Res* 2003, **27(12)**:1987-1992.
65. Niemela O, Parkkila S, Koll M, Preedy VR: **Generation of protein adducts with malondialdehyde and acetaldehyde in muscles with predominantly type I or type II fibers in rats exposed to ethanol and the acetaldehyde dehydrogenase inhibitor cyanamide.** *Am J Clin Nutr* 2002, **76(3)**:668-674.
66. Rintala J, Jaatinen P, Parkkila S, Sarviharju M, Kiianmaa K, Hervonen A, Niemela O: **Evidence of acetaldehyde-protein adduct formation in rat brain after lifelong consumption of ethanol.** *Alcohol Alcohol* 2000, **35(5)**:458-463.

Publish with **BioMed Central** and every scientist can read your work free of charge

"BioMed Central will be the most significant development for disseminating the results of biomedical research in our lifetime."

Sir Paul Nurse, Cancer Research UK

Your research papers will be:

- available free of charge to the entire biomedical community
- peer reviewed and published immediately upon acceptance
- cited in PubMed and archived on PubMed Central
- yours — you keep the copyright

Submit your manuscript here:
http://www.biomedcentral.com/info/publishing_adv.asp

