Temperature-induced selective death of the C-domain within angiotensin-converting enzyme molecule

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Abstract Somatic angiotensin-converting enzyme (ACE) consists of two homologous domains, each domain bearing a catalytic site. Differential scanning calorimetry of the enzyme revealed two distinct thermal transitions with melting points at 55.3 and 70.5°C. which corresponded to denaturation of C- and N-domains, respectively. Different heat stability of the domains underlies the methods of acquiring either single active N-domain or active N-domain with inactive C-domain within parent somatic ACE. Selective denaturation of C-domain supports the hypothesis of independent folding of the two domains within the ACE molecule. Modeling of ACE secondary structure revealed the difference in predicted structures of the two domains, which, in turn, allowed suggestion of the region 29–133 in amino acid sequence of the N-part of the molecule as responsible for thermostability of the N-domain. © **2002 Published by Elsevier Science B.V. on behalf of the Federation of European Biochemical Societies.**

Key words: Peptidyl dipeptidase A; Domain; Enzyme stability; Differential scanning calorimetry; Circular dichroism; Secondary protein structure

1. Introduction

Angiotensin-converting enzyme (ACE, peptidyl dipeptidase A, EC 3.4.15.1) is a zinc-metallopeptidase known to play a remarkable role in blood pressure control and water–salt metabolism [1]. There are several ACE forms produced in an organism. Somatic ACE (150–180 kDa) is mainly located in endothelial, epithelial and neuronal cells. This enzyme consists of two homologous domains (N- and C-domains) within a single polypeptide sequence, each domain containing its own catalytic site [2]. Molecular cloning shows that this structure resulted from gene duplication [2], the overall sequence similarity between the two domains is 60% in both nucleotide and amino acid sequences but increases to 89% in a sequence of 40 amino acids in each domain containing essential residues of the active site. The three-dimensional structure of the ACE molecule is still unknown. A smaller ACE isoform (90–100 kDa) is present in mature germinal cells. Testicular isoenzyme is identical to the C-domain of somatic ACE, except for a small N-terminal region, and contains a single active site [3]. The form of ACE that corresponds to the N-terminal domain was found in ileal fluid of patients undergoing surgical operation [4]. This enzymatic form probably originates in vivo from limited proteolysis of intestinal somatic ACE.

It was shown, however, that the 'bridge sequence' between the two ACE domains can also be cleaved in vitro within the native enzyme with endoproteinase Asp-N [5] and in slightly denatured ACE molecule with trypsin, plasmin, kallikrein [4,6]. It is remarkable that the last approach always results in the isolation of only the ACE N-domain whereas the remaining part of the molecule is digested by proteinases after denaturing procedures. This fact was attributed to different sensitivity of ACE N- and C-domains to proteolysis and/or to different stability of the two domains upon denaturation [5]. It is worth noting that different denaturation treatments of the enzyme, e.g. exposure to alkali, acid, urea or moderate heat, made the C-domain susceptible to cleavage by proteinases, this fact indicating different sensitivity of the two domains to the proteolytic digestion. The possibility exists that the C-domain is inaccessible to proteolytic digestion in native ACE and is only exposed to proteinases in slightly denatured enzyme. It was suggested earlier on the basis of different immunogenicity of the two ACE domains [7] that the enzyme molecule can be folded in such a way that the main part of the solvent-accessible area is provided by the amino acids of the N-domain, and the C-domain is buried under the N-domain.

However, the two domains within the ACE molecule can also differ in their stabilities with respect to denaturation. Rat testicular ACE, corresponding to the C-domain of somatic ACE, was shown to be less stable at high temperatures than the somatic enzyme [8]. Comparison of the heat stability of human recombinant full-sized ACE and separate domains showed that the wild-type ACE and separate N-domain demonstrated similar stability at 47°C (54–55% retained activity after 15 min incubation) whereas the separate C-domain was less stable (only 23% retained activity) [9].

