Human inhibin genes

Genomic characterisation and sequencing

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Inhibin is a gonadal hormone involved in the non-steroidal regulation of follicle stimulating hormone (FSH) secretion. Using the cDNAs coding for bovine inhibin A and B subunits we have identified inhibin genes within the human genome using Southern blot hybridisation techniques. The genes are likely to be present as single copies. Cloning and sequencing inhibin genes obtained from lambda libraries of human genomic DNA provide structural and sequence data on the human A and B genes. Comparison of the known inhibin gene sequences showed, in particular, that the B subunits have identical sequences in man, pigs and cattle thus demonstrating a remarkable evolutionary conservation in these genes.

Inhibin *DNA hybridization* *Gene cloning* *Reproductive hormone*

1. INTRODUCTION

The controversy surrounding the existence of inhibin, a hormone postulated to be produced in the gonads and involved in the feedback control of FSH secretion [1,2], has been clarified with the purification of inhibin from two animal species [3,4]. Bovine follicular fluid yielded a protein with a molecular mass of 58 kDa composed of two disulphide-linked subunits termed A and B of 43 and 15 kDa, respectively [3]. Subsequently a smaller active form (31–32 kDa) was isolated from both porcine and bovine follicular fluids with subunits A_c and B of 20 and 15 kDa, respectively [4–8]. These observations were confirmed by more detailed information on the structures of inhibin, its subunits and precursors obtained by the recent cloning of the cDNAs for the subunits of bovine, porcine, human and bovine inhibins [9–11].

This study describes the cloning and characterisation of genomic sequences of human inhibin A and B genes and examines the homologies between three mammalian inhibin species.

2. MATERIALS AND METHODS

2.1. *Southern blot analysis*

Human genomic DNA from a human macrophage cell line U937 (ATCC CRL 1539) was digested with various restriction enzymes, subjected to electrophoresis on agarose gels and transferred to nitrocellulose membranes [12]. These were prehybridised by incubating in 50% formamide, 5 × SSPE, 5 × Denhardt's solution and 25 μg/ml denatured herring sperm DNA for 4 h at 42°C, then probed with [^{32}P]cDNA coding for substantial parts of the 43 and 15 kDa bovine inhibin subunits. For the A subunit a 725 base pair (bp) fragment of the bovine inhibin cDNA A chain gene was used (bases 242–967 [11]). For the B subunit a 358 bp fragment of the bovine cDNA B chain gene was used (bases 1–358 [11]). Labelling was performed by the oligonucleotide method [13]

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and hybridisation was as for the prehybridisation but in the added presence of 10% dextran sulphate and only 1 × Denhardt's solution. Filters were washed in 0.2 × SSC and 0.1% SDS at 50°C [12] and radioactive bands detected by autoradiography.

2.2. *Isolation of human inhibin genes*

Two λ bacteriophage libraries of human genomic DNA were used. One, in λL47 [14] was used for the isolation of the A gene, the other, in the λEMBL3 vector [15] was used for the preparation of the B gene. Plaques were transferred to nitrocellulose filters and probed with bovine inhibin cDNA fragments as described for the Southern Blot analysis. Positive plaques were purified, the DNA extracted and the insert mapped for restriction sites and partially sequenced by the dideoxynucleotide chain-termination method [16].

3. RESULTS

3.1. *Inhibin sequences in the human genome*

Sequences similar to or homologous with the genes coding for the large (43 kDa) or the small (15 kDa) subunits of bovine inhibin were identified in human genomic DNA by standard Southern Blot hybridisation techniques [12]. A number of fragments hybridised to cDNA coding for the large or small subunits of bovine inhibin as shown in table 1. The conditions of hybridisation and washing were such that the hybridising human DNA must be at least 75% homologous overall with the bovine cDNA. Digestion of the DNA with most of the enzymes (except *Pst*I) resulted in the detection of a single fragment which hybridised to either the large or the small subunit probes. Human DNA, therefore, contains sequences which are at least 75% homologous with bovine inhibin genes and may represent the human inhibin genes. These genes are present in low copy number, probably unique and may not be adjacent since the probes for the large and small subunits did not hybridise to similar sized fragments.

Table 1

Hybridisation of bovine inhibin cDNA to human genomic DNA

Probe	Enzymes	Fragment sizes (bp)
Bovine large subunit cDNA	*Pst*I	2450, 1200, 480
	*Pvu*II	700
	*Bam*HI	18000
	*Eco*RI	15000
	*Hin*dIII	20000
Bovine small subunit cDNA	*Pst*I	650
	*Eco*RI	9000
	*Hin*dIII	11000

3.2. *Isolation of inhibin genes from human genomic DNA*

Phages hybridising to the bovine inhibin cDNAs were isolated from libraries of human genomic DNA in bacteriophage λ. One clone, λBTA1406, contained an insert of human DNA of 11.5 kb. Digestion with *Pst*I yielded three fragments of about 2550, 1160 and 480 bp, respectively, which hybridised to the bovine inhibin A chain gene. These are the same fragments observed by Southern blot analysis of *Pst*I digested genomic DNA (table 1). The nucleotide sequence of part of this insert was determined and identified it as the gene for the preproA subunit of human inhibin by homology with the bovine sequence (fig.1). In this gene there is one intron inserted in the codon for amino acid 29 which is surrounded by typical intron/exon boundaries [17]. This intron is in a similar position to one found in an unspliced bovine inhibin A chain mRNA [11]. The complete coding sequence of the human preproinhibin A chain gene is shown in fig.1. This data extends the previously published sequence which differs slightly in the N-terminal region [10]. Also shown is a comparison between the amino acid sequences of human, bovine and porcine preproinhibins which are about 84% homologous. The overall structure of the A gene is shown in fig.2.

