Nucleotide Sequence of Cloned Complementary Deoxyribonucleic Acid for the α Subunit of Bovine Pituitary Glycoprotein Hormones†

Christopher R. Erwin, Michelle L. Croyle, John E. Donelson, and Richard A. Maurer*

ABSTRACT: Recombinant DNA plasmids containing sequences coding for the α subunit of the bovine pituitary glycoprotein hormones have been isolated. The nucleotide sequences of three different cDNA clones have been determined. The largest α-subunit cDNA clone was found to contain 713 bases including 77 nucleotides from the 5'-untranslated region, 72 nucleotides coding for a precursor segment, 288 nucleotides coding for the mature α subunit, and 276 nucleotides from the 3'-untranslated region of the mRNA followed by a poly(A) segment. This cDNA likely represents most of the bovine α-subunit mRNA sequence. Nucleotide sequences were obtained from the cDNA inserts of two other α-subunit clones, and several differences among the three cDNA sequences have been detected. These differences in nucleotide sequence may represent either individual variation in genomic sequence or cloning artifacts. Comparison of the bovine α-subunit cDNA sequence to the sequences of human, rat, and mouse α-subunit cDNAs reveals that the bovine sequence has greater than 70% homology with the other cDNAs. The cloned α-subunit cDNA should provide a useful probe for further studies of the structure and expression of this interesting gene.

The glycoprotein hormones of the pituitary are a family of structurally related, subunit proteins. This family includes luteinizing hormone, follicle stimulating hormone, and thyroid stimulating hormone. In addition, the placenta produces a related hormone, chorionic gonadotropin, which is also a member of the hormone family. Each member of this family contains two nonidentical, noncovalently linked subunits (Pierce et al., 1971; Ward et al., 1973). The subunits are designated α and β, and both subunits are glycosylated. Within a species, the α subunits of all the pituitary glycoprotein hormones are very similar, if not identical, and the β subunits differ considerably. Dissociation and reassociation experiments have shown that the β subunit specifies the biological activity of an $\alpha\beta$ dimer (Pierce et al., 1971; Ward et al., 1973). Thus, the β subunit is the hormone-specific subunit while the α subunit appears to be common for all of the hormones.

These related hormones provide an interesting model for studies of the regulation of gene expression. The physiological regulation of these hormones has been extensively studied, and a number of regulators have been identified. Recent studies using cell-free translation have suggested that regulation of the production of the pituitary glycoproteins involves changes in specific mRNA levels (Alexander & Miller, 1982; Godine et al., 1980). For further studies of the structure and expression of the glycoprotein hormone genes, it would be desirable to identify specific cloned cDNAs. We report here the construction and analysis of a cloned cDNA containing sequences coding for the bovine α subunit. One of the cloned cDNAs contains sequences representing the complete coding sequence of the α-subunit mRNA, as well as the complete 3'-untranslated region and a substantial portion of the 5'-untranslated region. The nucleotide sequence of the bovine α subunit has been compared to the recently determined sequence of cloned cDNAs for the human (Fiddes & Goodman, 1979), mouse (Chin et al., 1981), and rat (Godine et al., 1982) α subunit.

Experimental Procedures

Isolation of mRNA. Bovine pituitaries were obtained from a local abattoir and immediately frozen in liquid nitrogen. Total pituitary RNA was prepared by sedimentation through CsCl as described previously (Maurer, 1980a). Poly(A)-containing RNA was obtained by chromatography of the total RNA on oligo(dT)–cellulose as described previously (Maurer, 1980b). The poly(A)-containing RNA was further fractionated by sedimentation through a 5–20% sucrose gradient (Gubbins et al., 1979). The mRNA activity of gradient fractions was determined by using a nuclease-treated reticulocyte lysate cell free translation system followed by specific immunoprecipitation (Maurer, 1980b). RNA that sedimented at approximately 9 S was found to be enriched for α-subunit mRNA activity.

Synthesis of cDNA. Single-stranded cDNA was synthesized from either poly(A)-containing RNA or specific sucrose gradient fractions with viral reverse transcriptase as described previously (Gubbins et al., 1979). After removal of the mRNA by alkaline hydrolysis, a dCMP homopolymer extension was added to the 3'-terminus of the cDNA by using terminal transferase (Land et al., 1981). Then, a second strand of cDNA was synthesized as described previously (Gubbins et al., 1979) except the reaction included oligo(dG) as a primer. This procedure has been shown to facilitate the cloning of sequences representing the 5'-portion of an mRNA (Land et al., 1981). A dCMP homopolymer extension was then added to the 3'-termini of the duplex cDNA as described above.

