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The cDNA of the two isoforms of bovine cGMP-dependent protein kinase

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cDNAs encoding the isoform Iα of the cGMP-dependent protein kinase were isolated from a bovine trachea smooth muscle cDNA library constructed in λgt10. The deduced protein sequence is identical with the protein sequence obtained by Edman degradation of the bovine lung enzyme [(1984) Biochemistry 23, 4207–4218]. Alternate cDNA clones were isolated which code for a protein slightly different within the aminoterminal part from the known amino acid sequence. These alternate cDNAs contain the sequence of a peptide identified in the isoform Iβ of cGMP-dependent protein kinase. Northern blot analysis of poly(A)$^+$ RNA from bovine trachea smooth muscle indicated the presence of two different mRNA species of about 6.2 kb.

Protein kinase, cyclic GMP-dependent; cDNA cloning; Smooth muscle

1. INTRODUCTION

Cyclic GMP-dependent protein kinase (cGMP-kinase) is one of the main intracellular receptors for cGMP [1]. The native enzyme consists of two identical 76331 Da subunits [2] and is activated by the binding of 4 mol cGMP per mol holoenzyme [3,4]. Each subunit of cGMP-kinase contains a regulatory part – the aminoterminus including the autophosphorylation site and two distinct cGMP binding sites – and a catalytic part: the ATP binding site and the catalytic center. Sequence analysis of various protein kinases and cyclic nucleotide binding proteins has led to the hypothesis that cGMP-kinase is the product of a fusion between two ancestral genes which code for a cyclic nucleotide binding protein and a protein kinase [2].

Cyclic GMP-kinase is present in high concentrations in smooth muscle [5–8]. The active enzyme decreases cytosolic levels in tracheal [7] and vascular [8] smooth muscle by an unidentified mechanism and thereby induces relaxation. Two isoforms (Iα and Iβ) which apparently are functionally identical but may differ in the expression of their mRNA have been identified in bovine aorta [9,10]. To facilitate further studies on the physiological role of cGMP-kinase, the cDNA of cGMP-kinase mRNA has been cloned from bovine trachea smooth muscle.

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2. MATERIALS AND METHODS

2.1. *Construction and screening of cDNA libraries*

Poly(A)$^+$ RNA was isolated from bovine trachea smooth muscle according to [11], followed by oligo(dT)-cellulose column chromatography [12]. Specific and random primed cDNA libraries were constructed in λgt10 according to [13]. 2 μg poly(A)$^+$ RNA yielded 5×10^6 independent recombinant phages.

The libraries were screened without prior amplification by synthetic oligonucleotide probe mixtures (fig.1, inset) and isolated cDNA fragments. The 5′ ends of the oligonucleotides were labeled using T_4 polynucleotide kinase. Isolated cDNA fragments were labeled by random priming [14].

2.2. *Subcloning and sequencing of cDNA inserts*

Positive clones were plaque purified, their cDNA inserts isolated and subcloned into the *Eco*RI or *Sma*I site of pUC18. Plasmid DNA suitable for sequencing was prepared by the alkaline lysis method [15]. Sequence analysis was performed by

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the dideoxy chain termination method [16] using [α-^{35}S]dATP and modified T7 DNA-polymerase (USB). Most of the sequence was obtained by sequencing both strands of the cDNA independently.

2.3. *Northern blot analysis*

Poly(A)$^+$ RNA was denatured with glyoxal (1 M) and 50% dimethylsulfoxide, electrophoresed on 1.5% agarose gels and transferred to Biodyne Nylon membranes [17]. Blot hybridization was carried out with cDNA fragments labeled by the random priming method [14]. If not stated otherwise all other cloning procedures were carried out according to [17].