Highly homologous proteins possess, as a rule, similar overall structure. Despite this, differences in α-helix content, hydrophobic regions, proline residue content, the number of disulfides and salt bridges can highly influence the observed stability of the homologues. For instance, glyceraldehyde-3-phosphate dehydrogenase from yeast irreversibly loses its activity at 40°C, whereas the homologous enzyme from thermophilic bacterium *Thermotoga maritima* maintains its structure

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Abbreviations: ACE, angiotensin-converting enzyme; DSC, differential scanning calorimetry; SDS, sodium dodecyl sulfate; PMSF, phenylmethylsulfonyl fluoride; FA-Phe-Gly-Gly, furanacryloyl-L-phenylalanyl-glycyl-glycine; FA-Phe-Ala-Ala, furanacryloyl-L-phenylalanyl-alanyl-alanine; Hip-His-Leu, *N*-benzoyl-glycyl-histidyl-leucine; Cbz-Phe-His-Leu, *N*-carbobenzoxy-phenylalanyl-histidyl-leucine

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and retains catalytic activity even at 100°C [10]. The thermophilic enzyme was shown to have a low flexibility of polypeptide chain and enhanced content of both hydrophobic amino acids and salt bridges. Proline residues are also believed to be responsible for high stability at high temperatures as, for example, stability of human protein RAD52 [11]. The interesting feature of the ACE molecule is that the two homologues apparently differing in their stability are included in a single polypeptide chain.

Here, we demonstrate different heat stability of the domains within bovine somatic ACE molecule, which allows one to obtain single active N-domain or active N-domain with an inactive C-domain within parent somatic ACE. We have also tried to describe the structural differences between the two domains responsible for higher stability of the N-domain.

2. Materials and methods

2.1. Reagents

The substrates FA-Phe-Gly-Gly (furanacryloyl-L-phenylalanyl-glycyl-glycine), Hip-His-Leu (*N*-benzoyl-glycyl-histidyl-leucine) and Cbz-Phe-His-Leu (*N*-carbobenzoxy-phenylalanyl-histidyl-leucine) were obtained from Sigma (USA), FA-Phe-Ala-Ala (furanacryloyl-L-phenylalanyl-alanyl-alanine) was kindly given by Dr. M. Ovchinnikov from the Cardiology Research Center, Moscow, Russia. Phenylmethylsulfonyl fluoride (PMSF) was from Sigma, EDTA-free proteinase inhibitor cocktail was obtained from Roshe Diagnostics (Germany), Triton X-100 was from Ferak (Germany).

2.2. Enzyme purification

Soluble forms of somatic and testicular ACEs were isolated from bovine lung and bovine testicles, respectively, by Triton X-100 extraction and were further purified by lisinopril affinity chromatography as described in [12]. Membrane form of somatic ACE was isolated from bovine lung by Triton X-100 extraction in the presence of 1 mM EDTA as described in [13]; the enzyme was purified by hydrophobic and affinity chromatography according to [14]. Electrophoretic homogeneity of ACE preparations was proved by electrophoresis by the Laemmli method [15] in polyacrylamide gel in the presence of 0.1% sodium dodecyl sulfate (SDS) and β-mercaptoethanol. Proteins were stained with Coomassie brilliant blue G-250.

2.3. Enzyme activity measurements

The rates of catalytic hydrolysis of FA-Phe-Gly-Gly and FA-Phe-Ala-Ala were monitored spectrophotometrically as described in [16]. The rates of catalytic hydrolysis of Hip-His-Leu and Cbz-Phe-His-Leu were determined fluorometrically by *o*-phtalaldehyde modification of His-Leu as a product of the reaction [17]. The reactions were carried out in 50 mM HEPES buffer, pH 7.5, containing 0.15 M NaCl and 1 μM $ZnCl_2$, at 25°C. Kinetic parameters of the hydrolysis were determined based on the concentration of enzyme active molecules in ACE preparation. Stoichiometric titration of ACE active molecules was performed with the specific competitive inhibitor lisinopril as in [6].