Construction of Recombinant DNA. The cDNAs were inserted at the *Pst*I site of plasmid pBR322 with oligo(dC)-oligo(dG) homopolymer extensions and used to transform *Escherichia coli* strain HB101 as described previously (Gubbins et al., 1979). For some studies, a derivative of pBR322 was used as a vector. This vector was constructed by insertion of an oligonucleotide linker into the *Eco*RI site of pBR322 so that a unique *Sma*I site was created that is flanked on either side by an *Eco*RI site. The insertion of cDNAs at the *Sma*I site was performed by the poly(dG)·

† From the Departments of Biochemistry (C.R.E. and J.E.D.) and Physiology and Biophysics (M.L.C. and R.A.M.), University of Iowa, Iowa City, Iowa 52242. *Received February 23, 1983.* This work was supported by National Science Foundation Grants PCM 76-13461 and 82-16123, Diabetes and Endocrine Core Center Grant AM 25295, and Research Career Development Award AM 00841. C.R.E. was supported by National Institutes of Health Predoctoral Training Grant GM07091.

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poly(dC) technique as described above.

DNA Sequence Determinations. The nucleotide sequence of several cloned cDNAs was determined by using the modification and cleavage reactions of Maxam & Gilbert (1977).

Analysis of α-Subunit Chromosomal Gene. DNA was prepared from bovine liver tissue by the method of Blin & Stafford (1976). After digestion with restriction endonucleases, the DNA was electrophoresed in an agarose gel and transferred to nitrocellulose (Southern, 1975). The filter-immobilized DNA was then hybridized to radiolabeled α subunit cDNA as described previously (Jeffreys & Flavell, 1977; Wahl et al., 1979).

Results and Discussion

Identification of an α-Subunit cDNA Clone. The strategy for isolating an α-subunit cDNA clone relied on the published amino acid sequence of the bovine α subunit (Liao & Pierce, 1971). First, bovine pituitary cDNA libraries enriched for α-subunit sequences were constructed, and then a specific α subunit clone was identified by sequencing portions of the cDNA inserts of random clones. Several different pituitary cDNA libraries were constructed and analyzed. Initial studies utilized two different cDNA libraries, each consisting of about 1000 clones. One library was constructed from total bovine pituitary poly(A)-containing RNA, and the other library was constructed from size-fractionated RNA enriched in α subunit mRNA activity. It was anticipated that both cDNA libraries would likely contain a high percentage of clones for abundant mRNAs such as prolactin and growth hormone. Therefore, the libraries were initially screened by the colony hybridization procedure (Grunstein & Hogness, 1975) with cloned bovine prolactin and growth hormone cDNAs that had been previously identified (C. Erwin, unpublished data). It was found that 21% of all clones contained prolactin mRNA sequences and 2.6% contained growth hormone mRNA sequences. Then, mRNA enriched in α-subunit mRNA activity by sucrose density gradient fractionation was used to synthesize a radiolabeled cDNA that could be used to screen the colonies. Out of the total or nearly 2000 clones, only 70 hybridized to the α-enriched probe and did not also hybridize to the prolactin or growth hormone probe. Random clones were selected from this reduced library of 70 clones and the cDNA inserts sequenced. The amino acid sequence predicted by the DNA sequence was then compared to the amino acid sequence of the bovine α subunit. Sequence data were obtained for the cDNA inserts of eight different clones. In seven clones, no known protein was predicted by the DNA sequence. In the other clone, one reading frame of the DNA sequence predicts the known amino acid sequence of the α subunit. This provided unequivocal evidence that an α-subunit cDNA clone had been isolated. It is referred to as pALPHA-3 in Figure 1.

The pALPHA-3 cDNA insert was then used as a hybridization probe to screen all of the clones in the bovine pituitary cDNA libraries. In addition to the approximately 2000 clones from the first two cDNA libraries, an additional 500 clones that had been constructed later were screened. Of the 2500 clones, seven or approximately 0.3% were found to contain α-subunit mRNA sequences. Four of the clones contained very small cDNA inserts and were not characterized further. Two clones with large cDNA inserts were chosen for further study; they are designated pALPHA-1 and pALPHA-2 as shown in Figure 1.