3. RESULTS

3.1. *Isolation of cDNA clones for isoform Iα*

The cloning strategy is summarized in fig.1. Twelve 14-mer oligodeoxynucleotides complementary to all possible codons for the carboxyterminal amino acid sequence Trp-Asp-Ile-Asp-Phe (excluding the third nucleotide residue of the Phe-codon) of cGMP-kinase were synthesized and used for construction of a primer extension library. About 5 × 10^5 recombinant phages were screened with two different probes. One probe (A) contained an equimolar mixture of 64 oligonucleotides (21-mer) representing all the possible cDNA sequences corresponding to amino acid 618–624 (fig.1, amino acid numbers (fig.2) correspond to those in [2] + 1 since the sequence of [2] lacks the initiation amino acid Met). The second probe (B) (42 bp, aa 648–661) was designed according to codon usage [18]. The clone λcGpeI-6.2 was isolated, subcloned and sequenced. The cDNA sequence (215 bp, nt 1799–2014 of the complete sequence, fig.2) contained the sequence of the primer and of both probes. The insert of this clone was used to rescreen the same library and to isolate clone λcGpeI-8.2. Sequencing of this insert (nt 601–2010, fig.2) showed that it contained an open reading frame coding for an amino acid sequence corresponding to residues 200 (Ile) to 670 (Asp) of cGMP-kinase [2]. Using the 5′-terminal *Hin*dIII (nt 695)–*Hin*dIII (nt 1316) fragment one clone λcGrp-5.1 was isolated by screening approx. 1 × 10^6 independent clones of a random primed cDNA library. The cDNA insert of this clone (1810 bp) contained the sequence from nucleotide 959 to 2769 (fig.2).

A synthetic primer complementary to nt 828–846 (fig.2) was used to construct a second primer extension library (fig.1). One million recombinant phages of this library were screened using the nt 601–695 fragment as probe. Eleven

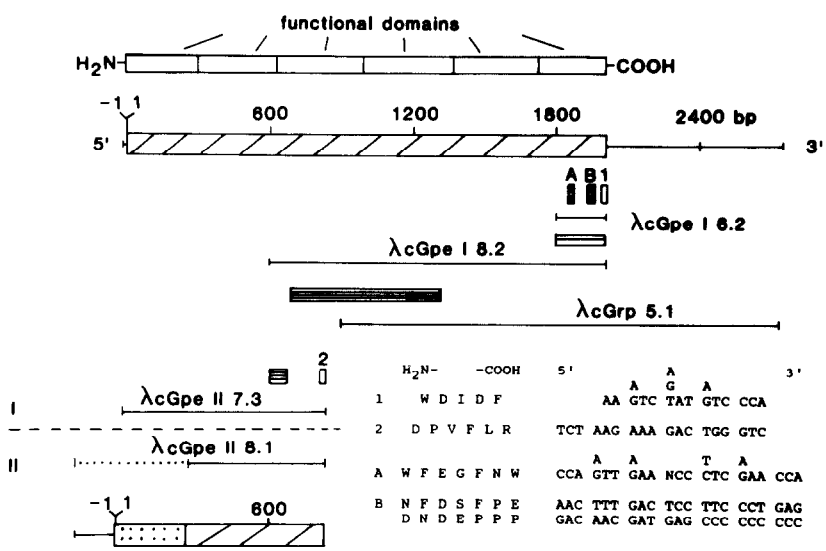

Fig.1. Cloning strategy. (Top) Functional domains of the amino acid sequence of cGMP-kinase according to [2]. Crossed bar and solid line represent the protein coding and the 5′- and 3′-noncoding region of the nucleotide sequence, respectively. (Bottom) The length of the cDNA inserts of individual clones are shown by lines. The open and hatched bars indicate the position of primers 1 and 2 and the screening probes, respectively. (I) Isoform Iα; (II) isoform Iβ; the dotted part of Iβ represents that part which is not identical with Iα. (Inset) Amino acid sequences and derived synthesized nucleotide sequences of primers 1 and 2 and screening probes A and B.