2.4. ACE thermoinactivation kinetics

Enzyme samples (10^{-8}–2×10^{-6} M) were incubated with or without proteinase inhibitors in 0.025 M phosphate, 0.025 M borate buffer, pH 6.5, containing 0.15 M NaCl and 1 μM $ZnCl_2$ (PB buffer), at 55°C. After required periods of time, aliquots were taken and cooled on ice. Residual enzymatic activity was assayed with 5×10^{-5} M Cbz-Phe-His-Leu as a substrate.

2.5. Differential scanning calorimetry (DSC)

ACE solutions were dialyzed against PB buffer with proteinase inhibitors and then were concentrated to 0.4 mg/ml on Amicon YM-30 ultrafiltration membrane. The protein concentration was determined from a stock solution of protein using the Lowry method [18]. Calorimetric experiments were performed on a DASM-4 differential adiabatic scanning microcalorimeter (Biopribor, Russia) equipped with a 0.47 ml capillary platinum cell interfaced with an IBM-compatible computer. An external pressure of two atmospheres was applied with nitrogen gas. The sample was scanned over a temperature range of 20–100°C at a rate of 60°/h. The reversibility of the thermal transitions was checked by reheating the samples after cooling from the first scan. DSC measurements on buffer alone had no transitions in the temperature range 20–100°C. As the ACE thermal transition was completely irreversible, enzyme calorimetric traces were corrected for the instrumental baseline by subtracting scans for the reheating of the samples. Total enthalpy of ACE denaturation was calculated according to the molecular mass of somatic enzyme equal to 180 kDa.

2.6. Circular dichroism (CD) spectroscopy

Far UV CD spectra from 200 to 250 nm were recorded in the modified dichrograph Jobin Ivon Mark V (France) interfaced with an IBM-compatible PC. Measurements were performed in 1 mm cells in PB buffer at ACE concentrations of 0.2–0.4 mg/ml.

2.7. Modeling of ACE secondary structure

Secondary structure of ACE domains was predicted on the basis of the amino acid sequence of bovine somatic ACE [19] using the standard range of the service Consensus method [20] available through web-server Network Protein Sequence Analysis http://npsa-pbil.ibcp.fr/. This approach allows to perform modeling by four different algorithms simultaneously on the basis of statistics methods, homology, as well as empirical and self-learning methods. The final prognosis was developed by correlation of the results obtained by all algorithms used.

3. Results and discussion

3.1. ACE thermoinactivation

Somatic and testicular forms of bovine ACE exhibited different kinetics of thermoinactivation. Single-domain testicular ACE inactivated at 55°C in a first-order kinetic reaction with the value of $k_{in} = (1.9 \pm 0.2)\times10^{-3}$ s^{-1} (Fig. 1, curve 1). Two-domain somatic ACE, however, revealed two distinctive fractions differing in their stability: the first fraction inactivated with first-order kinetics with $k_{in} = (1.7 \pm 0.2)\times10^{-3}$ s^{-1}, whereas the second one retained catalytic activity for at least several hours (Fig. 1, curve 2). The existence of these fractions could not be explained by the presence of a native hydrophobic anchor in some portion of ACE molecules in the enzyme preparation as there was no difference to be found in observed kinetics of inactivation of ACE with and without anchor (data not shown). Variation of ACE concentration from 10^{-8} to 2×10^{-6} M in reaction medium also did not cause any alteration in the value of k_{in} and the ratio of the fractions (data not shown). Subsequent incubation of the thermoinactivated enzyme at room temperature up to 24 h, or at 4°C up to 1 week, did not restore enzymatic activity. Repeated heating of once heated enzyme did not expose the first labile fraction on the curve of enzyme inactivation but revealed the presence of only a stable ACE form. Thus, the thermoinactivation of somatic ACE is irreversible and there is no equilibrium between two enzyme fractions. Thus, these fractions cannot be attributed to different enzyme conformers or different oligomeric forms differing in stability. The coincidence of the value of k_{in} of labile fraction in somatic enzyme preparation with k_{in} value of testicular enzyme indicates that the existence of the two fractions could be assigned to different stabilities of C- and N-domains within somatic ACE globule.