Sequence Analysis of Cloned α-Subunit cDNAs. The sequences of the three α-subunit cDNAs were determined as shown in Figure 1. After digestion with a number of different restriction endonucleases, the 3-termini of cleaved cDNA inserts were labeled with the appropriate [α-^{32}P]dNTP and *E. coli* DNA polymerase I (Nichols & Donelson, 1978) for sequence determination by the Maxam & Gilbert (1977) chemical cleavages. For pALPHA-1, *Eco*RI sites outside of the cDNA insert were labeled for sequence analysis across the poly(dG)·poly(dC) boundaries and into the cDNA. Similarly, *Hpa*II sites outside of the cDNA inserts were used for sequence analysis of pALPHA-2 and pALPHA-3. The complete nucleotide sequence of all three cDNA inserts was determined. The cDNA insert of pALPHA-1, the largest, contains 77 nucleotides of 5′-nontranslated sequences, 360 nucleotides of coding sequence, and 276 nucleotides of 3′-nontranslated sequence followed by poly(A). The coding sequence is terminated by two contiguous termination codons in phase with the translation reading frame. A comparison of the amino acid sequence predicted from the cDNA sequence and the published sequence of the mature protein (Liao & Pierce, 1971) suggests the presence of a 24 amino acid precursor segment. The positions of leucine residues within the deduced precursor sequence are identical with those determined by Guidice et al. (1979) from cell-free translation experiments. The predicted sequence of the mature α subunit is the same as the published sequence except at position 28, at which pALPHA-1 predicts an alanine instead of the published proline. However, as discussed below, the sequences of pALPHA-2 and pALPHA-3 cDNAs do predict proline at this position.

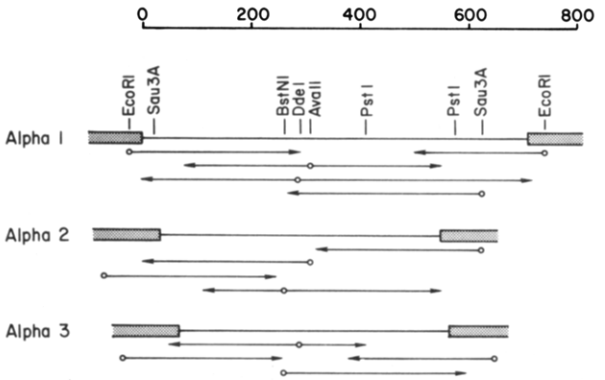

FIGURE 1: Diagram of cDNA inserts of plasmids pALPHA-1, pALPHA-2, and pALPHA-3. Cleavage sites for the restriction endonucleases *Ava*II, *Bst*NI, *Dde*I, *Eco*RI, *Pst*I, and *Sau*3A are indicated. The stippled regions flanking the cDNAs are vector sequences and the poly(dG)·poly(dC) homopolymer extension used for cloning. Nucleotide length is indicated by numbers across the top. Restriction sites that were radiolabeled for sequence analysis are indicated (O), as well as the extent of sequence analysis (arrows).

The cDNA inserts of pALPHA-2 and pALPHA-3 are smaller than the inserts of pALPHA-1 and contain several differences from the nucleotide sequence of pALPHA-1. The cDNA insert of pALPHA-2 extends between nucleotide positions 38 and 550 of the pALPHA-1 sequence and includes the complete coding sequence and portions of the 5′- and 3′-nontranslated regions. pALPHA-3 cDNA contains the region between positions 67 and 564 of pALPHA-1 and thus also includes the complete coding sequence and portions of the 5′- and 3′-nontranslated sequence. A comparison of the three cDNAs reveals several base differences as indicated in Figure 2. Position 114 is adenine in pALPHA-1 and guanine in both pALPHA-2 and -3; position 231 is a guanine in pALPHA-1 and cytosine in both pALPHA-2 and -3; position 162 is a guanine in pALPHA-1 and -2 while pALPHA-3 is missing a nucleotide altogether at this position (which destroys the translation reading frame beyond this point). Nucleotide 439, the third position of the termination codon, is adenine in pALPHA-1 and -3 and guanine in pALPHA-2. Position 501