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-6 aaaaacATGAGCGAGCTGGAGGAAGACTTTGCCAAGATTCTCATGCTCAAGGAGGAGAGGATCAAAGAGCTGGAGAAG
        M  S  E  L  E  E  D  F  A  K  I  L  M  L  K  E  E  R  I  K  E  L  E  K   24
73 CGGCTGTCAGAGAAGGAGGAAGAAATCCAGGAGCTGAAGAGGAAACTCCATAAATGCCAGTCAGTGCTGCCCGTGCCC
    R  L  S  E  K  E  E  E  I  Q  E  L  K  R  K  L  H  K  C  Q  S  V  L  P  V  P   50
151 TCGACCCACATCGGCCCCCGGACCACCCGGGCACAGGGCATCTCGGCCGAGCCGCAGACCTACAGGTCCTTCCACGAC
    S  T  H  I  G  P  R  T  T  R  A  Q  G  I  S  A  E  P  Q  T  Y  R  S  F  H  D   76
229 CTCCGACAGGCATTCCGGAAGTTCACCAAATCCGAAAGGTCCAAGGATCTCATAAAGGAGGCCATCCTTGACAATGAC
    L  R  Q  A  F  R  K  F  T  K  S  E  R  S  K  D  L  I  K  E  A  I  L  D  N  D  102
307 TTTATGAAGAACTTGGAGCTGTCACAGATCCAAGAGATTGTGGATTGTATGTACCCAGTGGAGTACGGCAAAGACAGC
    F  M  K  N  L  E  L  S  Q  I  Q  E  I  V  D  C  M  Y  P  V  E  Y  G  K  D  S  128
385 TGCATCATCAAAGAAGGAGATGTGGGGTCACTGGTGTATGTCATGGAAGATGGTAAGGTTGAAGTTACAAAAGAAGGC
    C  I  I  K  E  G  D  V  G  S  L  V  Y  V  M  E  D  G  K  V  E  V  T  K  E  G  154
463 GTGAAGCTGTGCACAATGGGTCCTGGTAAAGTGTTTGGAGAGTTGGCTATCCTTTACAACTGTACCCGGACGGCGACC
    V  K  L  C  T  M  G  P  G  K  V  F  G  E  L  A  I  L  Y  N  C  T  R  T  A  T  180
541 GTCAAAACTCTTGTAAATGTGAAACTCTGGGCCATTGATCGACAATGTTTTCAGACGATAATGATGAGGACAGGACTT
    V  K  T  L  V  N  V  K  L  W  A  I  D  R  Q  C  F  Q  T  I  M  M  R  T  G  L  206
619 ATCAAGCATACCGAGTATATGGAATTTTTAAAAAGCGTTCCAACATTCCAGAGCCTTCCTGAAGAGATCCTCAGTAAG
    I  K  H  T  E  Y  M  E  F  L  K  S  V  P  T  F  Q  S  L  P  E  E  I  L  S  K  232
697 CTTGCTGACGTCCTTGAAGAGACCCACTATGAAAATGGGGAATATATCATCAGGCAAGGTGCAAGAGGGGACACCTTC
    L  A  D  V  L  E  E  T  H  Y  E  N  G  E  Y  I  I  R  Q  G  A  R  G  D  T  F  258
775 TTTATCATCAGTAAAGGAAAGGTTAATGTCACTCGTGAAGACTCGCCCAATGAAGACCCAGTCTTTCTTAGAACCTTA
    F  I  I  S  K  G  K  V  N  V  T  R  E  D  S  P  N  E  D  P  V  F  L  R  T  L  284
853 GGAAAAGGAGATTGGTTTGGAGAGAAAGCCTTGCAGGGGGAAGATGTGAGAACAGCGAATGTAATTGCGGCAGAAGCT
    G  K  G  D  W  F  G  E  K  A  L  Q  G  E  D  V  R  T  A  N  V  I  A  A  E  A  310
931 GTAACCTGCCTTGTGATCGACAGAGACTCTTTCAAACATTTGATTGGAGGATTAGATGATGTTTCTAATAAAGCATAT
    V  T  C  L  V  I  D  R  D  S  F  K  H  L  I  G  G  L  D  D  V  S  N  K  A  Y  336
1009 GAAGATGCAGAAGCTAAGGCAAAATATGAAGCTGAAGCTGCTTTCTTCGCCAACCTGAAGCTGTCTGATTTCAACATC
     E  D  A  E  A  K  A  K  Y  E  A  E  A  A  F  F  A  N  L  K  L  S  D  F  N  I  362
1087 ATTGACACCCTTGGAGTTGGAGGTTTCGGACGCGTAGAACTGGTCCAGTTAAAAAGTGAAGAATCCAAAACCTTTGCA
     I  D  T  L  G  V  G  G  F  G  R  V  E  L  V  Q  L  K  S  E  E  S  K  T  F  A  388
1165 ATGAAGATTCTCAAGAAACGGCACATCGTGGATACAAGACAGCAGGAACACATCCGCTCGGAGAAGCAGATCATGCAG
     M  K  I  L  K  K  R  H  I  V  D  T  R  Q  Q  E  H  I  R  S  E  K  Q  I  M  Q  414