3.2. Characteristics of thermostable ACE fraction

Thermoinactivation of somatic ACE was accompanied by the appearance of additional protein bands with molecular masses 100–150 kDa as seen by SDS–PAGE (Fig. 2, lane

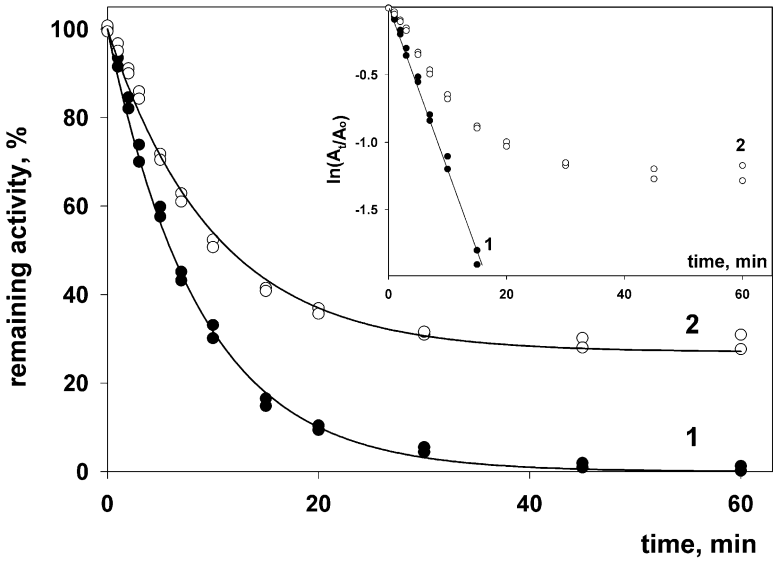

Fig. 1. Thermoinactivation of bovine testicular (1) and somatic (2) ACE in PB buffer, pH 6.5, at 55°C. Inset – the data in first-order coordinates.

3). We have attributed this fact to the presence of a contaminating proteinase of a certain kind in stock ACE preparations which is able to cleave denatured ACE molecules. Subsequent treatment of thermoinactivated, for 1 h, ACE preparation with trypsin resulted in the formation of a single protein band with a molecular mass of about 100 kDa (Fig. 2, lane 4). The determination of the first six N-terminal amino acids of the resulting fragment (NH_2-Glu-Leu-Asp-Pro-Ala-Leu) revealed their identity with the first six amino acids of native bovine somatic ACE [19]. Since this sequence is absent in the other parts of the primary structure of the somatic enzyme, we concluded that the resulting fragment represents the N-domain of ACE molecule.

Presence of proteinase inhibitors (10^{-5} M PMSF and proteinase inhibitor cocktail as directed by the supplier) in ACE solution during thermoinactivation did not influence the observed kinetics of inactivation but preserved the enzyme against proteolytic degradation; electrophoresis revealed in this case the only protein band with a molecular mass of 180 kDa corresponding to native enzyme (Fig. 2, lane 5).

Kinetic parameters of the hydrolysis of synthetic tripeptide substrates by both individual N-domain and denatured enzyme with a molecular mass of 180 kDa are given in Table 1. The values obtained are very similar, and coincide with the kinetic parameters of the hydrolysis of these substrates by individual bovine ACE N-domain obtained by limited proteolysis of the parent somatic form after NH_4OH treatment [6]. Thus, we can conclude that the thermostable 180 kDa enzymatic form represents an enzyme with active N-domain but inactive C-domain.

