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Sequence Homology between Human α1-Antichymotrypsin, α1-Antitrypsin, and Antithrombin III†

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ABSTRACT: α1-Antichymotrypsin mRNA was isolated by specific polysome immunoprecipitation from turpentine-treated baboon liver. The highly enriched mRNA was used for synthesis and cloning of the corresponding cDNA. Baboon α1-antichymotrypsin cDNA clones were identified by hybrid-selected translation, and the insert DNA fragment from one of the putative clones was used as a probe to screen a human liver cDNA library comprised of 40 000 independent transformants. One of the human cDNA clones was unambiguously identified to contain α1-antichymotrypsin DNA sequences by comparison of its 5′-terminal nucleotide sequence with the N-terminal amino acid sequence of the protein. This cDNA clone, designated phACT235, contains 1524 base pairs of human DNA, which was sequenced in its entirety. The inserted DNA codes for a 25 amino acid signal peptide sequence and the entire mature α1-antichymotrypsin of 408 amino acid residues. Comparison of the amino acid sequence of α1-antichymotrypsin with that of the human α1-antitrypsin has revealed a homology level similar to that between chymotrypsin and trypsin.

α1-Antichymotrypsin is a plasma protease inhibitor synthesized in the liver. It is a single glycopeptide chain of approximately 68 000 daltons and belongs to a class of serine protease inhibitors with an apparent affinity toward chymotrypsin-like enzymes (Travis et al., 1978a,b). Its target substrates include neutrophil cathepsin G and mast cell chymase, which are capable of converting angiotensin I to the biologically active vasoconstrictor angiotensin II in vitro (Reilly et al., 1982; Wintroub et al., 1981; Tonnensen et al., 1982). α1-Antichymotrypsin is structurally related to α1-antitrypsin (Morii & Travis, 1983a,b), which is a specific inhibitor of neutrophil elastase and protects the lung elastin fibers from degradation by this protease (Olsen et al., 1975; Tuttle & Jones, 1975). It has recently been shown that neutrophil cathepsin G enhances the rate of elastin digestion by neutrophil elastase (Reilly & Travis, 1978). Thus, although the physiological function of α1-antichymotrypsin has not yet been clearly defined, it could be involved in the maintenance of the overall protease–antiprotease balance in the lung. Indeed, α1-antichymotrypsin was found to be selectively concentrated in the bronchial lumen of patients with chronic infections (Ryley & Brogan, 1972).

In man, the normal serum level of α1-antichymotrypsin is only about one-tenth that of α1-antitrypsin, and both inhibitors are members of acute-phase reactants, in that under inflammatory states or during infection the plasma concentration of α1-antichymotrypsin increases to 4 times its basal level within 8 h, while that of α1-antitrypsin increases slowly and doubles in 3 days (Laurell, 1972). Another structurally and functionally related plasma protease inhibitor, antithrombin III, is not induced under acute-phase conditions. Limited N-terminal amino acid sequence analysis of α1-antichymotrypsin has revealed some homology with α1-antitrypsin and antithrombin III, suggesting a common genetic ancestry between these plasma serine–protease inhibitors (Morii & Travis, 1983a,b). We have recently reported the complete amino acid sequences of the latter two inhibitors by cloning and sequencing of their respective cDNAs (Kurachi et al., 1981; Chandra et al., 1981). In this paper we report the isolation of a full-length human α1-antichymotrypsin cDNA clone and the determination of its nucleotide sequence. The deduced amino acid sequence for human α1-antichymotrypsin showed limited sequence homology with human antithrombin III but extensive homology with α1-antitrypsin.

Experimental Procedures

Materials

α1-Antichymotrypsin was prepared from spent human plasma by column chromatography using Bio-Rad Affi-Gel

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blue (Gianazza & Arnaud, 1982). The partially purified α1-antichymotrypsin was covalently linked to cyanogen bromide activated Sepharose purchased from Pharmacia. Specific immunoglobulin molecules against human α1-antichymotrypsin were purified from the crude rabbit antiserum obtained from Cappel Laboratory and rendered ribonuclease free by antigen affinity column chromatography. The mRNA-dependent rabbit reticulocyte lysate for cell-free translation was purchased from New England Nuclear. Restriction enzymes were obtained from Bethesda Research Labs and New England Biolabs. S1 nuclease was from Miles Laboratories. Bacterial alkaline phosphatase was purchased from Worthington Biochemicals and T4 polynucleotide kinase from Boehringer-Mannheim. [γ-^{32}P]ATP[1] (3000 Ci/mmol) and deoxynucleoside [α-^{32}P]triphosphates were purchased from Amersham. Hydrazine was purchased from Pierce Chemical Co. Piperidine (Fisher Scientific) was redistilled prior to use. Avian myeloblastosis virus reverse transcriptase was supplied by J. W. Beard, Life Sciences, Inc., St. Petersburg, FL. Calf thymus terminal deoxynucleotidyl transferase was a generous gift of R. Ratliff, University of California, Los Alamos, NM.