1243 GGGGCCCATTCGGACTTCATAGTGAGATTATACAGAACATTTAAGGACAGCAAATATTTGTATATGTTGATGGAAGCT
     G  A  H  S  D  F  I  V  R  L  Y  R  T  F  K  D  S  K  Y  L  Y  M  L  M  E  A  440
1321 TGCCTAGGTGGAGAGCTCTGGACCATTCTCAGGGATCGGGGGTCATTTGAAGATTCTACAACCAGATTTTATACAGCA
     C  L  G  G  E  L  W  T  I  L  R  D  R  G  S  F  E  D  S  T  T  R  F  Y  T  A  466
1399 TGTGTGGTAGAAGCTTTTGCCTATCTGCATTCCAAAGGAATCATTTACAGGGACCTCAAGCCTGAAAATCTCATCCTA
     C  V  V  E  A  F  A  Y  L  H  S  K  G  I  I  Y  R  D  L  K  P  E  N  L  I  L  492
1477 GATCACCGAGGTTATGCCAAACTGGTTGATTTTGGCTTTGCAAAGAAAATAGGATTTGGAAAGAAAACATGGACTTTT
     D  H  R  G  Y  A  K  L  V  D  F  G  F  A  K  K  I  G  F  G  K  K  T  W  T  F  518
1555 TGTGGGACTCCAGAATATGTAGCCCCAGAGATCATCCTGAACAAAGGCCATGACATTTCAGCCGACTATTGGTCACTG
     C  G  T  P  E  Y  V  A  P  E  I  I  L  N  K  G  H  D  I  S  A  D  Y  W  S  L  544
1633 GGAATCCTCATGTATGAGCTTCTGACTGGCAGCCCACCTTTCTCAGGCCCAGATCCTATGAAAACCTATAACATCATA
     G  I  L  M  Y  E  L  L  T  G  S  P  P  F  S  G  P  D  P  M  K  T  Y  N  I  I  570
1711 TTGAGGGGGATTGACATGATAGAGTTTCCAAAGAAGATTGCCAAAAATGCTGCTAATTTAATTAAAAAACTATGCAGG
     L  R  G  I  D  M  I  E  F  P  K  K  I  A  K  N  A  A  N  L  I  K  K  L  C  R  596
1789 GATAATCCATCAGAAAGATTAGGGAATTTGAAAAACGGAGTGAAAGACATTCAAAAGCACAAATGGTTTGAGGGCTTT
     D  N  P  S  E  R  L  G  N  L  K  N  G  V  K  D  I  Q  K  H  K  W  F  E  G  F  622
1867 AATTGGGAAGGCTTAAGAAAAGGCACCTTGACACCTCCTATAATACCAAGTGTTGCATCACCCACAGACACAAGCAAT
     N  W  E  G  L  R  K  G  T  L  T  P  P  I  I  P  S  V  A  S  P  T  D  T  S  N  648
1945 TTTGACAGTTTCCCTGAGGACAATGATGAACCGCCACCTGATGACAACTCAGGATGGGACATAGACTTCTAAtgtatt
     F  D  S  F  P  E  D  N  D  E  P  P  P  D  D  N  S  G  W  D  I  D  F          671
     tctcttacctgcttctgccttgctgaagacagcttttctaagacacagctgccagcaaacctgaaggaaagagagaa
2101 gctgagtgcttggggtcaccatgatgcctttgatcgatgctgctccagtaactacagtggcattaggacttattgctt
     agatgacagtagtgctctttacatgttttctgtttcaacctaaatatagcagttgacatggtggtcctgaagcaaagc
2257 ctttcaccactaaagagatgttctccactgttgcaatgatcttgctttgctctgattatattgaaagactgtaagaac
     cacttcaatctagtaaaagagtcagtaccttgctagaattatcaagaagatcaaaaaataatatattgggtacgatag
2413 attactatggtacaaaaactggactcttccttcttcaggtgagggttgtcggttctgtttctgcaagccagtgtatat
     accgtacacaagaggaccacacatctgttggtcacagaggtcatgtcaaaccagtgctagaagtttcatgattttatt
2569 tcccagcagtgctgatgacgagtctgaatgttacctttcctttctgacagattttaaaaattggtatgataaaagcac
     aactgctatggattctgctgagaaatttcatagcaggtacatacgtgttttcacagaggactgaagaaaaatcaacat
2725 gcatgtatctgttcatttctttttgataaattggcatgacagaat
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Fig.2. Nucleotide and deduced amino acid sequences of isoform Iα of cGMP-kinase. The translational initiation signal ATG and the termination signal TAA are underlined.