It was shown previously [9] in the experiments on recombinant human ACE that the part of the N-domain of 141 amino acids but not its active site linked to the active C-domain affected the characteristics of the C-domain active center. The properties of this construct differed from the properties of both wild-type ACE and separate C-domain in reactivity to Cl^- and SO_4^{2-} ions, hydrolysis ratios of substrates and heat stability. These findings are consistent with observations in [21] where chimeric N- and C-domains of human ACE demonstrated the importance of the sequence surrounding the central regions in each domain on their catalytic characteristics. The properties of the active N-domain within the molecule of bovine somatic ACE, however, seem to be not affected by the presence of the thermodenatured C-domain. The properties of structural domains differing in stability within a single protein globule are generally determined by mutual influence of the domains in the protein molecule. Strong interaction between domains often diminishes the difference in stability of separate domains, as happens in case of γ-crystallin from human eye lens in which an intermediate consisting of denatured labile domain and intact stable domain is not detected [22]. The existence of a bovine somatic ACE intermediate consisting of both denatured C-domain and intact N-domain is a strong evidence of independent folding of the two domains within a protein globule.

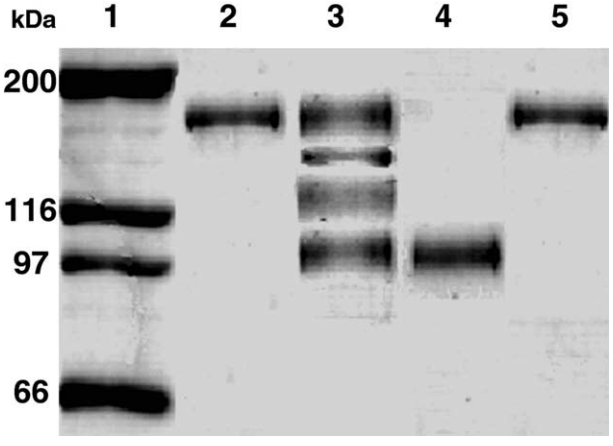

Fig. 2. SDS–PAGE (8%, Coomassie-stained), illustrating denaturation and digestion of ACE C-domain. 1, molecular mass standards; 2, somatic ACE; 3, somatic ACE after 1 h incubation without proteinase inhibitors at 55°C, pH 6.5; 4, trypsin digestion (trypsin: ACE = 1:20, M/M) of ACE preparation shown on lane 3; 5, somatic ACE after 1 h incubation at 55°C, pH 6.5, in the presence of 10^{-5} M PMSF and proteinase inhibitor cocktail.

Table 1
Kinetic parameters for the hydrolysis of specific substrates by different ACE forms

Enzyme	k_{cat} (s^{-1})	K_m (mM)
	Fa-Phe-Gly-Gly	
Native somatic	280 ± 16	0.70 ± 0.06
N-domain (ammonia-treated)	279 ± 10 (6)	1.40 ± 0.02 (6)
N-domain (heated)	270 ± 10	1.00 ± 0.10
Heated somatic	270 ± 10	1.00 ± 0.10
	Fa-Phe-Ala-Ala	
Native somatic	108 ± 11	0.050 ± 0.005
N-domain (ammonia-treated)	35 ± 3 (6)	0.050 ± 0.005 (6)
N-domain (heated)	45 ± 10	0.050 ± 0.005
Heated somatic	50 ± 12	0.04 ± 0.01
	Cbz-Phe-His-Leu	
Native somatic	56 ± 5	0.25 ± 0.02
N-domain (ammonia-treated)	122 ± 10 (6)	0.15 ± 0.02 (6)
N-domain (heated)	115 ± 12	0.15 ± 0.02
Heated somatic	135 ± 15	0.12 ± 0.02
	Hip-His-Leu	
Native somatic	12 ± 1	0.90 ± 0.10
N-domain (ammonia-treated)	12 ± 1 (6)	0.50 ± 0.05 (6)
N-domain (heated)	15 ± 1	0.40 ± 0.05
Heated somatic	13 ± 1	0.50 ± 0.05

Conditions: 0.05 M HEPES buffer, pH 7.5; 0.15 M NaCl, 1 μM $ZnCl_2$, 25°C.