Methods

Purification of α1-Antichymotrypsin mRNA by Specific Polysome Immunoprecipitation. Polysomes were extracted from turpentine-treated baboon liver as previously described (Stackhouse et al., 1983). Polysomes synthesizing nascent α1-antichymotrypsin chains were enriched by immunoprecipitation using affinity-purified antibody against α1-antichymotrypsin and *Staphylococcus aureus* cells (Gough & Adams, 1978). RNA was released from the cell pellet by $NaDodSO_4$/EDTA and subsequently isolated by oligo(dT)–cellulose column chromatography. The poly(A+) RNA was analyzed by the mRNA-dependent cell-free translation system derived from rabbit reticulocytes (Pelham & Jackson, 1976) with [^{35}S]methionine as the tracer. Immunoprecipitation of the radioactive α1-antichymotrypsin from total translation products was performed by the procedure of Rohrschneider et al. (1979). The total and immunoprecipitated translation products were then analyzed by $NaDodSO_4$–polyacrylamide gel electrophoresis (Laemmli, 1970), followed by fluorography (Bonner & Laskey, 1974).

Synthesis and Cloning of Baboon α1-Antichymotrypsin cDNA. Previously reported procedures were used for the synthesis of cDNA from enriched α1-antichymotrypsin mRNA and its insertion into the *Pst*I site of pBR322 (Chandra et al., 1981). Identification of α1-antichymotrypsin clones by hybrid-selected translation was carried out as previously described (Stackhouse et al., 1983).

Identification and Sequence Analysis of Human α1-Antichymotrypsin cDNA Clones from a Human Liver cDNA Library. The construction of the human liver cDNA library has been reported (Chandra et al., 1983). The library was screened by colony hybridization (Grunstein & Hogness, 1975) using a nick-translated *Pst*I fragment of a baboon α1-antichymotrypsin cDNA clone. Recombinants containing human α1-antichymotrypsin DNA sequences were identified and isolated. The lengths of the inserted DNAs were determined by agarose and polyacrylamide gel electrophoresis. The cDNA clone with the longest DNA insert was designated phACT235 and used for DNA sequence analysis (Maxam & Gilbert, 1977). All experiments were performed according to the guidelines for recombinant DNA research from the National Institutes of Health.

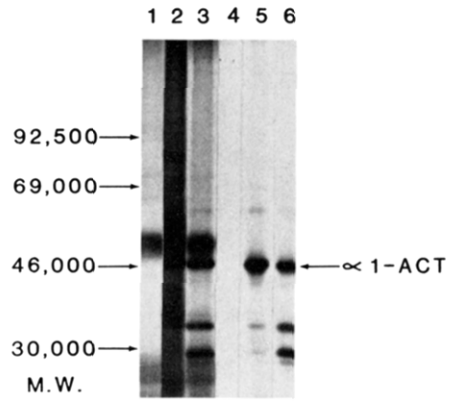

FIGURE 1: Enrichment of α1-antichymotrypsin mRNA by polysome immunoprecipitation as analyzed by cell-free translation, followed by polyacrylamide gel electrophoresis and fluorography. Lanes 1–3 show total translation products; lanes 4–6 show translation products after immunoprecipitation with the specific antibody against human α1-antichymotrypsin. Lanes 1 and 4, mock translation; lanes 2 and 5, translation products synthesized with total baboon liver mRNA; lanes 3 and 6, translation products synthesized in response to polysome-enriched mRNA.

Results

Enrichment of Baboon α1-Antichymotrypsin mRNA by Polysome Immunoprecipitation. α1-Antichymotrypsin mRNA was enriched by specific immunoprecipitation of total liver polysomes from a turpentine-induced baboon. The purity of the enriched α1-antichymotrypsin mRNA preparation was determined by cell-free translation. The translation products directed by total liver mRNA showed multiple protein bands as expected (Figure 1, lane 2). A significant amount of α1-antichymotrypsin in the translation products was detected by immunoprecipitation using the specific antibody against human α1-antichymotrypsin (Figure 1, lane 5), suggesting the presence of a relatively high level of α1-antichymotrypsin mRNA in the acute-phase baboon liver. Although serum α1-antichymotrypsin has a molecular weight of 68 000, the cell-free translation product is only about 46 000 daltons due to the lack of carbohydrate side chains. Translation of the mRNA preparation after polysome immunoprecipitation showed predominantly the 46 000-dalton band plus two others of smaller molecular weights, in addition to the 50 000-dalton band endogenous to the system (Figure 1, lane 3). All three bands were immunoprecipitated by the specific antibody to human α1-antichymotrypsin, suggesting that the two smaller molecular weight bands are truncated translation products (Figure 1, lane 6). Thus, the polysome-enriched mRNA preparation is mostly comprised of α1-antichymotrypsin mRNA.