positive clones including clone λcGpeII-7.3 were isolated. This clone contained the cDNA sequence from nucleotide −6 to 827 (numeration starts with +1 at the A of the initiation codon ATG) of cGMP-kinase.

Fig.2 shows the composed nucleotide sequence and the deduced amino acid sequence of the cDNA of isoform Iα of the cGMP-kinase from bovine trachea smooth muscle. The composed nucleotide sequence consisted of 2775 bp and contained an open reading frame of 2013 bp coding for a protein with a calculated molecular mass of 76418 Da and also part of the 5′- and 3′-noncoding sequence. The deduced amino acid sequence is identical to the sequence of the bovine enzyme [2] with the addition of the initiation Met.

3.2. *Isolation of cDNA clones for isoform Iβ*

In addition to λcGpeII-7.3 the clone λcGpeII-8.1 was isolated from the second primer extension library (fig.1). The cDNA insert of this clone (1042 nt, fig.3) contained an open reading frame of 879 bp including the initiation codon ATG (A = +1 in the numeration) and 163 bp of the 5′-noncoding region of the isoform Iβ mRNA. From nucleotide 312 to the 3′-end of the clone (nt 879) the nucleotide and the deduced amino acid sequence were identical to the corresponding part of the isoform Iα (nt 267 to nt 834). To confirm the second isoform the sequence nt 312–879 of clone λcGpeII-8.1, which is present in both isoforms, was used to rescreen the same library. Ten clones were isolated and sequenced. Seven and 3 clones coded for isoform Iα and Iβ, respectively.

