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CONTRIBUTION OF ALDH1A1 ISOZYME TO DETOXIFICATION OF ALDEHYDES PRESENT IN FOOD PRODUCTS

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Abstract: Even though food awareness is so developed and more and more people pay attention to what their diet is composed of, it is not possible to exclude all potentially dangerous substances present in our diet. One group of such compounds may be aldehydes as several studies indicate that they can be mutagenic, carcinogenic, genotoxic and cytotoxic. These relatively reactive organic molecules are natural constituents of food. They are also extensively used by food industry as additives giving aroma and taste. Fortunately many enzyme systems were developed to protect us against these toxic compounds, one of which is aldehyde dehydrogenase enzyme superfamily. As mouth is the first part of digestive system it seems crucial for detoxifying toxic substances introduced with our diet. The only ALDH isozyme present in saliva is ALDH3A1, which has very high affinity towards aromatic aldehydes commonly found in food. However, because of hyposalivation, which is not uncommon nowadays, the effectiveness of this barrier can be drastically diminished. As another member of this enzyme family, isozyme ALDH1A1 is also present in digestive system its possible contribution to detoxification of 'food' aldehydes was addressed. Kinetic parameters (K_M, V_{max}) of recombinant ALDH1A1 towards several aliphatic and aromatic aldehydes occurring in food products (vanillin, citral, furfural, cinnamaldehyde, anisaldehyde, benzaldehyde and *trans*-hexenal) were determined by measuring the increase of NADH fluorescence after adding various concentrations of aldehyde substrates. Rates were used to construct the Lineweaver-Burk plot from which K_M and V_{max} (measured relative to that of benzaldehyde which was assigned the value of 100) values were calculated. The following results were obtained: 0.04 ± 0.06 μM and 277 ± 81 for anisaldehyde, 0.86 ± 0.03 μM and 50 ± 3 for vanillin, 0.18 ± 0.05 μM and 93 ± 9 for *trans*-2-hexenal, 0.17 ± 0.03 μM and 201 ± 32 for cinnamaldehyde, 5.8 ± 0.3 μM and 281 ± 59 for furfural, 0.65 ± 0.05 μM and 139 ± 9 for citral, 0.4 ± 0.2 μM and 100 for benzaldehyde. It turned out that this ubiquitous member of ALDHs superfamily, has very good affinity for examined aldehydes. The resulting Michaelis – Menten constant values are even lower than the corresponding values for ALDH3A1 enyzme. Thus supporting role of ALDH1A1 in the protection of organisms against these dangerous compounds from food can be suggested.

Keywords: food, aldehydes, aldehyde dehydrogenases, enzyme kinetics, ALDH1A1

Not many people are aware of the danger aldehydes can impose. First of all, the presence of a polarized carbon-oxygen double bond makes them relatively reactive enabling adduct formation with proteins and DNA, what is responsible for their toxicity (1). Secondly they are widespread in nature. They can be formed endogenously by biotransformation of amino acids, neurotransmitters, carbohydrates and lipids (2). They are present in the outdoor (motor vehicle exhaust, smog) and indoor air (furniture, paints, cooking fumes, cigarette smoke, hair salons). They are generated *in vivo* from drugs (diphenylhydramine, codeine) and food additives (preservatives –hexamethylenetetramine) (3). They are found in over 300 different foods as natural constituents or flavoring additives and aromas (1). For many dietary aldehydes there is still insufficient data available to evaluate their potential risks. However, it was confirmed that formaldehyde, acetaldehyde and furfural are carcinogenic towards experimental animals (1).

Due to the fact that ALDH3A1 is probably the only isozyme of aldehyde dehydrogenase superfamily present is saliva (4, 5) it can be regarded as a very important factor in the protection of organisms against various aldehydes contained in food.

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Another argument supporting this notion is its high affinity towards these chemical compounds. Another member of this enzyme superfamily, ALDH1A1 is ubiquitously distributed in the adult epithelium of e.g., testis, brain, eye lens, kidney, lungs. It is also found in the liver, pancreas and stomach mucosa. Its presence in digestive system could indicate that it is also engaged in the process of detoxifying aldehydes from alimentary products. However, so far there were no data available about its affinity towards aldehydes frequently used as flavors and aromas. The purpose of this study was to determine kinetic parameters of recombinant ALDH1A1 (K_M, V_{max}) towards several aldehydes commonly found in food by observing the increase in NADH fluorescence.

EXPERIMENTAL

Materials

Benzaldehyde was purchased from Fluka. Anisaldehyde, cinnamaldehyde, vanillin, furfural, 2-hexenal, citral, coenzymes NAD^+ and NADH, as well as dithiothreitol were purchased from Sigma – Aldrich.