Cloning and Identification of Baboon α1-Antichymotrypsin cDNA. The purified α1-antichymotrypsin mRNA was used for synthesis of its cDNA and insertion into the *Pst*I site of pBR322 DNA by the dG/dC homopolymer addition method. Recombinants were initially identified by screening the transformants with [^{32}P]cDNA synthesized from purified α1-antichymotrypsin mRNA. Candidate recombinants were further analyzed by hybrid-selected translation. Immunoprecipitation of the translation products yielded a strong band for α1-antichymotrypsin for most of the clones tested (Figure 2A, lanes 3–10), while protein synthesized from RNA selected by the cloning vector pBR322 DNA showed no immunopre-

[1] Abbreviations: $NaDodSO_4$, sodium dodecyl sulfate; ATP, adenosine 5′-triphosphate; EDTA, ethylenediaminetetraacetic acid; poly(A+), poly(adenylic acid) containing.

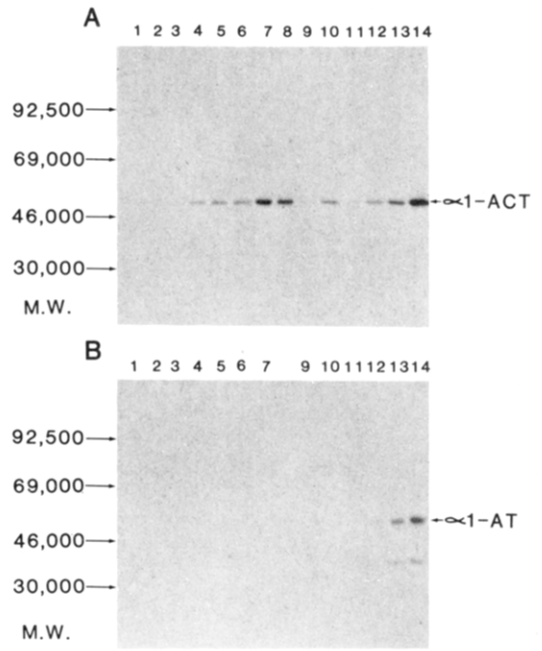

FIGURE 2: Analysis of candidate α1-antichymotrypsin cDNA clones by hybrid-selected translation, followed by immunoprecipitation, gel electrophoresis, and fluorography. Panel A shows [^{35}S]methionine-labeled products immunoprecipitated with the specific antibody to human α1-antichymotrypsin. Panel B shows the same translation products immunoprecipitated with a specific antibody to human α1-antitrypsin. Lane 1, mock translation; lane 2, translation products directed by RNA selected with pBR322 DNA; lanes 3–10, products from RNA selected with candidate α1-antichymotrypsin cDNA clones; lanes 11–14, translation products synthesized in response to 62.5, 125, 250, and 500 ng of total baboon liver RNA, respectively.

cipitable α1-antichymotrypsin (Figure 2A, lane 2). Since α1-antichymotrypsin and α1-antitrypsin share a certain amount of structural homology and the cell-free translation products migrate at the same position in the denaturing polyacrylamide gel, the total translation products directed by RNA selected with the putative α1-antichymotrypsin cDNA clones were also immunoprecipitated with an antibody against human α1-antitrypsin and analyzed in the same experiment. None of the clones that yielded an immunoprecipitable band for α1-antichymotrypsin also yielded a band for α1-antitrypsin, demonstrating the specificity of the hybrid-selection procedure (Figure 2B, lanes 3–10). However, when cell-free translation products directed by various concentrations of total baboon liver mRNA were immunoprecipitated separately with the two antibody preparations, both α1-antichymotrypsin and α1-antitrypsin were readily detected (Figure 2, lanes 11–14). Taken together, these data strongly suggest that the cDNA clones identified by hybrid-selected translation indeed contain baboon α1-antichymotrypsin DNA sequences.

