Isoenzymatic forms of human cytidine deaminase

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Cytidine deaminase (CDA) purified from human placenta revealed the presence of five isoenzymatic forms that differ only in their isoelectric point. Since human cytidine deaminase exists in two variants (CDA 1 and CDA 2) with a non-conservative amino acid substitution at codon 27, in this work we demonstrate that these two variants may combine together \textit{in vitro}, giving five CDA isoforms as observed \textit{in vivo} from human placenta. For this purpose, each of the two forms of CDA was purified close to homogeneity and dissociated into monomers in the presence of a small amount of sodium dodecyl sulfate as a dissociating agent. The monomers were mixed together and subjected to anion-exchange chromatography and to chromatofocusing analysis in order to visualize the formation of the five isoforms. Furthermore, for both CDA 1 and CDA 2 some substrates and inhibitors of CDA were assayed, with the aim of demonstrating different kinetic behavior between the two natural variants.

\textit{Keywords:} cytidine deaminase/isoforms/subunit dissociation

Introduction

Cytidine deaminase (CDA; EC 3.5.4.5) is an enzyme of the pyrimidine salvage pathways catalysing the hydrolytic deamination of cytidine and deoxyctydine to the corresponding uridine and deoxyuridine. The clinical interest of the human enzyme is due to its capability to deaminate several antitumoral and antiviral cytosine nucleoside analogs, such as the anti-leukemic agent 1-β-d-arabinofuranosycytosine (ara-C) and the anti-cancer agent 5′-azadeoxycytidine (aza-CdR, Decitabine), leading to their pharmacological inactivation (Cameneri, 1967; Chabot \textit{et al.}, 1983; Laliberté \textit{et al.}, 1992; De Clerq, 2001). Furthermore, it has been shown that increased levels of cytidine deaminase in leukemic cells or in cell lines infected with the human immunodeficiency virus (HIV) are responsible for the resistance to Ara-C or to antiviral cytosine analogs (Balzarini \textit{et al.}, 1998), respectively. Several authors have suggested different ways to overcome the negative effect of human CDA on chemotherapy. Studies were performed with the objective of finding an efficient and non-toxic CDA inhibitor to co-administer with the cytosine nucleoside analogs to patients with solid tumors in order to increase the plasma level of these antineoplastic agents (Wentworth and Wolfenden, 1975). Other approaches consisted of designing new cytosine nucleoside analogs not susceptible to the deaminating action of human CDA (Shafiee \textit{et al.}, 1998). Finally, some studies suggested ways to exploit the CDA drug resistance: the cytidine deaminase gene could be used in cancer gene therapy to protect normal cells against the cytotoxic effects of cytosine nucleoside analogs (Eliopoulos \textit{et al.}, 1998). It is in this context that knowledge of the human CDA mechanism of action and also its expression in different human tissues has great importance.

Human CDA has been studied in several tissues, e.g. liver (Chabot \textit{et al.}, 1983), spleen (Vita \textit{et al.}, 1989), granulocytes (Teng \textit{et al.}, 1975; Bøyum \textit{et al.}, 1994) and lymphocytes (Pérignon \textit{et al.}, 1985). Studies on human cytidine deaminase purified from human placenta (hpCDA) have been performed, leading to the determination of the kinetic and molecular characteristics of the enzyme (Cacciamani \textit{et al.}, 1991).

Teng \textit{et al.} (1975) observed the presence of three electrophoretic CDA phenotypes in human granulocytes and supposed them to be the homozygous and heterozygous expression of two alleles: CDA 1 and CDA 2. Thereafter, the cloning of human CDA cDNA by two different research groups permitted the identification of two cDNA sequences coding for two CDAs that revealed one non-conservative amino acid substitution at position 27 of the amino acid sequence: CDA 1 carrying a Gln residue (Kühn \textit{et al.}, 1993) and CDA 2 that presents a Lys residue (Laliberté and Momparler, 1994).

The presence of two isoforms has also been identified by other research groups (Watanabe and Uchida, 1996; Kirch \textit{et al.}, 1998). A comparison of the enzymatic properties of the two variants has been performed but only in the case of Ara-C as substrate and tetrahydrodridine (THU) as inhibitor (Kirch \textit{et al.}, 1998). Comparative studies between hpCDA and recombinant human CDA 2 over-expressed in \textit{Escherichia coli} (Vincenzetti \textit{et al.}, 1996) have been performed, revealing that the kinetic properties of both substrates and inhibitors and the pH activity profile were very similar. Further studies on recombinant human CDA 2 (Cambi \textit{et al.}, 1998) revealed that the enzyme is a tetramer of 16 kDa subunits, each containing an essential zinc atom in the active site. Each subunit binds one molecule of substrate and acts independently of each other (Vincenzetti \textit{et al.}, 2000).