Another clone, λBTA1407, which hybridised to the bovine B subunit gene, contained an insert of human DNA of about 18 kb. The nucleotide sequence of part of this insert coded for the B chain of human inhibin by its homology with bovine and porcine inhibin genes (fig.3). There is a 9.7 kb intron 542 bp upstream from base position 1 as shown in fig.2. The B proteins encoded by all three genes are identical.

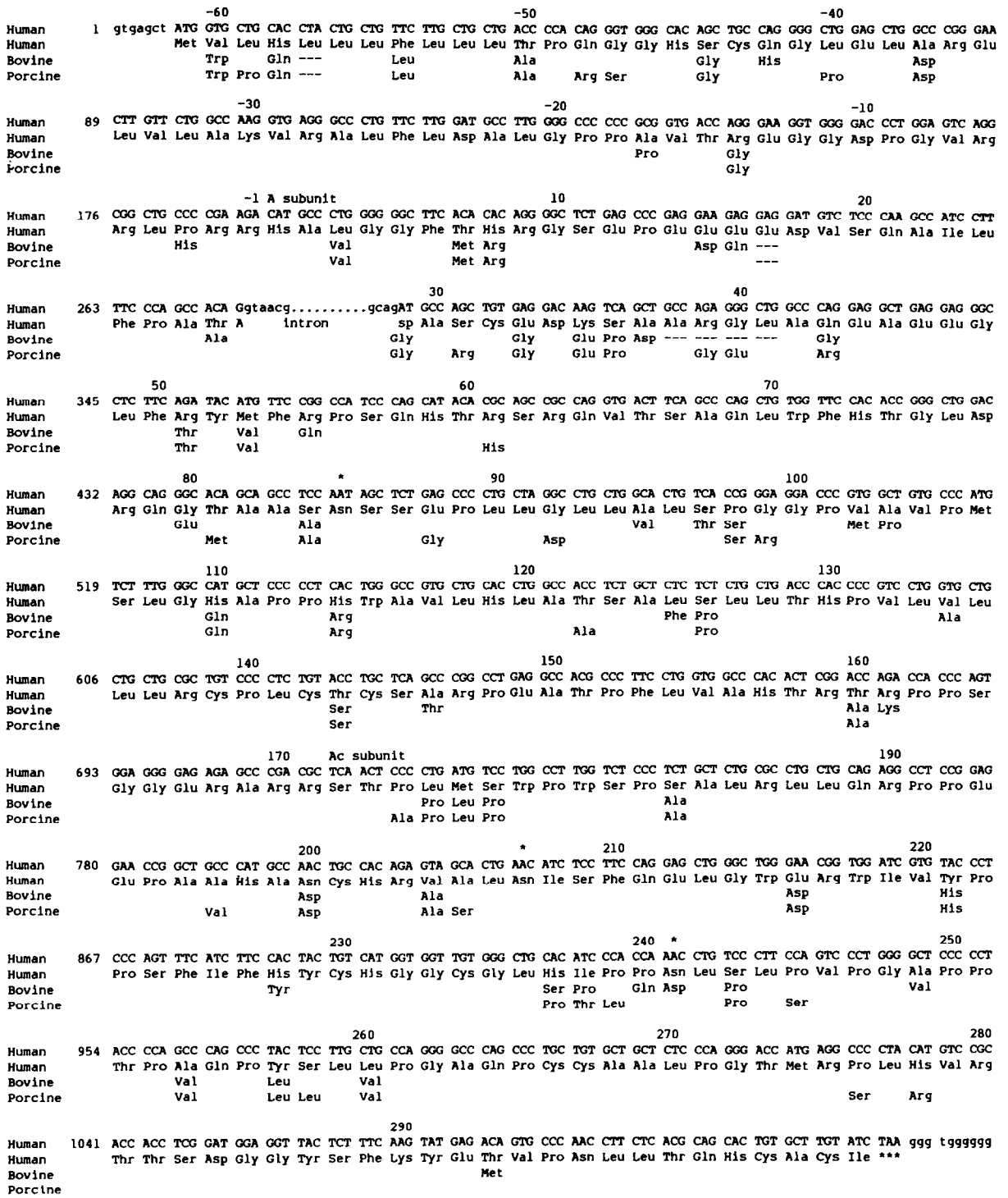

Fig.1. The nucleotide and encoded amino acid sequences of the human preproinhibin A gene. For comparison, where the bovine and porcine amino acid sequences differ from that of human inhibin the amino acid is shown. (*) Putative *N*-glycosylation sites. (–) Deleted amino acids.