Enzyme assays

All assays were run in 50 mM pyrophosphate buffer, pH 8.1 at 25°C, in the presence of 0.5 mM EDTA and 0.5 mM DTT using HITACHI F – 7000 Fluorescence Spectrophotometer. Concentration of NAD^+ was 100 μM. The enzymatic reaction was initiated by the addition of various concentrations of aldehyde substrates. Fluorescence of NADH was followed at 460 nm with excitation at 340 nm and spectral bandwidths 5 and 10 nm. Purified reaction product (NADH) at the concentration 2 – 5 μM was added as internal standard to obtain absolute reaction rates, which were calculated according to the formula:

$$v = \frac{dF}{dt} \cdot \frac{C_{st}}{F_{st}}$$

where C_{st} is standard concentration, F_{st} its fluorescence and dF/dt slope of the fluorescence time dependence. The K_M and V_{max} values for the oxidation were calculated from the Lineweaver-Burk plot.

Cloning of the cDNA for ALDH1A1 and its overexpression

The full-length human ALDH1A1 gene was PCR amplified from "TrueClone", cDNA clone in pCMV6-AC vector purchased in OriGene (Rockville, MD, U.S.A.). The PCR primers:

CTAGCTAGCATGTCATCCTCAGGCACGCCAG

CCGGAATTCTTATGAGTTCTTCTGAGAGAT

complementary to 5' and 3' ends of the ALDH1A1 gene contained NheI and EcoRI restriction site, respectively.

The 1.5-kb PCR-amplified fragment was digested with NheI and EcoRI, then purified from agarose gel using the QIAquick Gel Extraction Kit (Qiagen), and ligated with vector – pET-28a (Novagene). Vector DNA had been digested with the same enzymes and gel-purified. The ligation reaction was used to transform *E. coli* DH5α competent cells. The nucleotide sequence of the entire inset of the pET28a(ALDH1A1) plasmid was analyzed, it is identical with sequence of ALDH1A1 gene present in GenBank NCBI (acc. no. NM_00689).

Then, *E. coli* BL21(DE3) competent cells (Invitrogen) were transformed using plasmid pET28a(ALDH1A1). The cultures of the overproducing strain were grown at 37°C in LB broth (35 g/L tryptone, 20 g/L yeast extract, 5 g/L NaCl) supplemented with 50 μg/mL kanamycin to an OD_{600} of 0.6. The expression was induced by adding IPTG to a final concentration of 1 mM. Recombinant ALDH1A1 was isolated with Ni-NTA Fast Start Kit (Qiagen) and dialyzed overnight to 50 mM pyrophosphate buffer containing 1 mM EDTA and 1 mM DTT (Fig. 1).

RESULTS

From Table 1 it is evident that ALDH1A1 is very effective catalyst of aldehydes commonly found in food products. The submicromolar and low micromolar K_M values indicate that all of examined aldehydes are very good substrates for this isozyme, better than for ALDH3A1 which is characterized by higher K_M values. What is more, the maximal rates of enzymatic oxidation of selected aldehydes by ALDH1A1 are greater than that of ALDH3A1. It is worth to notice that furfural, known to be rapidly eliminated from the human organism and surprisingly poor substrate for ALDH3A1, is a good substrate for ALDH1A1.

DISCUSSION AND CONCLUSION

Many aldehydes are natural constituents of food. Because of their sensory properties (taste and aroma) they are very often used as approved additives to improve attractiveness of dietary products (ice-creams, sweets, meat preparations (1). What is more, they are also formed during deterioration of aliments (for example 2-furfural – the main product

Table 1. Kinetic parameters of recombinant ALDH1A1 and ALDH3A1. Maximal rates were measured relative to that of benzaldehyde (100).

Aldehyde	ALDH1A1		ALDH3A1	
	K_M [μM] (standard error)	V_{max} (relative)	K_M [μM]	V_{max} (relative)
Anisaldehyde	0.40 ± 0.06[#]	277 ± 81	19[(4)]	73[(4)]
Vanillin (pH = 8.1)	0.86 ± 0.03	50 ± 3	152[(4)]	6[(4)]
trans-2-Hexenal	0.18 ± 0.05	93 ± 9	162[(6)], 155 ± 26[(7)]	72[(6)]
Cinnamaldehyde	0.17 ± 0.03	202 ± 32	6[(4)]	160[(4)]
Furfural	5.8 ± 0.3	281 ± 59	> 1000[(6)]	nd[(6)]
Citral	0.65 ± 0.05	139 ± 9	218[(6)]	~4[(6)]
Benzaldehyde	0.4 ± 0.2	100	148[(6)]	100[(6)]
4-Hydroxynonenal (HNE)	4.8[(8)], 17.9[(2)]	-	45 ± 18[(7)]	-
Malonaldehyde (MDA)	3.5[(8)], 15[(9)], 114.4[(2)]	-	0.0[(9)] 6550 ± 501[(7)]	-
Acetaldehyde	117[(9)], 50-180[(2)]	-	67000[(9)] 7034 ± 635[(7)]	-