3.3. *Northern blot analysis*

The 3′-noncoding and the protein coding sequence of isoform Iα were used for Northern blot analysis of poly(A)$^+$ RNA from bovine trachea smooth muscle (fig.4). These probes hybridized to a mRNA species of approx. 6.2 kb suggesting that the mRNA for cGMP-kinase contains approx. 4.2 kb noncoding sequences. The 5′-noncoding and that part of the coding sequence of λcGpeII-8.1 which was not identical to isoform Iα also hybridized to a 6.2 kb mRNA species (fig.4). In agreement with the isolation frequency of the cDNA clones the message for the isoform Iβ was consistently less (approx. 10%) than that of isoform Iα (fig.4) suggesting that each isoform of cGMP-kinase is coded by a distinct mRNA species of approximately identical size.

```
-163  gcagactgggcatgctcagaagccaagcctggctttggtctcaagtcggaagctttcg
-105  cactcgggaggcagcagcgactttgggggaaagttgatcggagaggggaggaagccccaag
 -45  acgcggagcagcggcaggaaggagccccccggcagcccggagggagcATGGGCACCCTGCGG
                                                    M  G  T  L  R    5
  16  GATTTACAGTATGCGCTCCAGGAGAAGATCGAGGAGCTGAGGCAGCGGGATGCTCTCATC
       D  L  Q  Y  A  L  Q  E  K  I  E  E  L  R  Q  R  D  A  L  I   25
  76  GACGAGCTGGAGCTGGAGTTGGATCAGAAGGACGAACTGATCCAGAAGCTGCAGAACGAG
       D  E  L  E  L  E  L  D  Q  K  D  E  L  I  Q  K  L  Q  N  E   45
 136  CTGGACAAGTACCGCTCGGTGATCCGGCCGGCCACCCAGCAGGCGCAGAAGCAGAGCGCG
       L  D  K  Y  R  S  V  I  R  P  A  T  Q  Q  A  Q  K  Q  S  A   65
 196  AGCACCTTGCAAGGCGAGCCGCGCACCAAGCGGCAGGCGATCTCCGCTGAGCCCACCGCC
       S  T  L  Q  G  E  P  R  T  K  R  Q  A  I  S  A  E  P  T  A   85
 256  TTTGACATCCAGGATCTCAGCCATGTGACCCTGCCCTTCTACCCCAAGAGTCCACAGTCC
       F  D  I  Q  D  L  S  H  V  T  L  P  F  Y  P  K  S  P  Q  S  105
 316  AAGGATCTCATAAAGGAGGCCATCCTTGACAATGACTTTATGAAGAACTTGGAGCTGTCA
       K  D  L  I  K  E  A  I  L  D  N  D  F  M  K  N  L  E  L  S  125
 376  CAGATCCAAGAGATTGTGGATTGTATGTACCCAGTGGAGTACGGCAAAGACAGCTGCATC
       Q  I  Q  E  I  V  D  C  M  Y  P  V  E  Y  G  K  D  S  C  I  145
 436  ATCAAAGAAGGAGATGTGGGGTCACTGGTGTATGTCATGGAAGATGGTAAGGTTGAAGTT
       I  K  E  G  D  V  G  S  L  V  Y  V  M  E  D  G  K  V  E  V  165
 496  ACAAAAGAAGGCGTGAAGCTGTGCACAATGGGTCCTGGTAAAGTGTTTGGAGAGTTGGCT
       T  K  E  G  V  K  L  C  T  M  G  P  G  K  V  F  G  E  L  A  185
 556  ATCCTTTACAACTGTACCCGGACGGCGACCGTCAAAACTCTTGTAAATGTGAAACTCTGG
       I  L  Y  N  C  T  R  T  A  T  V  K  T  L  V  N  V  K  L  W  205
 616  GCCATTGATCGACAATGTTTTCAGACGATAATGATGAGGACAGGACTTATCAAGCATACC
       A  I  D  R  Q  C  F  Q  T  I  M  M  R  T  G  L  I  K  H  T  225
 676  GAGTATATGGAATTTTTAAAAAGCGTTCCAACATTCCAGAGCCTTCCTGAAGAGATCCTC
       E  Y  M  E  F  L  K  S  V  P  T  F  Q  S  L  P  E  E  I  L  245
 736  AGTAAGCTTGCCGACGTCCTTGAAGAGACCCACTATGAAAATGGGGAATATATCATCAGG
       S  K  L  A  D  V  L  E  E  T  H  Y  E  N  G  E  Y  I  I  R  265
 796  CAAGGTGCAAGAGGGGACACCTTCTTTATCATCAGTAAAGGAAAGGTTAATGTCACTCGT
       Q  G  A  R  G  D  T  F  F  I  I  S  K  G  K  V  N  V  T  R  285
 856  GAAGACTCGCCCAATGAAGACCCA
       E  D  S  P  N  E  D  P                                      293
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Fig.3. Nucleotide and deduced amino acid sequences of the aminoterminal part of isoform Iβ of cGMP-kinase. The nucleotide sequence of λcGpeII-8.1 which is identical to the isoform Iα-sequence is underlined (Ser^{105} of isoform Iβ = Ser^{90} of isoform Iα). The initiation codon ATG and the upstream termination codon TGA are also underlined.

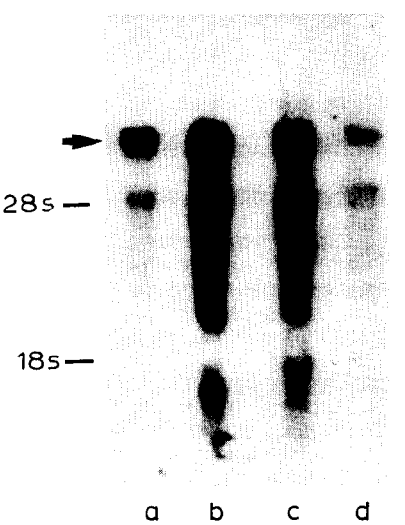

Fig.4. Northern blot analysis of RNA from bovine trachea smooth muscle. (a,b) 5 and 20 μg of poly(A)$^+$ RNA were hybridized with a probe for isoform Iα (nt 959–2769). Identical results were obtained when the sequence nt 695–1316 (Iα) was used as a probe (not shown); (c,d) 20 and 5 μg of poly(A)$^+$ RNA were hybridized with a probe specific only for isoform Iβ (λcGpeII-8.1, nt −163 to 312). The probes were labeled to the same specific activity.

4. DISCUSSION

Two distinct cDNAs for cGMP-kinase were identified. The derived amino acid sequence for isoform Iα is identical with the known sequence of bovine lung cGMP-kinase [2]. The second derived amino acid sequence (Iβ) had an extended aminoterminus which differs from that of isoform Iα. No evidence was obtained for any differences between the two isoforms in the remaining part of the coding region which contains the two cGMP- and the ATP-binding sites and the catalytic center. The in vivo occurrence and identity of isoform Iβ is further supported by (i) the identity of aa 66–75