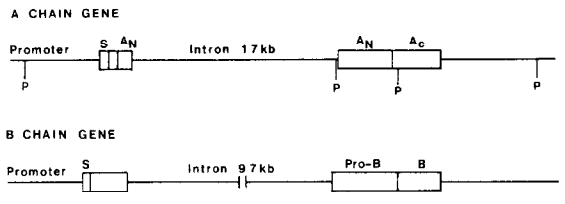

Fig.2. Structures of the human A and B inhibin genes. The regions coding for the preproinhibin chains are boxed. P, *PstI* restriction sites of the A chain gene. S, putative signal sequences.

4. DISCUSSION

Protein purification studies and DNA cloning techniques have now established the protein structure of bovine, porcine and human inhibins. Mature inhibin is most likely a protein of two disulphide-linked chains A_C and B of molecular masses 20 kDa and 15 kDa, respectively, whereas precursors of inhibin of various molecular masses, notably 58 kDa, have been detected in follicular fluids [3,4]. The cloning of the cDNA genes coding

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                                                    10
Human    Gly Leu Glu Cys Asp Gly Lys Val Asn Ile Cys Cys Lys Lys Gln Phe
Human    GGC TTG GAG TGT GAT GGC AAG GTC AAC ATC TGC TGT AAG AAA CAG TTC
Bovine       C           C
Porcine      C         C   C                                       G

                        20                                  30
Human    Phe Val Ser Phe Lys Asp Ile Gly Trp Asn Asp Trp Ile Ile Ala Pro
Human    TTT GTC AGT TTC AAG GAC ATC GGC TGG AAT GAC TGG ATC ATT GCT CCC
Bovine         T                     T
Porcine                                        C               C       G

                                40
Human    Ser Gly Tyr His Ala Asn Tyr Cys Glu Gly Glu Cys Pro Ser His Ile
Human    TCT GGC TAT CAT GCC AAC TAC TGC GAG GGT GAG TGC CCG AGC CAT ATA
Bovine     C       C   C                   T                   C       C
Porcine    C       C   C                               C       C       C

             50                                                 60
Human    Ala Gly Thr Ser Gly Ser Ser Leu Ser Phe His Ser Thr Val Ile Asn
Human    GCA GGC ACG TCC GGG TCC TCA CTG TCC TTC CAC TCA ACA GTC ATC AAC
Bovine            A   G   C   A   C   C       T       G   G
Porcine    G          G   C       G   C   G           G   G

                                70                                      80
Human    His Tyr Arg Met Arg Gly His Ser Pro Phe Ala Asn Leu Lys Ser Cys
Human    CAC TAC CGC ATG CGG GGC CAT AGC CCC TTT GCC AAC CTC AAA TCG TGC
Bovine                           C           C               G
Porcine                    C     C           C               G

                                        90
Human    Cys Val Pro Thr Lys Leu Arg Pro Met Ser Met Leu Tyr Tyr Asp Asp
Human    TGT GTG CCC ACC AAG CTG AGA CCC ATG TCC ATG TTG TAC TAT GAT GAT
Bovine                                                           C
Porcine    C   C                   G               C         C   C   C

                    100                                     110
Human    Gly Gln Asn Ile Ile Lys Lys Asp Ile Gln Asn Met Ile Val Glu Glu
Human    GGT CAA AAC ATC ATC AAA AAG GAC ATT CAG AAC ATG ATC GTG GAG GAG
Bovine     G   G               G           C
Porcine    G   G               G           C

Human    Cys Gly Cys Ser ***
Human    TGT GGG TGC TCA TAG
Bovine         T
Porcine    C           C
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Fig.3. The nucleotide and encoded amino acid sequences of human inhibin B gene. For comparison, where the nucleotide sequence of the bovine or porcine cDNAs differs the base is shown.

for each of these species of inhibin shows that the two subunits arise from separate genes, as well as identifying the possible precursors of each subunit and the proteolytic cleavage sites [9–11].

Our studies confirm and extend these observations by reporting the first characterisation of human inhibin gene structure at the genomic level. From the Southern blot data the genes coding for the A and B subunits appear to be present as single copies and are not adjacent.

The β_B gene of human inhibin [10] will be described in a later publication. Its low homology with the bovine inhibin B would not permit its identification under the conditions of hybridisation used here.

A striking feature of the similarities between human, bovine and porcine inhibins is that the amino acid sequence of all three B subunits is identical: this represents an extraordinary degree of conservation. The B chains also show similarities to the A chains as well as to Mullerian inhibiting substance, a gonadal protein of importance in sexual differentiation [18], and human transforming growth factor β (TGFβ) [19] suggesting a common ancestral gene.

The isolation of genes coding for animal and human inhibins has helped in our understanding of the inhibin structure. The expression of these genes in a suitable host should provide quantities of the protein hitherto unobtainable from the natural source except in trace amounts. This inhibin will be used to give a greater understanding of its physiological role in vivo, to explore the practical applications of inhibin in the manipulation of fertility and to develop sensitive diagnostic systems to evaluate the role of inhibin in human reproductive disorders.

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