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                                                                                                  -24
                                                                                                  Met Asp Tyr Tyr Arg Lys Tyr Ala
5' ...AAAAACTAAAATTCTTCTTCAGATCCACAGTCAACTGCCCTGACTACATTCTGCAAAAATCCAGAGGACGAAGAGCC ATG GAT TAC TAC AGA AAA TAT GCA
                                                                                                                          101

                          -10                                                -1  +1
Ala Val Ile Leu Thr Ile Leu Ser Leu Phe Leu Gln Ile Leu His Ser Phe Pro Asp Gly Glu Phe Thr Met Gln Gly Cys Pro Glu
GCT GTC ATT CTG ACC ATT TTG TCT CTG TTT CTG CAA ATT CTC CAT TCC TTT CCT GAT GGA GAG TTT ACA ATG CAG GGC TGT CCT GAA
                G                                                                  G                                  188
                G                                                                  ()

                          +20                                                                                  +40
Cys Lys Leu Lys Glu Asn Lys Tyr Phe Ser Lys Pro Asp Ala Ala Ile Tyr Gln Cys Met Gly Cys Cys Phe Ser Arg Ala Tyr Pro
TGC AAG CTA AAA GAA AAC AAA TAC TTC TCC AAG CCA GAT GCT GCA ATC TAT CAG TGC ATG GGG TGC TGC TTC TCC AGG GCA TAC CCC
                                           C                                                                       275
                                           C
                                                                  +60
Thr Pro Ala Arg Ser Lys Lys Thr Met Leu Val Pro Lys Asn Ile Thr Ser Glu Ala Thr Cys Cys Val Ala Lys Ala Phe Thr Lys
ACT CCA GCG AGG TCT AAG AAG ACA ATG TTG GTC CCC AAG AAC ATC ACC TCG GAA GCT ACA TGC TGT GTG GCC AAA GCA TTT ACC AAG
                                                                                                                      362

                                    +80                                                                   +96
Ala Thr Val Met Gly Asn Val Arg Val Glu Asn His Thr Glu Cys His Cys Ser Thr Cys Tyr Tyr His Lys Ser *** ***
GCC ACA GTG ATG GGA AAT GTC AGA GTG GAG AAC CAC ACC GAG TGC CAC TGC AGC ACT TGT TAT TAT CAC AAA TCC TAA TAG TTTGCAG
                                                                                                        G             450
                                                                                                        A

TGGGCCTTGCTGATGATGGCTGACTTGCTCAAAAGGAAAATTAATTTGTCCAGTGTCTATGGCTTTGTGAGATAAAACCCTCCTTTTCCTTGCCATACCATTTTTAACCTGCTTT
                                           A                                                                          565
                                           C

GAGAATATACTGCAGCTTTATTGCTTTTCTCCTTATCCTACAATATAATCAGTAGTCTTGATCTTTTCATTTGGAATGAAATATGGCATTTAGCATGACCATAAAAAGCTGATTC
                                                                                                                      680

CACTGGAAATAAAGTCTTTTAAATCATCACTCT poly(A)
                                  713
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FIGURE 2: Nucleotide sequence of pALPHA-1 cDNA and corresponding amino acid sequence. Several differences were observed in the pALPHA-2 and pALPHA-3 cDNA sequences. Nucleotides located one line underneath the sequence are positions at which pALPHA-2 differs from pALPHA-1; nucleotides two lines underneath are where pALPHA-3 differs from pALPHA-1. The parentheses at nucleotide position 162 indicate the absence of a nucleotide at this position in pALPHA-3. The two codons with stars above them are termination codons. The signal-peptide amino acids are indicated by negative numbers; the mature α subunit is indicated by positive numbers.

is cytosine in pALPHA-1 and -3 and adenine in pALPHA-2. The significance of these differences is not clear. One possibility is that errors occurred during the construction and/or cloning of the cDNA molecules. This is probably the most feasible explanation for the missing nucleotide in pALPHA-3 that destroys the translation reading frame. It has been reported that reverse transcriptase accumulates copying mistakes in vitro at the rate of about 1/1000 nucleotides (Gopinathan et al., 1979). Furthermore, there are other reports of single nucleotides missing in cloned cDNA molecules (Hartley et al., 1982). The other differences in the cDNA comparisons are nucleotide replacements. These could also be reverse transcription errors, or they may represent heterogeneity among individual animals from which pituitaries were obtained. In the one case where the difference occurs in a region of the known mature α-subunit sequence (nucleotide position 231), two of the three cDNAs (pALPHA-2 and -3) contain a cytosine such that the codon predicts a proline (consistent with the published α-subunit amino acid sequence) while the other cDNA (pALPHA-1) has a guanine so that the alanine codon occurs. Since the presence or absence of proline can have a distinct effect on the folding of a peptide chain, this difference may also be a reverse transcriptase error. On the other hand, the difference at position 439 merely substitutes one termination codon for another, which presumably would have little or no effect on translation or subsequent α-subunit function. This difference could be individual heterogeneity.