In this paper, we demonstrate that the two variants of recombinant human cytidine deaminase may combine together and produce \textit{in vitro} five enzymatic isoforms as observed in the CDA purified from human placenta. For this purpose, the two recombinant variants CDA 1 and CDA 2 were overexpressed in \textit{E.coli}, purified close to homogeneity and dissociated into monomers, mixed together and finally subjected to anion-exchange chromatography and chromatofocusing analysis in order to verify the combination of the two variants in the five isoforms. Moreover, further kinetic characterization of the two CDA variants was performed with various substrates and inhibitors.
Materials and methods

Chemicals

Nucleosides, nucleotides, bases, tris(hydroxymethyl)aminomethane (Trizma base), sodium dodecyl sulfate (SDS) and isopropyl-1-thio-β-galactopyranoside (IPTG) were obtained from Sigma Chemical, dithiothreitol (DTT) from ICN Biochemicals (Aurora, OH), ampicillin from USB (Cleveland, OH) and other chemical reagents from J.T. Baker Chemicals (Deventer, The Netherlands). PM 10 membranes were purchased from Amicon and protein markers from Bio-Rad Laboratories (Hercules, CA). Oligodeoxyribonucleoside primers were synthesized by DNA Technology (Aarhus, Denmark). PTrc99-A vector was supplied by Pharmacia (Uppsala, Sweden). Restriction nucleases were obtained from either Promega (Madison, WI) or New England Biolabs (Boston, MA).

DNA techniques

The cDNA coding for cytidine deaminase 1 (CDA 1), cloned in pCDNA3 vector, was a kind gift from Professor H.Christoph Kirch (University of Essen Medical School, Essen Germany). Escherichia coli DH5α was used as the host for cloning (Sambrook et al., 1989). For the complementation test, the pyrimidine requiring the cytidine deaminase negative derivative of MC1061, SO5201 (MC1061::pyrD::Kan) was employed. The minimal medium was phosphate-buffered AB (Clark and Maaløe, 1967), supplemented with 0.2% glucose and 0.2% vitamin-free casamino acids (Norite treated). L-Broth was used as rich medium (Miller, 1972). Thiamine 1 μg/ml, uracil 20 μg/ml, deoxyctydine 40 μg/ml, ampicillin 100 μg/ml, kanamicin 30 μg/ml and tetracycline 10 μg/ml (final concentrations) were added when required. Plasmid DNA was isolated using a Qiagen DNA kit and PCR products were purified with a Qiagen DNA kit and PCR products were purified with a Qiagen Purification Kit. Endonuclease digestion and ligation of DNA were carried out according to the suppliers and the transformation was performed according to Sambrook et al. (1989).

The sequence of the primers used for PCR sub-cloning of CDA 1 cDNA into pTrc99-A vector was: 5'-primer (PnocI), CAGACCATGGCCAGAAAGGCTG, containing the CDA start codon (italics) and an overlapping Ncol site (underlined); 3'-primer (PBam), CCGGATCCAGGTGCTGTTAC, including the BamHI site (underlined) located 39 bp downstream of the CDA stop codon. The resulting plasmid was named pTrcHUMCDA 1. DNA sequence analysis performed as described by Sanger et al. (1977) confirmed the primary structure of the insert.

Enzyme preparation and CDA assay

Escherichia coli SO5201 (MC1061 cdd::pyrD::Kan) containing the plasmid pTrcHUMCDA 2 (Vincenzetti et al., 1996) was used as a source of cytidine deaminase 2 (CDA 2). Both recombinant CDA 1 and CDA 2 were prepared as described previously (Vincenzetti et al., 1996). Briefly, cells were grown in L-broth supplemented with 100 μg/ml ampicillin. CDA expression was induced during late exponential growth (A436 nm = 1.0) by the addition of 0.6 mM IPTG. After 19 h of shaking at 37°C, the cells were harvested by centrifugation at 5000 g and washed with 0.9% NaCl. The cells were resuspended in buffer A (Tris–HCl 50 mM, pH 7.5, 1 mM DTT, 1 mM EDTA) and disrupted by sonication. The crude extracts were loaded on to an affinity column containing the cytidine analog 6-[3-(5-cytidyl)acyrloylamino]hexanoic acid (CV-6) bound to Affi-Gel 102 agarose resin, as reported previously (Vincenzetti et al., 1996). The pooled active fractions were collected and dialyzed against buffer A by ultrafiltration using a PM-10 membrane (Amicon). Cytidine deaminase from human placenta (hpCDA) was purified as described by Cacciamani et al. (1991) with minor modifications to the last step of purification.