3.3. DSC

We then used DSC to probe the stability of somatic ACE in the presence of proteinase inhibitors. The thermal stability profile was composed of two transitions with melting temperatures (T_m) of 55.3 and 70.5°C (Fig. 3, curve 1). Total enthalpy of ACE denaturation was found to be equal to 62.9 ± 3.2 kJ/mol/K, transition at 55.3°C taking 48.8% and transition at 70.5°C taking 51.2% of total enthalpy, respectively. Reheating the samples after cooling from the first scan did not show any thermal transitions indicating irreversibility of melting. An ACE preparation preliminarily heated at 55°C for 1 h demonstrated a single transition at higher temperature of 70.7°C (Fig. 3, curve 2). Individual N-domain obtained by trypsin treatment of heated somatic ACE as described above also showed only one transition at the same temperature (Fig. 3, curve 3). Thus, this transition should be attributed to denaturation of N-domain both in its individual state and within the parent molecule of somatic ACE. The results obtained confirm the hypothesis of independent folding of the two domains within the somatic ACE molecule.

Thus, we suggest quick methods of obtaining active individual N-domain, or active N-domain within parent somatic molecule with inactive C-domain, based on the different temperature stability of the two domains and their apparent independent folding. Individual domain can be obtained by the treatment of the enzyme at 55°C for 1 h with subsequent proteolysis, while active N-domain within parent ACE molecule with inactive C-domain can be obtained by the same denaturing treatment but in the presence of proteinase inhibitors.

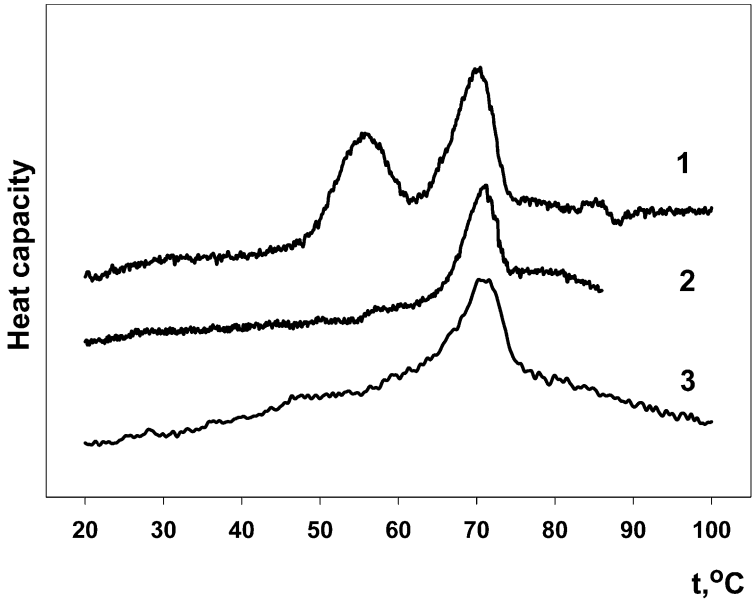

Fig. 3. DSC scans of different ACE forms (PB buffer with proteinase inhibitors, scan rate 60°/h). 1, somatic ACE; 2, somatic ACE preheated for 1 h at 55°C; 3, individual N-domain.