Analysis of Chromosomal Gene for α Subunit. pALPHA-1 cDNA was used as a hybridization probe for analysis of the organization of the bovine chromosomal gene (Figure 3). Bovine genomic DNA was digested with *Eco*RI, *Xba*I, or a mixture of these two enzymes. The DNA fragments were separated on an agarose gel and transferred to nitrocellulose. The cDNA probe hybridized to a single large *Eco*RI fragment

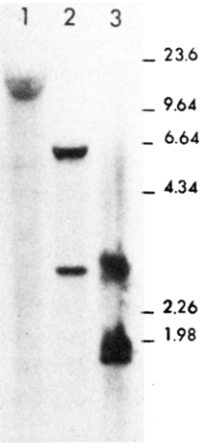

FIGURE 3: Analysis of bovine chromosomal α-subunit gene. Bovine DNA (20 μg) was digested with *Eco*RI (lane 1), *Xba*I (lane 2), or *Eco*RI plus *Xba*I (lane 3), electrophoresed on an agarose gel, and transferred to nitrocellulose. After hybridization to radiolabeled pALPHA-1 DNA, the migration of DNA fragments containing α-subunit sequences was determined by autoradiography. Numbers along the side indicate the size in kilobases of *Hin*dIII-digested λ DNA fragments that were used as standards to determine the size of genomic fragments.

and two smaller *Xba*I fragments. One of the *Xba*I fragments was cleaved by *Eco*RI. Since the cDNA does not contain an *Xba*I site, either the haploid genome must contain a single α-subunit gene with one or more introns or these are isogenes for the α subunit. Without further analysis it is not possible to distinguish between these possibilities. However, it should be pointed out that the α-subunit gene structure has been reported for the human (Fiddes & Goodman, 1981), and in this case, there appears to be one gene with three introns. Construction of a bovine genomic library and identification

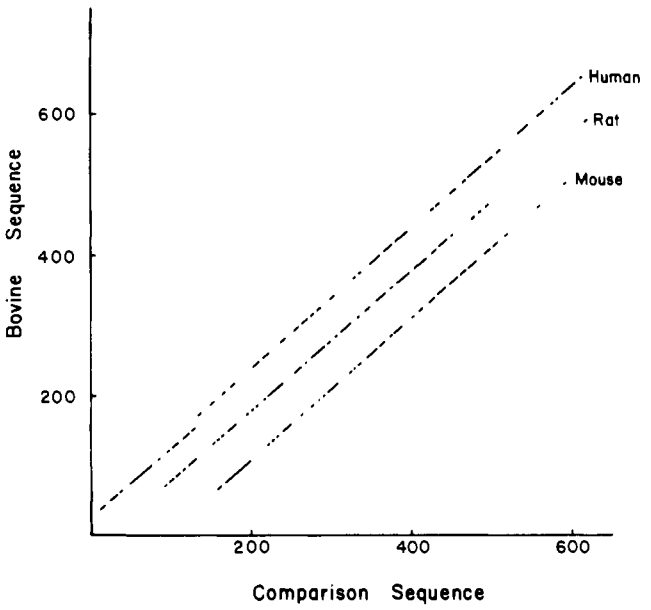

FIGURE 4: Dot-matrix analysis for comparison of nucleotide sequence of bovine α-subunit cDNA sequence to human, rat, and mouse α-subunit cDNAs. Each dot represents the position at which seven nucleotides were identical for two different sequences. The vertical axis indicates the bovine sequence; 5′-nontranslated sequences are at the bottom and 3′-nontranslated at the top. The horizontal axis indicates the human, rat, and mouse sequences; 5′-nontranslated sequences start at the left. The rat sequence is offset to the right of the human sequence by 50 nucleotides, and the mouse sequence is offset to the right of the rat sequence by 50 nucleotides. In the preparation of a composite of the three dot-matrix comparisons, only those dots on the diagonal (indicating collinear sequence identity) are shown. Small homologies scattered randomly throughout the comparison are not shown.

of cloned fragments containing the α-subunit coding sequence are in progress.