The final step of purification for CDA 1, CDA 2 and hpCDA was anion-exchange chromatography on Mono Q HR 5/5 (Amersham Biosciences) connected to an HPLC system (ÄKTA Purifier, Amersham Biosciences). The column was equilibrated in buffer A and eluted with a linear gradient between buffer A and buffer B (buffer A containing 0.5 M KCl).

The final preparations of CDA 1, CDA 2 and hpCDA were dialyzed by ultrafiltration on PM-10 membranes (Amicon) against buffer A and, therefore, analyzed by 15% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE). Cytidine deaminase activity was measured spectrophotometrically as described previously (Vincenzetti et al., 1996). In kinetic studies with inhibitors, deoxycytidine (0.018–0.085 mM) was used as substrate. One enzyme unit is defined as the amount of enzyme that catalyzes the deamination of 1 μmol of cytidine per minute at 37°C.

Chromatofocusing

Chromatofocusing was performed on an HPLC system using a Mono P HR 5/5 column (Amersham Biosciences). The buffer system was 0.025 M bis-tris–HCl, pH 5.5, for column equilibration and Polybuffer 74 (Amersham Biosciences), pH 4, as eluent (diluted 1:10 with distilled water). Fractions of 0.5 ml were collected and immediately tested for pH and enzymatic activity. The p/ corresponds to the pH of the fraction showing the highest activity.

Subunit dissociation and re-association

To dissociate both CDA 1 and CDA 2 into subunits, 0.15 mg of each protein sample was incubated separately in the presence of 1.75 mM sodium dodecyl sulfate (SDS), in order to reach a molar SDS/enzyme ratio of about 600. After 4 h of incubation at 25°C, the samples were mixed together and dialyzed extensively by ultrafiltration on PM 10 membranes (Amicon) against buffer A (Tris–HCl 50 mM, pH 7.5, 1 mM DTT, 1 mM EDTA) without SDS. After dialysis, the mixture was analyzed for enzymatic activity and protein content. After the dialysis process, 0.09 mg of the mixture that was recovered was loaded on to a Mono Q column, equilibrated and eluted as described under Enzyme preparation and CDA assay, or on a Mono P column treated as described under Chomatofocusing.

The dissociation of CDAs into monomers was verified by a series of 15% PAGEs carried out in the presence of an increasing concentration of SDS, ranging from 0.35 mM (active tetrameric CDA) to 1.75 mM (completely dissociated into inactive monomers), as described in a previous paper (Vincenzetti et al., 2003). CDA activity on the gel after the electrophoresis was performed by cutting the polyacrylamide gel into 2 mm slices and incubating each slice with buffer A in the presence of the same SDS concentration as used during the electrophoresis.

Other analytical procedures

Protein concentration was determined by the method of Bradford (1976). The molecular mass and the purity of the
proteins were estimated by 15% SDS–PAGE (Laemmli, 1970); the low-range molecular weight markers were from Bio-Rad (phosphorylase b, 97.4 kDa; bovine serum albumin, 66.2 kDa; ovalbumin, 45.0 kDa; carbonic anhydrase, 31.0 kDa; soybean trypsin inhibitor, 21.5 kDa; lysozyme, 14.4 kDa). A 13% PAGE of pure recombinant CDA 1 and CDA 2 was performed, as described by Laemmli (1970), in the presence of 3 M urea. The electrophoretic course was performed at 4°C with a constant voltage of 200 V. After the electrophoresis, the gel was stained by silver staining or with Coomassie Brilliant Blue and the relative migration of both CDA 1 and CDA 2 was determined. The relative migration (rm) of a band is the ratio between the migration in cm of a sample band and the migration in cm of the dye in the gel.

