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Heterogeneity in human soluble guanylate cyclase due to alternative splicing

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Two forms of the smaller subunit of the human soluble guanylate cyclase enzyme have been cloned by using PCR. One of the clones (HSGC-1) is identical to bovine and rat lung smaller subunit cyclase. However, the other (HSGC-2) is lacking 33 amino acids. Comparison of its sequence with published partial genomic sequences of bovine guanylate cyclase indicates that HSGC-2 is formed due to alternative splicing.

Human guanylate cyclase; Polymerase chain Reaction; cDNA; Alternative splicing

1. INTRODUCTION

Cyclic GMP is a second messenger found in virtually all tissues. It is formed from GTP by guanylate cyclases which exist in membrane-bound and soluble forms. The soluble cyclase (EC 4.6.1.2) is composed of 2 subunits. The smaller subunit (619 amino acids) has been previously cloned from rat [1] and bovine [2] lung. Recently the larger subunit (691 amino acids) was also cloned from bovine lung [3]. Northern blot analysis indicates that the enzyme is ubiquitously distributed, being present in tissues such as cerebrum, cerebellum, liver, heart, kidney and muscle [1]. The role of cyclic GMP as intracellular second messenger is well established for smooth muscle, where it acts as a regulator of muscle tone [4]. The function of cyclic GMP is also well established in the retina, where it participates in visual transduction [5]. However, recent findings that nitric oxide (which is a potent stimulator of the soluble guanylate cyclase) is produced endogenously and that nitric oxide may participate in intracellular and intercellular communications, has implicated a wider role for the cyclic GMP system [6]. For example, cyclic GMP and nitric oxide are suggested to play important roles in neuronal signalling and in immunomodulation [7]. Previous studies have shown that rat and bovine smaller subunits of guanylate cyclase are highly conserved [1,2] but recent studies have also shown that molecular heterogeneity may exist in both membrane-bound and soluble forms of guanylate cyclase [8]. Using PCR we now report the isolation of alternatively spliced forms of the smaller subunit of soluble human guanylate cyclase.

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2. MATERIALS AND METHODS

2.1. *Oligonucleotides*

The oligonucleotide primers used were specific for previously cloned rat and bovine smaller subunit guanylate cyclase. The primers were designed with restriction endonuclease linkers *Eco*RI and *Bam*HI at the 5′ ends to facilitate cloning of amplified fragments:

Primer 1	5′ CCGGAATTCGGCCTGTACCTGAGTGACATCCC
Primer 2	5′ CCGGATCCGTTTCCGAAGAGACAGTACCGAGGCAT

2.2. *PCR amplification of human guanylate cyclase*

Approximately 2×10^6 phage in 2 μl (corresponding to 3 times the number of independent phage in library-Clonetech 1066b, human lung cDNA) were mixed with 32 μl dH_2O and freeze-thawed twice. The liberated DNA was then subjected to PCR with the specific primers described above. The PCR was performed with Gene AMP DNA amplification reagent kit from Perkin Elmer Corp. USA, and the thermal profile used as 93°C for 30 s, 55°C for 30 s and 72°C for 1 min for a total of 30 cycles. The amplified products were separated by agarose gel electrophoresis and cloned into PGEM7Zf(+) vector between the *Eco*RI and *Bam*HI restriction enzyme sites and sequenced by the dideoxy chain termination method [9].

3. RESULTS AND DISCUSSION

Using a strategy based on the polymerase chain reaction (PCR) and oligonucleotide primers corresponding to consensus sequences of rat [1] and bovine [2] soluble guanylate cyclase enzymes, we obtained 2 clones (HSGC-1 and HSGC-2) encoding the smaller subunit of human soluble guanylate cyclase from human lung cDNA. Sequence comparison showed that one of the clones (HSGC-1) was completely identical to the published sequences, whereas the other (HSGC-2) was not. Alignment of the latter sequence showed that it was lacking 33 amino acids (see Fig. 1).