Cloning and Sequence Analysis of Human α1-Antichymotrypsin cDNA. Screening the human cDNA library of 40 000 transformants by colony hybridization (Grunstein & Hogness, 1975) using a nick-translated *Pst*I fragment from a baboon α1-antichymotrypsin cDNA clone yielded a number of positive clones. Analysis of the plasmid DNAs isolated from several of these clones by a minilysis procedure (Birnboim & Doly (1979) followed by gel electrophoresis showed that they shared common internal restriction fragments (data not shown). The cDNA clone containing the longest DNA insert, designated phACT235, was sequenced in its entirety according to the strategy shown in Figure 3. In most cases, both strands of the inserted DNA fragments were subjected to sequence analysis. When only one strand was sequenced, at least two

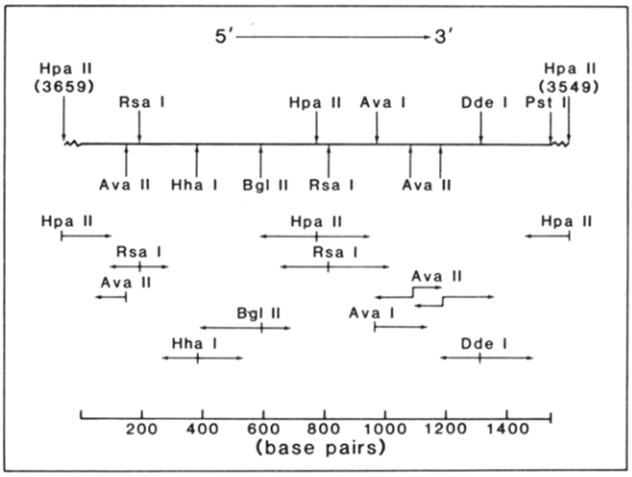

FIGURE 3: Sequence analysis strategy of the human α1-antichymotrypsin cDNA clone. The restriction sites used for labeling are shown, but they do not represent all such sites present on the inserted human DNA.

independent experiments were performed with different labeling sites. All restriction sites used for labeling and recutting were sequenced across to ensure the accuracy of the nucleotide sequence.

The inserted human DNA in phACT235 is 1524 base pairs in length. Comparison of the amino acid sequence deduced from the nucleotide sequence with the known N-terminal sequence of human α1-antichymotrypsin (Morii & Travis, 1983a) has demonstrated that asparagine, the first amino acid residue in the mature protein, is encoded by the codon AAC at nucleotides 88–90 of the cDNA clone (Figure 4). The amino acid sequence following this asparagine residue agrees well with the rest of the N-terminal amino acid sequence reported by Morii and Travis with only minor deviations, providing unambiguous evidence for the identity of the human α1-antichymotrypsin cDNA clone. From the remaining nucleotide sequence, it is apparent that the mature human α1-antichymotrypsin is comprised of 408 amino acids encoded by nucleotides 88–1311 of the cDNA clone (Figure 4). The amino acid residue immediately preceding the first residue in the mature protein is not a methionine but a proline. Human α1-antichymotrypsin, being a secretory protein, apparently contains a signal peptide (Blobel et al., 1979) of either 22 or 25 amino acids, due to the presence in the signal peptide region of two potential methionine start sites at −22 and −25.

Sequence Homology between Human α1-Antichymotrypsin, α1-Antitrypsin, and Antithrombin III. A comparison of the amino acid sequence of α1-antichymotrypsin with that of α1-antitrypsin and antithrombin III revealed overall homology levels of 42% and 33%, respectively. Comparison of the nucleotide sequences of the cDNAs for these proteins reveals a homology level of 56% for α1-antichymotrypsin and α1-antitrypsin and a homology level of 46% for α1-antichymotrypsin and antithrombin III. Previously, we reported 33% homology of the amino acid sequences (Kurachi et al., 1981) and 46% homology of the nucleotide sequences between α1-antitrypsin and antithrombin III (Chandra et al., 1983). It is interesting to note that the level of homology between α1-antitrypsin and antithrombin III is identical with the level of homology between α1-antichymotrypsin and antithrombin III.

The regions of homology and nonhomology between the three plasma protease inhibitor sequences were further analyzed by computer-assisted dot-matrix analysis (Novotny, 1982; Dayhoff, 1978), in which each dot represents a perfect