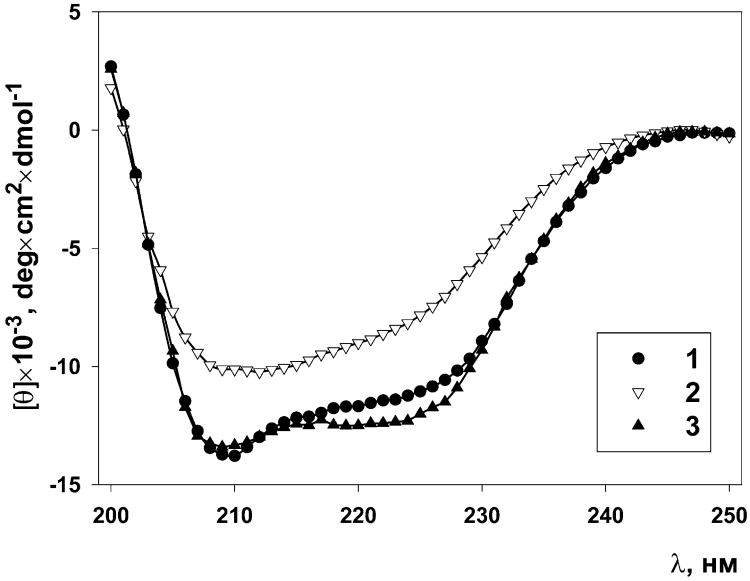

Fig. 4. CD spectra of different ACE forms: 1, somatic ACE (37% of α-helices); 2, somatic ACE heated for 1 h at 55°C (24% of α-helices); 3, individual N-domain (39% of α-helices).

3.4. CD spectroscopy

Structural differences between the domains within ACE molecule were demonstrated by CD spectroscopy of ACE preparations. We detected the changes in CD spectra of somatic ACE upon denaturation and the difference between somatic ACE spectrum and N-domain spectrum. Thermodenaturation of somatic ACE caused alterations in the intensity and the form of CD spectrum, indicating the damage in secondary structure of the protein (Fig. 4). Removal of the denatured part of the molecule by trypsin with subsequent isolation of the N-domain did not result in restoration of the initial spectrum, the N-domain possessing higher intensity at 220 nm as compared to that of somatic ACE (Fig. 4). This observation indicates a higher content of α-helix in N-domain versus that in parent somatic form and, in turn, a higher content of α-helix in N-domain versus that in C-domain. Estimation of α-helix content in ACE molecule using the Greenfield and Fasman equation [23] gives the value about 40%, the difference in α-helix content between the two domains does not exceed 2–3%.

3.5. Modeling of a secondary structure

Evaluation of theoretical secondary structures of the two ACE domains was carried out by simultaneous use of different approaches [20] and was based on the primary structure of the enzyme [19]. We also took into consideration the extent of domain homology in the investigated regions. So, if the results on secondary structure of some region in one domain predicted by different methods markedly diverged we could use the results obtained for the corresponding region in another domain, provided homology of the regions was more than 90%. This approach gave us the possibility to reveal the possible secondary structure of the enzyme and to find structurally different regions in homologous domains (Fig. 5). According to structural modeling of the ACE molecule both domains contain about 40% of α-helix, about 60% of random

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N ----LDPALQPGNFPADEAGAQIFAASFNSSAEQVLFQSTAASWAHDTNI 75
      *   :  :: :**  *: *   ::  :: *  : * * : *:*
C RPPMPDNYPEGIDLVSDEDEARKFVEEYDRRSQVVWNEYAEANWNYSTDI 679

N TEENARLQEEAALLSQEFSEAWGQKAKDLFDPVWQNFTDPTLLRIIGAVR 125
  : :*::*  *  *   : :  :*  *   **    ** : *: *:*  ::
C STDNSKLLMEKNLQMANHTVKYGTWAS-KFD--VTNFQNATMKRMIKKIQ 726

N TLGPANLDLEKRQKYNSLLSNMSRIYSTAKVCFPNKTAPCWSLDPELTNI 175
  *  * *  :: ::** :* :*  :** * **  *    *  *:*:***:
C DLERAALPTKELEEYNQILLDMETVYSVASVCHEN--GTCLRLEPDLTNL 774

N LASSRSYTLLLYAWEGWHNAAGIPLKPLYQDFTALSNEAYKQDGFSDTGA 225
  :*:** *  * :**: *::  *  : * :  :  *:*:* : :*: * *
C MATSRNYQDLAWAWKSWRDKVGRSILPYFPKYVELTNKAARLNGYQDGGD 824

N YWRSWYDSPTFTEDLERLYQQLEPLYLNLHAYVRRALHRRYGDRYINLRG 275
   *** *: * : *:**:*:*:*:****************:**   *** *
C SWRSMYEMPFLEEELEQLFQELQPLYLNLHAYVRRALHRHYGPDVINLEG 874