Results

Purification of human recombinant CDA 1 and CDA 2

Human CDA 1 cDNA was sub-cloned from pCDNA3 to pTrc99-A in order to obtain the same expression system as for CDA 2 (Vincenzetti et al., 1996). For this purpose, the coding region of pCDNA3HUMCDA 1 was amplified by PCR, using PNCO and PBAM as 5’- and 3’- primers, respectively. The amplicon was digested with NcoI and BamHI and the resulting fragment cloned into NcoI–BamHI-digested Ptrc99-A, yielding pTrcHUMCDA 1. The recombinant plasmid was transformed into SØ5201 and the resulting strain was tested for ability to grow in the presence of deoxycytidine as the sole pyrimidine source (complementation test), as described previously (Vincenzetti et al., 1996). Both recombinant CDAs were purified by the same procedure: human CDA 1 or CDA 2 was loaded on to a CV-6 affinity chromatography column, as described (Vincenzetti et al., 1996). The active fractions were pooled and dialyzed against buffer A by ultrafiltration. After dialysis, 0.16 mg of CDA 2 or 0.19 mg of CDA 1 was loaded on the Mono Q HR5/5 anion-exchange chromatography column equilibrated and eluted as described under Materials and methods. Human recombinant CDA 1 was eluted from Mono Q at about 38% of buffer B, while CDA 2 was eluted at about 28% (Figure 1). According to the 15% SDS–PAGE analysis, the CDAs purified were judged more than 98% pure and had the same molecular weight (Figure 1, inset).

Purification of CDA from human placenta (hpCDA)

Cytidine deaminase was purified from human placenta in order to verify which of the two variants is expressed in the tissue. The purification protocol was as described by Cacciamani et al. (1991), except for the last step, where 0.76 mg of placental CDA eluted from the CV6 affinity column was loaded on to the Mono Q HR5/5 column equilibrated and eluted as described above. The result of this chromatographic step showed the presence of five active peaks (Figure 2), peak 1 eluting at about 30% of buffer B and peak 5 eluting at about 40% of buffer B. The active fractions of each peak were collected and loaded on to
15% SDS–PAGE: all of them resulted in one electrophoretic band corresponding to cytidine deaminase (Figure 2, inset). Furthermore, each of the five peaks re-loaded on to the Mono Q column was eluted at the same percentage of eluent B with respect to the previous chromatographic course (data not shown). The pool of each peak had the same specific activity (about 60 U/mg) and revealed the same $K_m$ value ($3.0 \times 10^{-5}$ M), determined with deoxycytidine as substrate.

**Molecular and kinetic characterization of CDA 1 and CDA 2**

Gel filtration chromatography and electrophoresis under denaturing conditions on pure recombinant CDAs suggested that both variants are oligomers, having four identical subunits of 15 kDa (data not shown). Several attempts were necessary to obtain an acceptable focused band of CDAs on native PAGE. The best condition was obtained at 13% PAGE in the presence of 3 M urea. Under this condition, the two variants showed a native conformation, since they are completely active. The difference in relative migration, therefore, was due also to the net charge of the proteins. As shown in Figure 3, CDA 1 and CDA 2 had a relative migration of 0.27 and 0.22, respectively; the mixture of CDA 1 and CDA 2 showed a diffuse band having a relative migration between these two values. Since the two CDAs have the same molecular weight (see Figure 1, inset), the results suggested that the different net charge of the
The enzymatic activity of both CDA 1 and CDA 2 on CdR as substrate was assayed between pH 3.0 and 10 (data not shown). Beyond these values, irreversible inactivation occurs. The resulting profile was very similar for the two variants and the $pK_v$ values were identical with those previously reported for CDA purified from human placenta (Cacciamani et al., 1991).

Mono P analysis
Chromatofocusing of recombinant CDAs on a Mono P HR 5/5 column (Amersham Biosciences), as described under Materials and methods, resulted in a $pI$ value of $4.50 \pm 0.01$ for CDA 1 and $5.00 \pm 0.02$ for CDA 2. Each of the five peaks of hpCDA eluted by Mono Q chromatography was subjected to Mono P analysis under the same conditions as described above. The results, reported in Table II, showed $pI$ values of the five peaks ranging from $4.90 \pm 0.01$ (peak 1) to $4.54 \pm 0.02$ (peak 5).