[#]average, standard error

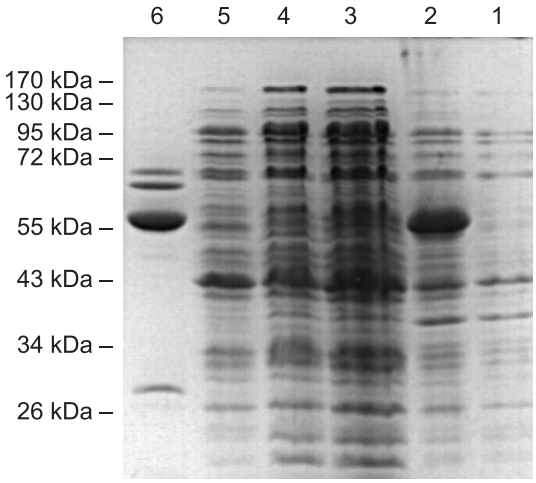

Figure 1. SDS/PAGE of recombinant ALDH1A1. Lysed bacterial culture before (1) and after (2) induction. Solution after applying crude lysate on the Ni-NTA column (3), first (4), and second (5) washing of the column. Solution from the first elution of recombinant ALDH1A1 (6). The gel was stained with Coomassie Brilliant Blue

of the hydrolysis of ascorbic acid (10). The most common are: furfural – present naturally in some fruits and vegetables (apples, cherries, carrots, potatoes), used as flavoring agent and formed during thermal processing of food containing sugars (cocoa, coffee, tea, beer, wine) with the highest concentration of 255 ppm in coffee, anisaldehyde – commonly found in fruits (apricots, cranberries), alcoholic beverages and anise (25000 ppm), vanillin – with the highest concentration in vanilla (23200 ppm), found also in fruits, asparagus and spices and trans-2-hexenal, responsible for the characteristic aroma of bananas (76 ppm). Their content is usually small but in some products can reach even 90% like in case of cinnamaldehyde in cinnamon oil (1, 3) which is often added to candies and chewing gums (700 ppm and up to 4900 ppm, respectively (11). Because of their widespread occurrence in a diet it is important to realize their effects on human health. In general, they can be mutagenic, carcinogenic, genotoxic and cytotoxic (12). That is why several enzyme systems were developed to protect us against these reactive compounds, like: alcohol dehydrogenases, aldo-ketoreductases, aldehyde oxidases, catalases, short-chain dehydrogenases/reductases, xanthine oxidase) (2). Another route of aldehyde detoxification is their reversible oxidation to carboxylic acids by aldehyde dehydrogenases, a superfamily of $NAD(P)^+$-dependent enzymes. They are encoded by 19 putatively functional genes with distinct chromosomal location (13). Their contribution to protection of organisms from aldehydes is crucial as evidenced by several studies in which they protected cells against aldehyde-induced cytotoxity (14). Some of these enzymes have high substrate specificity and are selectively expressed in particular human organs while others are ubiquitously spread. In the previous paper (4), it was shown that the only aldehyde dehydrogenase present in saliva is ALDH3A1, for which aldehydes commonly found in food products (anisaldehyde, benzaldehyde, cinnamaldehyde, vanillin) are good substrates. That is

why it was suggested that poor activity of this aldehyde dehydrogenase isozyme in saliva can be considered as predisposing factor for cancer of oral cavity. Here we showed that another isozyme, namely ALDH1A1 has even better affinities towards 'food' aldehydes, which were under investigation (submicromolar or low micromolar K_M values). This, together with the fact that it is expressed in the digestive system (esophagus, stomach, liver) makes it an important factor in food safety considerations as it can also play a role in the detoxification of aldehydes from food supporting the activity of ALDH3A1. What is more the contribution of ALDH1A1 to exogenous aldehyde metabolism is crucial in people suffering from hyposalivation which is not uncommon nowadays. It can be caused by many commonly used medications like tricyclic antidepressants, some antihypertensive drugs (α-adrenergic agonist antagonists), antispasmodic drugs (barbiturates) (15), radiotherapy for head and neck or dehydration. To conclude, the supporting role of ALDH1A1 in detoxification of aldehydes form food can be suggested.

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