Comparison of α-Subunit Sequence in Different Species. The nucleotide sequence of cloned α-subunit cDNAs has been reported for three other species, including human (Fiddes & Goodman, 1979), rat (Godine et al., 1982), and mouse (Chin et al., 1981). Figure 4 presents a dot-matrix analysis (Novotny, 1982) for the comparison of the bovine cDNA sequence with the other sequences. In the coding regions, the bovine sequence displays between 71% (human) and 80% (rat and mouse) homology with the others. Curiously, although the bovine–human comparison has the least homology in the coding region, it has the most homology in the 3′-nontranslated region (note the top portions of the diagonal comparisons). Very little homology exists in this region in the bovine–rat and bovine–mouse comparisons.

A small discontinuity occurs in the bovine–human comparison that is caused by a four-codon deletion in the human sequence relative to the others. Previous studies of amino acid sequence have shown that the human α subunit contains four fewer amino acids at the amino terminus than does the α subunit from the bovine (Liao & Pierce, 1971) and ovine (Liu et al., 1972; Papkoff et al., 1971). The present study demonstrates that this difference is due to a difference in mRNA nucleotide sequence rather than a difference in processing of the protein. Furthermore, this deletion in the human sequence occurs precisely at the point where an intervening sequence interrupts the coding sequence of the human chromosomal gene (Fiddes & Goodman, 1981). This suggests that the deletion in the human gene may have involved changes in splicing sites at intron–exon boundaries. Future studies of the structure of the bovine chromosomal gene may provide some insight into this possibility.

Conclusions

A cloned cDNA likely representing most of α-subunit mRNA sequences has been isolated and its nucleotide sequence determined. This cDNA is longer than any of the α-subunit cDNAs isolated from other species. The construction of this large cDNA clone was probably facilitated by the use of a procedure that preserves sequences representing the 5′-termini of the mRNA (Land et al., 1981).

The α-subunit cDNA should provide a useful hybridization probe for further studies of the pituitary glycoprotein hormones. There are several features that suggest that analysis of the glycoprotein hormone genes should be interesting. A recent study (Boorstein et al., 1982) suggests that the human genome contains a cluster of tandem and inverted pairs of chorionic gonadotropin β-subunit genes and luteinizing hormone β-subunit genes. The significance of the unusual organization of these genes is not yet clear. Any possible physical linkage between α- and β-subunit genes has not yet been determined. Analysis of the organization of the glycoprotein hormone genes in a number of different species may yield some insight into the possible functional significance of their structures. Furthermore, the presence of multiple, related genes presents several questions concerning the regulation of these genes. The availability of cloned cDNAs for the glycoprotein hormones should facilitate future studies of the possible coordinate regulation of this gene family. Recently, Nilson et al. (1983) reported the identification of an α-subunit cDNA clone very similar to that described in this paper.

Registry No. pALPHA-1 cDNA, 86846-24-4; pALPHA-2 cDNA, 86846-25-5; pALPHA-3 cDNA, 86846-26-6; pituitary hormone (ox clone pALPHA-1 α-subunit precursor reduced), 86846-27-7; pituitary hormone (ox clone pALPHA-1 α-subunit reduced), 86846-28-8.

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Interaction of Human Plasma Kallikrein and Its Light Chain with $C\bar{1}$ Inhibitor†