Subunit dissociation and re-association
In this experiment, human recombinant CDA 1 and CDA 2 were separately dissociated into monomers, as described under Materials and methods, and mixed together. The mixture of CDA 1 and CDA 2 obtained had lost its activity since it corresponded to the monomeric form of cytidine deaminase (molecular weight 15 kDa), as verified by 15% PAGE in the presence of 0.35 mM SDS (see Figure 4, gel B). After extensive dialysis against buffer A without SDS, the mixture containing both CDA 1 and CDA 2 was re-activated and the enzymes were in tetrameric form (molecular weight 60 kDa), as demonstrated by running the samples in 15% PAGE in the presence of 0.35 mM SDS (Figure 4, gel A).

When 0.09 mg of the re-activated mixture was loaded on to a Mono Q column, equilibrated with buffer A and eluted under the same conditions as used for CDA 1, CDA 2 and hpCDA, five active peaks were obtained (Figure 5). Peak 1 eluted

Table I. Kinetic parameters for substrates and inhibitors of recombinant human CDA 1 and CDA 2

<table>
<thead>
<tr>
<th>Substrate</th>
<th>$K_m$ (10$^{-5}$ M)</th>
<th>$V_{max}$ (U/mg)</th>
<th>$V_{max}/K_m$</th>
<th>Inhibitor</th>
<th>$K_i$ (10$^{-5}$ M)$^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CDA 1</td>
<td>CDA 2</td>
<td>CDA 1</td>
<td>CDA 2</td>
<td>CDA 1</td>
</tr>
<tr>
<td>CdR</td>
<td>4.2</td>
<td>4.0</td>
<td>50.0</td>
<td>45.5</td>
<td>11.9</td>
</tr>
<tr>
<td>CR</td>
<td>4.0</td>
<td>4.2</td>
<td>52.0</td>
<td>53.0</td>
<td>13.0</td>
</tr>
<tr>
<td>5-I-CdR</td>
<td>8.7</td>
<td>10.0</td>
<td>45.0</td>
<td>91.0</td>
<td>5.2</td>
</tr>
<tr>
<td>Ara-C</td>
<td>15.0</td>
<td>11.0</td>
<td>30.0</td>
<td>31.0</td>
<td>2.0</td>
</tr>
<tr>
<td>5-Aza-CR</td>
<td>31.0</td>
<td>28.0</td>
<td>32.0</td>
<td>38.0</td>
<td>1.0</td>
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$^a$CdR, deoxycytidine; CR, cytidine; 5-I-CdR, 5-iododeoxycytidine; Ara-C, cytosine arabinoside; 5-Aza-CR, 5-azacytidine; FZEB, 5-fluorozebularine; THU, 3,4,5,6-tetrahydrouridine; 5,6-DHU, 5,6-dihydrouridine; CV6, (cytidyl)acryloylaminohexanoic acid; UR, uridine; CMP, cytidine monophosphate.
$^b$Vincenzetti et al. (1996).
$^c$Inhibition constants ($K_i$) were obtained from double-reciprocal plots of the deamination constants, in presence and in absence of inhibitors. All inhibitors were competitive.

![Active tetramer and Inactive monomer](image)

**Fig. 4.** 15% PAGE of the mixture of CDA 1 plus CDA 2. Gel A: the active tetrameric form in the presence of 0.35 mM SDS. Gel B: the inactive monomer in the presence of 1.75 mM SDS. St: low molecular weight standard (Bio-Rad).
at about 28% of buffer B and peak 5 eluted at about 38% of buffer B. These results are in accordance with the five peaks obtained with hpCDA (see Figure 2). The lower activity shown in Figure 5 with respect to Figures 1 and 2 is due to the loss of enzyme content during the dialysis in the re-association process. Each peak showed the same specific activity as before the dissociation and re-association process.

The mixture of CDA 1 and CDA 2 was loaded on to a Mono P column equilibrated and eluted as described under Materials and methods. Table II shows that five active peaks are formed having pI values similar to those of the five peaks isolated from human placenta.

Discussion

Cloning of human cytidine deaminase cDNA showed that there are two different variants with a non-conservative amino acid substitution at codon 27 resulting in a Gln (CDA 1) (Kühn et al., 1993) or a Lys (CDA 2) (Laliberté and Momparler, 1994).

In the present study, we undertook a molecular and kinetic characterization of the two recombinant CDA variants. Electrophoresis conducted under non-denaturing conditions (13% PAGE in the presence of 3 M urea) confirmed that CDA 1 and CDA 2 differ significantly in net charge since CDA 2, with a Lys residue at position 27 of the amino acid sequence, has a lower electrophoretic migration (see Figure 3) with respect to CDA 1 (Gln residue at codon 27).