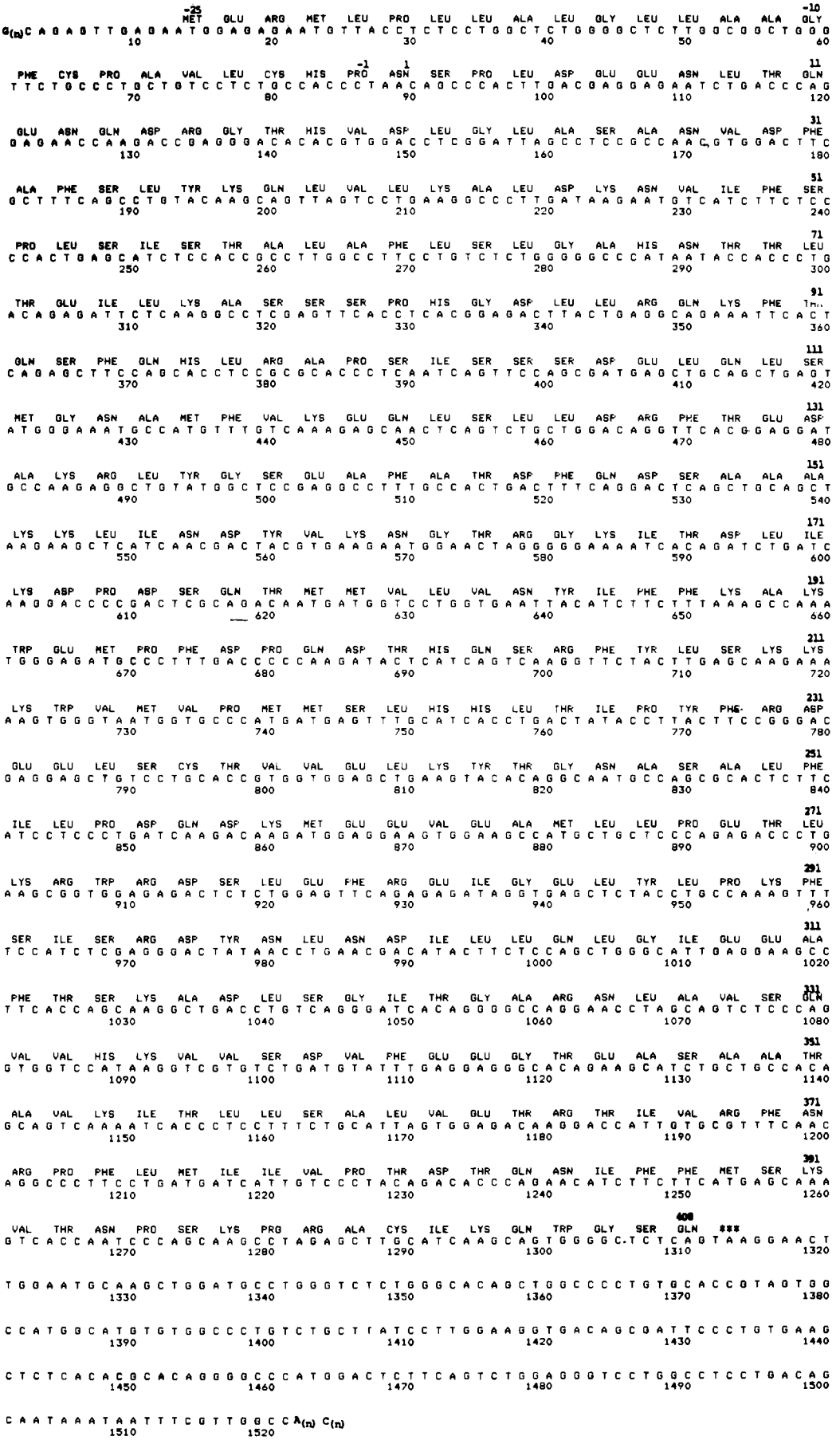

FIGURE 4: Complete nucleotide sequence of the human DNA insert in phACT235 and the amino acid sequence deduced from the DNA sequence. The amino acid residues in the mature protein are numbered 1 through 408, and those in the putative signal peptide are numbered −22 or −25 to −1, with the initiation codon for methionine as number −22 or −25. The numbering of the nucleotide sequence starts with the last G residue of the dG tails.