```
I                                                 M S E L E E D F A K I L M L -
II      M G T L R D L Q Y A L Q E K I E E L R Q R D A L I D E L E
III     ......27I Q A L L K D S I V Q L C T A R P E R P M A F L -
IV      ......25R P P D L V D F A V D Y F T R L R E A R S R A S -

I       K E E R I K E L E K R L S E K E E E I Q E L K R K L H K C
II      L E L D Q K D E L I Q K L Q N E L D K Y R S V I R P A T Q
III     R E Y F E K L E K E E A K Q I Q N L Q K A G S R A D S R E
IV      T P P A A P P S G S Q D F D P G A G L V A D A V A D S - E
V                                                 M T N N I S H N Q K

                                                  *
I       Q S V L P V P S T H I G - P R T T R A Q G I S A E P Q T Y
II      Q A Q K Q S A S T L Q G E P R - T K R Q A I S A E P T A F
III     D E I S P P P P N P V V K G R - R R R G A I S A E V Y - -
IV      S E D E E D L D V P I P - G R F D R R V S V C A E T Y N P
V       A T E K V E A Q N N N N I T R - K R R G A I S S E P - - -

I       R S F H D L - R Q A F R K F T K S E R S K D L 93 ....
II      D I Q - D L S H V T L P F Y P K S P Q S K D L 108 ...
III     T E E D A A S Y V R K V - I P K D Y K T M A A 127 ...
IV      D E E E E D T D P - R V I H P K T D Q Q R C R 126 ...
V       - L G D K P A T P - L P N I P K T V I T Q Q R 56 ....
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Fig.5. Comparison of the aminoterminal sequences of cGMP-kinases and regulatory subunits of cAMP-kinase. Amino acid sequence alignment of part of the regulatory domains of isoforms Iα and Iβ of cGMP-kinase and of part of the regulatory subunits of different types of the cAMP-kinase. The sequences were aligned by eye. Gaps are marked with a dash. Identical amino acids are shown in bold. The hinge region containing the autophosphorylation sites of cGMP-kinase Iα (* = autophosphorylated Thr) and RIIα is boxed. (I) aa 1–93 of isoform Iα of cGMP-kinase; (II) aa 1–108 of isoform Iβ of cGMP-kinase; (III) aa 27–127 of RIα of cAMP-kinase (bovine skeletal muscle) [26]; (IV) aa 25–126 of RIIα of cAMP-kinase (bovine heart) [27]; (V) aa 1–56 of R of cAMP-kinase (*Dictyostelium discoideum*) [28].

of the derived amino acid sequence with a peptide sequence published for isozyme Iβ [10], and (ii) the identification of two distinct mRNA species for isoforms Iα and Iβ. However, the concentration of the mRNA for isoform Iβ is about 10% of that of isoform Iα. The mRNA for both isoforms exceeds the coding region about 2-fold. This is not unusual since similar large mRNA species were described for the regulatory subunits RI (2.4–4.3 kb) [20–22] and RII (6.0 kb) [23,24] of cAMP-kinase.

The two isoforms from bovine trachea smooth muscle are very similar. Using the Dayhoff MDM-78 comparison matrix [25], the aminoterminus (aa 1–108) of the deduced amino acid sequence of isoform Iβ shows a high degree of similarity to isoform Iα (71.2%, aa 1–93) and to the corresponding parts of the regulatory subunits of cAMP-kinase (RIα from bovine skeletal muscle [26] 64.5% (aa 27–127); RIIα from bovine heart [27] 62.0% (aa 25–126) and R from *Dictyostelium discoideum* [28] 63.3% (aa 1–56)) (fig.5). The highest degree of similarity is found in the so-called 'hinge region' [29] which contains at Thr^{59} the autophosphorylation site of cGMP-kinase Iα [2,30]. The difference in the aminoterminus of isoforms Iα and Iβ is very interesting since it has been shown that the aminoterminus of Iα is that part of the enzyme which regulates binding of cGMP to and activation of the cGMP-kinase [4].

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