The kinetic parameters of the two recombinant CDAs of some substrates and inhibitors were also calculated. As shown in Table I, there were no significant differences in the deamination rate of the natural substrates cytidine and deoxycytidine. Also, the K_i values calculated for both CDA 1 and CDA 2 with respect to some inhibitors were not significantly different. In both recombinant CDAs, no cooperativity was observed between the subunits; in fact, all the inhibitions were competitive. The amino acid substitution at position 27 of the amino acid sequence lies in a protein region close to a highly conserved region in most of the tetrameric CDAs (Vincenzetti et al., 1999) and a comparison with the crystal structure of the tetrameric CDA from Bacillus subtilis (Johansson et al., 2002) showed that this amino acid at position 27 may be located on the α-helices α-1 and seems not to be directly involved in the construction of the active site.

In previous work (Vincenzetti et al., 2003), we described how recombinant human cytidine deaminase (CDA 2) can dissociate into monomers in the presence of SDS at concentrations ranging from 0.35 mM (active tetramer) to 1.75 mM (inactive monomers) and that the inactive monomers are able to re-associate into an active tetrameric form after the removal of the dissociating agent. In this paper, we demonstrated that a mixture of recombinant human CDA 1 and CDA 2, previously dissociated into inactive monomers by 1.75 mM SDS (Figure 4), was able to re-associate giving five isoforms, visible after a Mono Q analysis, each of them showing cytidine deaminase activity (Figure 5). These five isoforms obtained in vitro from the mixture of the two recombinant CDAs had the same characteristics of the five isoforms of CDA present in human placenta: they eluted on the Mono Q anion-exchange chromatographic column between 30% (peak 1) and 40% (peak 5) of eluent B. Similarities were also found in the pI values obtained for the five isoforms in vitro and those obtained from human placenta (Table II). In fact, the pI values observed for peaks 1 and 5 of the placental enzyme are 4.90 and 4.54, respectively, corresponding to the recombinant CDA 2 (pI 5.0) and CDA 1 (pI 4.50) respectively. Peaks 2, 3 and 4 of human placenta CDA showed intermediate pI values (4.76, 4.70 and 4.64, respectively). The pI values of the five isoforms obtained from the two natural variants ranged from 4.90, peak 1 (corresponding to CDA 2), to 4.35, peak 5 (corresponding to CDA 1), and were closely related to that obtained from human placenta CDA (Table II). From these data, it appears that the five isoforms observed in the cytidine deaminase extracted from human placenta may be a combination of the products of the two CDA genes. We suppose that the two genes of cytidine
deaminase designated cdd1 (Kühn et al., 1993) and cdd2 (Laliberté and Momparler, 1994) may be expressed in the somatic cells in the same amount and assembled into functional tetrameric forms by a combination of the two different monomers CDA 1 (A) and CDA 2 (B): A2B2; A2B2; A2B2; B2A2. A similar behavior was observed with lactate dehydrogenase (LDH). This tetrameric enzyme consists of two types of subunits, M and H, that can combine together casually giving five isoenzymes: M4; M3H1; M2H2; M1H3; H4 (Kopperschlager and Kirchberger, 1996). These isoenzymes are widely distributed in various organs and the appearance of some LDH isoenzymes in blood is indicative of tissue damage and is, therefore, useful as a diagnostic parameter (Xue and Yeung, 1996). Since, in the case of our isoenzymatic forms, we observed no important differences in kinetic properties or the absence of cooperativity between the subunits in both of CDA variants, the meaning of the two cytidine deaminase variants remains unclear at present; nor do we understand why the somatic cells differentially express the five combination of tetramers derived from the two CDAs. The presence of isofoms seems to be characteristic of some deaminases (Yeung et al., 1983; Mahnke-Zizelman et al., 1998). In particular, studies on adenosine deaminase (ADA) revealed that the activity of total ADA and its isoenzymes ADA1 and ADA2 is correlated with clinical disease in rheumatoid arthritis and lupus erythematosus (Stancikova et al., 1998; Hitoglou et al., 2001).

We are planning, therefore, to extend our investigations to other human tissues in order to discover if there is different expression of the isoforms in various human tissues and also to see if there is a relationship between different isoenzymatic expression and a particular pathology.

References


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