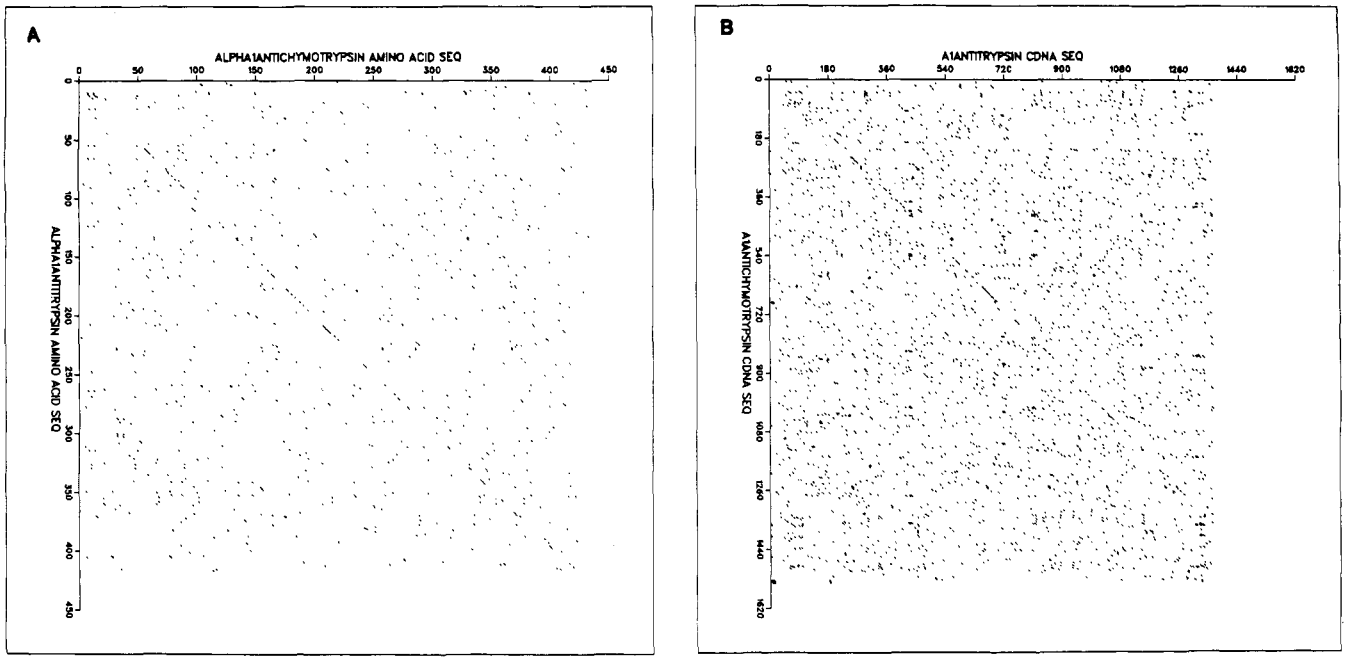

FIGURE 5: Dot-matrix analysis of the amino acid sequences (panel A) and nucleotide sequences (panel B) between human α1-antichymotrypsin and α1-antitrypsin. Each dot represents a perfect match of either two consecutive amino acid or five nucleotide residues, respectively.

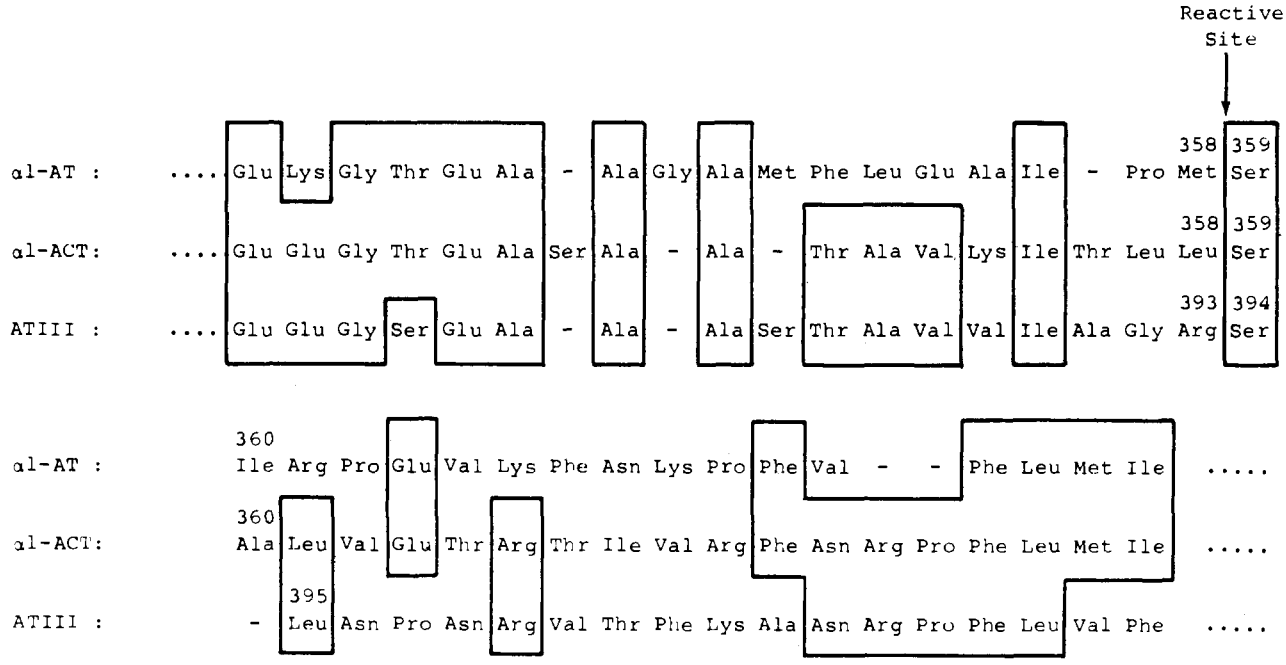

FIGURE 6: Comparison of active site sequences of α1-antichymotrypsin, α1-antitrypsin, and antithrombin III. The numbers refer to the amino acid residues of the mature proteins. The asterisks indicate the active serine residues in the three plasma serine protease inhibitors.

match of amino acid residues in the proteins or nucleotide residues in the cDNA clones. Comparison of the amino acid sequences between α1-antichymotrypsin and α1-antitrypsin results in a distinctive diagonal line (Figure 5A), indicating the existence of a high level of homology throughout the two sequences. Furthermore, there appears to be more homology between the N-terminal halves of the two protease inhibitors than between the C-terminal halves. A similar observation was also made between the plasma protease inhibitors at the nucleotide level (Figure 5B). Comparison of the amino acid and nucleotide sequences between α1-antichymotrypsin and antithrombin III shows a much more scattered plot (data not shown), indicating the two sequences are much less conserved.