N PIPAHLLGNMWAQSWENIYDTVVPFPDKPNLDVTDVMVQKGWNATHMFRV 325
  ***************  **** * ***  *::* *: *:::**   :**:
C PIPAHLLGNMWAQSWSNIYDLVAPFPSAPKMDATEAMIKQGWTPLRMFKE 924

N AEEFFTSLGLLPMPPEFWAESMLEKPSDGREVVCHASAWDFYNRKDFRIK 375
  *:;***************  :******;**************:* ******
C ADNFFTSLGLLPMPPEFWNKSMLEKPTDGREVVCHASAWDFFNGKDFRIK 974

N QCTRVTMDQLSTVHHEMGHVQYYLQYKGQHVSLRRGANPGFHEAIGDVLA 425
  *** * *::*   ******;**;::***   *::* ***************
C QCTSVNMEDLVVAHHEMGHIQYFMQYKDLPVTFREGANPGFHEAIGDVLA 1024

N LSVSTPAHLHKIGLLDQVTNDTESDINYLLKMALEKIAFLPFGYLVDQWR 475
  ******;***** **       * ***;*;*********:** :******
C LSVSTPTHLHKINLLSSGDGGYEEDINFLMKMALEKIAFIPFSFLVDQWR 1074

N WGVFSGRTRPSRYNYDWWYLRTKYQGICPPVVRNETHFDAGAKFHVPNVT 525
  * ** *      ** :** ** ****;***: * :  ** *****:*
C WRVFDGSVTRENYNQEWWSLRLKYQGVCPPLARSQDDFDPGAKFHIPASV 1124

N PYIRYFVSFVLQFQFHEALCKEAGHQGPLHQCDIYQSTQAGAKLRALLQA 575
  **;*******;*****:***: ********;****** :**  *   ::
C PYVRYFVSFVIQFQFHQALCQAAGHQGPLHKCDIYQSKEAGKLLADAMKL 1174

N GSSRPWQEVLKDMVGSDNLDARPLLSYFQPVTQWLEE 612
  * *:** * :: : *  *: *
C GFSQPWPEAMRLITGQSNMSASA 1197
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Fig. 5. Alignment and predicted locations of α-helices (underlined) in ACE domains; zinc-binding motif is shown in italics; '*' means identical residues in sequences; ':' indicates conserved substitutions. The region which shows remarkable structural differences is boxed.

coil, whereas the amount of β-sheets is negligible. These results are quite similar to those obtained by CD spectroscopy (taking into account the errors of both methods) which denotes the applicability of the suggested modeling of the enzyme structure. The main structural differences between domains were found in the region 29–133 of the N-domain and 633–734 of the C-domain, respectively, where both the position and length of α-helices differ. The α-helix content is anticipated to be higher in the N-domain (Fig. 5). This region in the N-domain contains two insertions (one and two amino acid residues) from three in the whole domain and, moreover, just this region exhibits a high content of proline residues, six residues in 29–133 amino acid sequence versus three proline residues in the corresponding 633–734 sequence of the C-domain.

The carbohydrate content of the N-domain would also contribute some difference to the stability of the two domains. It is known that the N-domain of the enzyme is highly glycosylated: the carbohydrate content in human somatic enzyme is about 18% [24], whereas the N-domain contains 37% of carbohydrates [4]. The deduced sequence of human somatic ACE has 17 potential sites for *N*-glycosylation of which 10 sites are arranged on the N-domain. Bovine ACE has 16 potential sites for *N*-glycosylation, the N-domain possessing 11 sites. The region 29–133 of the N-domain provides five potential sites of *N*-glycosylation, whereas the corresponding region of the C-domain contains only two sites.

Thus, we consider the enhanced stability of the N-domain within ACE molecule as a result of a higher rigidity of the enzyme polypeptide chain in the region of 29–133 amino acid residues and protected position of this region towards proteinase action compared to that in the homologous region in the C-domain.

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