Fedde van der Graaf, Johannes A. Koedam, John H. Griffin, and Bonno N. Bouma*

ABSTRACT: The light chain of human plasma kallikrein contains the enzymatic active site. The inactivation of kallikrein and of its isolated light chain by $C\bar{1}$ inhibitor was investigated to assess the functional contributions of the heavy-chain region of kallikrein and of high molecular weight kininogen to this reaction. The second-order rate constants for the inactivation of kallikrein or its light chain were respectively 2.7×10^6 and 4.0×10^6 M^{-1} min^{-1}. High molecular weight kininogen did not influence the rate of kallikrein inactivation. The nature of the complexes formed between kallikrein or its light chain and $C\bar{1}$ inhibitor was studied by using sodium dodecyl sulfate (SDS) gradient polyacrylamide slab gel electrophoresis. Kallikrein as well as its light chain combined with $C\bar{1}$ inhibitor to form stable stoichiometric complexes that were not dissociated by SDS and that exhibited apparent molecular weights (M_r's) of 185 000 and 135 000, respectively, on nonreduced SDS gels. Reduction of the kallikrein–$C\bar{1}$ inhibitor complex gave a band at M_r 135 000 that comigrated with the complex seen for the light chain–$C\bar{1}$ inhibitor complex. During the inactivation of both kallikrein and its light chain, a M_r 94 000 fragment of $C\bar{1}$ inhibitor was formed which was unable to inactivate or bind kallikrein or its light chain. Kallikrein inactivated by diisopropyl phosphofluoridate did not form SDS-stable complexes with $C\bar{1}$ inhibitor. These results demonstrate that the functional binding site for $C\bar{1}$ inhibitor is localized in the light chain of kallikrein. In addition, the mechanism of interaction between kallikrein or its isolated light chain with $C\bar{1}$ inhibitor appears identical, and the rate of inactivation of both forms of the enzyme by $C\bar{1}$ inhibitor is very similar. Neither the heavy-chain region of kallikrein nor high molecular weight kininogen is significantly involved in the inactivation of kallikrein by $C\bar{1}$ inhibitor.

Plasma kallikrein, which circulates in its inactive zymogen form prekallikrein, participates in the contact activation reactions of plasma. Activation of this system occurs upon exposure of blood to negatively charged surfaces and initiates the intrinsic pathway of blood coagulation (Ratnoff, 1966), the kinin-forming pathway (Margolis, 1958), and the fibrinolytic system (Niewiarowski & Prou-Wartelle, 1959). The mechanism of this surface-dependent activation probably involves the binding of blood coagulation factor XII to the surface followed by the reciprocal activation of factor XII and prekallikrein (Nagasawa et al., 1968; Kaplan & Austen, 1970; Cochrane et al., 1973; Revak et al., 1977; Griffin & Cochrane, 1979; Dunn et al., 1982). For optimal generation of factor XII_a and kallikrein activity, a third plasma protein, high molecular weight kininogen, is necessary. High molecular weight kininogen circulates in plasma in a noncovalent complex with prekallikrein (Mandle et al., 1976; Donaldson et al., 1977) and functions as a nonenzymatic cofactor in these reactions by linking prekallikrein to the surface, thereby bringing it adjacent to surface-bound Factor XII (Griffin & Cochrane, 1976b; Meier et al., 1977; Wiggins et al., 1977; Silverberg et al., 1980). Plasma kallikrein also cleaves high molecular weight kininogen to liberate the vasoactive peptide bradykinin (Nagasawa & Nakayasu, 1973; Habal et al., 1974).

Several studies have identified $C\bar{1}$ inhibitor as an important inhibitor of plasma kallikrein (Gigli et al., 1970; McConnell, 1972; Fritz et al., 1972; Trumpi-Kalshoven & Kluft, 1978; Gallimore et al., 1979; Schapira et al., 1981). Recently, a major role for $C\bar{1}$ inhibitor in the inactivation of kallikrein in plasma was demonstrated in a quantitative manner (Schapira et al., 1982; van der Graaf et al., 1983). $C\bar{1}$ inhibitor is a plasma protease inhibitor which operates as an important regulator of the complement and the contact activation system of plasma. It is an α_2-globulin with a high content of amino sugars (Pensky & Schwick, 1969) and is the only plasma proteinase inhibitor capable of inactivating $C\bar{1}$, the activated first component of complement (Donaldson, 1979; Ziccardi,

† From the Department of Haematology, University Hospital Utrecht, 3500 CG Utrecht, The Netherlands, and the Department of Immunology, Scripps Clinic and Research Foundation, La Jolla, California 92037 (J.H.G.). *Received December 6, 1982; revised manuscript received June 2, 1983.* This work was supported by Grant 13-30-33 from the Netherlands Organization for the Advancement of Pure Research (ZWO). Financial support was also provided by Research Grants HL-21544 and HL-16411 from the National Institutes of Health and by Grant RG-1695 from the North Atlantic Treaty Organization, Division of Scientific Affairs.

* Correspondence should be addressed to this author at the Department of Haematology, University Hospital Utrecht.

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