Comparison of Active Site Sequences of the Three Plasma Protease Inhibitors. The active sites are located near the carboxyl ends in α1-antitrypsin and antithrombin III, and their respective amino acid sequences are known to be Met-Ser and Arg-Ser (Johnson & Travis, 1978; Bjork et al., 1981). More recently, the active site residues in human α1-antichymotrypsin have been determined to be Leu-Ser (Morii & Travis, 1983b). The amino acid sequence surrounding the active site residues agrees well with a region located near the carboxyl terminus of α1-antichymotrypsin. It is interesting to note that the active site residues Leu-Ser in α1-antichymotrypsin are located at positions 358 and 359 of the mature protein, which is exactly the same position as those in α1-antitrypsin. The amino acid sequences surrounding the active sites of the three plasma protease inhibitors were then compared (Figure 6). It is apparent that the homologies at the active sites per se are much less extensive, and they appear to resume 5–10 residues away

from the active sites. This could be the result of more sequence divergence at the active sites of the inhibitors in order to generate target specificity.

Discussion

α1-Antichymotrypsin mRNA has been purified from baboon liver by specific polysome immunoprecipitation and used for cDNA cloning. By use of cloned baboon α1-antichymotrypsin cDNA insert as the hybridization probe to screen a human liver cDNA library (Chandra et al., 1983), a number of human α1-antichymotrypsin cDNA clones were identified. The recombinant plasmid containing the largest human DNA insert was sequenced in its entirety, and the amino acid sequence of human α1-antichymotrypsin has been deduced. Comparison of its amino acid sequence with those of α1-antitrypsin and antithrombin III (Kurachi et al., 1981; Chandra et al., 1983) shows that there is greater homology with α1-antitrypsin (42%) than with antithrombin III (33%). The amino acid sequence comparisons between the three plasma protease inhibitors described here support the hypothesis that they share a common genetic ancestry. In this context, it is interesting to note that the amino acid sequence homology between trypsin and chymotrypsin is also 42% (Dayhoff, 1978), which are the representative target proteases inhibited by α1-antitrypsin and α1-antichymotrypsin, respectively.

There is an additional member in this protein superfamily in chicken ovalbumin, and it has been estimated that antithrombin III, α1-antitrypsin, and chicken ovalbumin diverged approximately 500 million years ago (Hunt & Dayhoff, 1980). The elucidation of the amino acid sequence for α1-antichymotrypsin suggests that it and α1-antitrypsin diverged approximately 300 million years ago, providing strong evidence that the supergene family reported by Hunt & Dayhoff (1980) has a fourth member in α1-antichymotrypsin and that its divergence time from α1-antitrypsin is much more recent.

Although these genes appear to have evolved by divergent evolution from an ancestral gene, we have previously observed that the intron/exon structures of chicken ovalbumin and α1-antitrypsin are completely different (Leicht et al., 1982). Consequently, it has been suggested that introns could also be introduced into preexisting exons as an additional mechanism of gene evolution (Leicht et al., 1982). We have recently isolated the human chromosomal antithrombin III gene, and preliminary structural characterization has indicated that its intron/exon structure is different from both α1-antitrypsin and ovalbumin (V. J. Kidd, unpublished data). The higher level of amino acid homology observed between α1-antitrypsin and α1-antichymotrypsin would suggest that there might be some structural similarities between these two genes. Thus, the cloning and structural characterization of the human chromosomal α1-antichymotrypsin gene should provide further insight into the evolution of this superprotein family.

The plasma levels of α1-antichymotrypsin and α1-antitrypsin increase under acute-phase conditions such as inflammation, surgery, or infections, whereas the level of the third member of inhibitor family antithrombin III does not. It will be most interesting to characterize the signals in the genes for acute-phase response and to determine if they were acquired and retained by the α1-antitrypsin and α1-antichymotrypsin genes or lost by the antithrombin III gene during the divergent evolutionary process.

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Registry No. α1-Antichymotrypsin, 9004-07-3; α1-antitrypsin, 9041-92-3; antithrombin III, 9000-94-6; α1-antichymotrypsin (human liver precursor reduced), 87135-97-5; α1-antichymotrypsin (human liver reduced), 87135-98-6; DNA (human liver α1-antichymotrypsin messenger RNA complementary), 87135-96-4.