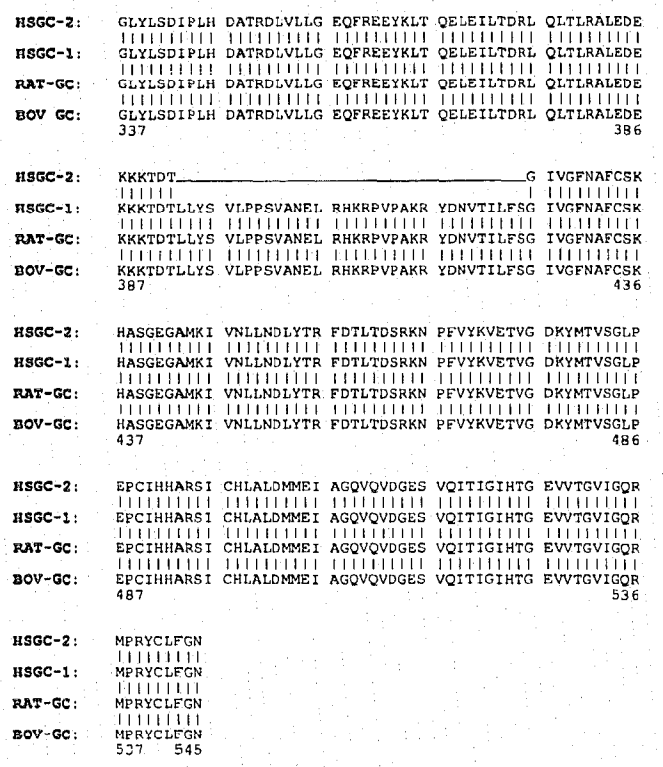

Fig. 1. Comparison of the amino acid sequences for rat (RAT-GC), bovine (BOV-GC) and two different forms of human (HSGC-1 and HSGC-2) soluble guanylate cyclase smaller subunits. The missing exon in clone HSGC-2 is marked as horizontal line.

Koesling et al. [2] have reported partial sequences for genomic clones of the bovine soluble guanylate cyclase enzyme. Koesling et al. [2] show that clone N2 (nucleotides 1950–2190) has an exon starting at nucleotide 2154. Upon comparison of our clone HSGC-2 with clone N2 it is clear that the 99 nucleotides (33 amino acids) lacking in HSGC-2 form the beginning of an exon in clone N2 [2]. The 5′ splice site GACTAT in the intron preceding this exon is very different to the consensus 5′ splice site GT(AG)AGT [10]. Such a different splice site may be responsible for its alternative recognition by splicing machinery. The HSGC-2 clone product does not represent an immature mRNA as it does not have anything extra in it; rather it is lacking 33 amino acids.

From our results it is clear that at least 2 forms of soluble guanylate cyclase smaller subunits are expressed in human lung, one lacking and other having a 99 nucleotide (33 amino acid) exon. It is interesting to note that a homologous region to this 33 amino acid sequence is present in the membrane-bound form of guanylate cyclase [11,12] where it is located between the protein kinase and catalytic domains. It is also notable that the HSGC-1 clone is exactly identical to the bovine and rat cyclase forms cloned before [1,2], indicating that the soluble enzyme is a very conserved protein. Recently Yuen et al. [13] have described a different form of guanylate cyclase preferentially expressed in rat kidney and liver. The sequence of this subunit of a guanylate cyclase is homologous to the smaller subunit of the soluble guanylate cyclase, but it nevertheless shows considerable diversity.

In summary we have shown the existence of 2 forms of human lung guanylate cyclase which seem to be generated due to alternative splicing. What function these alternatively spliced forms play remains to be seen. The presence of isoforms of soluble guanylate cyclases may potentially give rise to enzymes with different modes of regulation of catalytic activity as well as different tissue localizations due to differential levels of expression. This may find practical uses since soluble guanylate cyclases are targets for drugs such as organic nitroesters which are in wide use for the treatment of angina pectoris.

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