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Manganese(II) Electron Spin Resonance and Cadmium-113 Nuclear Magnetic Resonance Evidence for the Nature of the Calcium Binding Site in α-Lactalbumins†

Lawrence J. Berliner,* Paul D. Ellis, and Kentaro Murakami‡

ABSTRACT: Bovine and goat α-lactalbumins were substituted with $^{113}Cd(II)$ or Mn(II) at the strong calcium site [Murakami, K., Andree, P. J., & Berliner, L. J. (1982) *Biochemistry 21*, 5488–5494] and studied by ^{113}Cd NMR and electron spin resonance. The ^{113}Cd chemical shifts were in the −80 to −85 ppm range vs. $Cd(ClO_4)_2$, which was almost identical with that found for several nearly octahedral (oxygen-coordinated) calcium binding proteins such as calmodulin, parvalbumin, and troponin C. The electron spin resonance spectra of bound Mn(II)–α-lactalbumin complexes at 9 or 35 GHz were also confirmatory of a highly symmetric (cubic) environment around the Mn(II) with only slight distortions. The near identity of this site in α-lactalbumin to those of calcium binding proteins containing an "EF hand domain" was remarkable despite the absence of such a domain sequence in the α-lactalbumin structure.

The α-lactalbumins, which are "modifier" proteins in the biosynthesis of lactose in milk, have been found to have a very strong affinity for Ca(II) at a unique strong binding site. Murakami et al. (1982) have measured the equilibria between several α-lactalbumin species with a variety of di- and trivalent cations, all of which cause the same characteristic fluorescence changes upon binding to a specific α-lactalbumin (e.g., bovine, human, goat, guinea pig). These experiments above as well as more recent evidence verified a competition between Ca(II) and these other cations, for example, Mn(II) or Cd(II), for this site (Murakami & Berliner, 1983).

The substitution of $^{113}Cd(II)$ in several calcium binding proteins of known three-dimensional structure has been shown to be an excellent aid in predicting the coordination nature of the Ca(II) site. In particular, the ^{113}Cd NMR chemical shift is an accurate indicator of the liganding environment of the metal ion (Armitage & Otvos, 1982). Similarly, the success in predicting ligand nature and geometry by ESR[1] with Mn(II)-substituted proteins has been demonstrated by Reed and co-workers (Reed & Markham, 1984).

We present here the ^{113}Cd NMR and Mn(II) ESR of the respective metal–α-lactalbumin complexes as probes of the chemical structure of the α-lactalbumin calcium binding site.

Materials and Methods

Proteins. Electrophoretically pure bovine α-lactalbumin (lot 50F8105) was from Sigma Chemical Co. Other α-lactalbumin species were obtained or isolated as noted earlier (Berliner & Kaptein, 1981). Apo bovine α-lactalbumin was prepared by the procedures noted in our earlier paper (Murakami et al., 1982).

Chemicals. Ultrapure cation salts were from either Aldrich Chemical Co. or Alfa Products. ^{113}Cd metal was purchased from Prochem Isotopes and converted to the chloride salt. All other reagents were as reported earlier (Murakami et al., 1982).

Methods. ESR measurements were made in quartz capillaries or sealed Pasteur dispo-pipets on a Varian E-4 spectrometer at liquid nitrogen and room temperature (Berliner, 1977). Special care was taken with controls in the 77 K spectra by freezing aquomanganese(II) samples in the presence of Sephadex G-25 to avoid artifacts due to dipolar and exchange phenomena as noted by Leigh & Reed (1971). The spectra were processed on a Varian E-935 data system. ^{113}Cd NMR spectra were measured at the South Carolina Magnetic Resonance Laboratory on a Bruker WP-400 operating at 88.756 MHz. Spectral parameters were as follows: sweep width, 41 667 Hz; pulse width, 10 μs; line broadening, 100 Hz. The fractions of free and bound cations were calculated exactly from the single-site dissociation constants for bovine α-lactalbumin: $K_{Cd(II)} = 2.5$ μM; $K_{Mn(II)} = 31.7$ μM (Murakami et al., 1982). In the case of the exceptionally strong calcium dissociation constant ($K_{Ca(II)} = 0.2$ nM), the fraction of calcium-bound α-lactalbumin was estimated precisely by fluorescence (Murakami et al., 1982).

Results and Discussion

^{113}Cd NMR. Figure 1A shows the ^{113}Cd NMR spectrum of a 1:1 complex of 3.4 mM bovine α-lactalbumin–$^{113}Cd(II)$, pH 6.3, in 25 mM Tris-HCl and 20% D_2O at 25 °C. The

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[1] Abbreviations: ESR, electron spin resonance; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride.

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