ANALYSIS OF THE HUMAN 3'-PHOSPHOADENOSINE 5'-PHOSPHOSULFATE SYNTHASE GENES

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INTRODUCTION. PAPS is the universal sulfonate donor compound. PAPS synthase (PAPSS) catalyzes the formation PAPS in two concerted steps. In the first step SO_4^- combines with ATP to form adenosine 5'-phosphosulfate (APS) and PPi catalyzed by ATP sulfurylase domain. In the second step APS combines with another molecule of ATP to form PAPS and ADP catalyzed by APS kinase domain. In higher eukaryotes APS kinase domain (aa 1-268) and ATP sulfurylase domain (aa 220-623) are fused together into a single polypeptide termed PAPS synthase (PAPSS) (1). cDNA of the two isoforms PAPSS1 (accession # U53447) and PAPSS2b (accession # AF 150754) from human have been molecularly cloned and characterized[1]. PAPS synthase genes have been localized to chromosome 4q24 (PAPSS1) and chromosome 10q22.3-10q25.1 (PAPSS2)[1,2].

METHOD. Using the GenBank human genome depository from the NCBI server, DNA sequence analyses were performed. Exon-intron junctions were verified using consensus donor and acceptor splice sites.

PAPSS1	113	114	235	138	118	113	111	205	135	268	229	720 bp
PAPSS2b	137	117	235	138	118	113	111	205	135	268	229	573 bp
exon	1	2	3	4	5	6	7	8	9	10	11	12

RESULTS. The gene sequence analysis of PAPSS1 and PAPSS2b reveals conservation in the size of the coding regions of the exons (Figure). The number of nucleotides (nt) in PAPSS1 of the exons (ex) were (ex1 = 113, ex2 = 114, ex3 = 235, ex4 = 138, ex5 = 118, ex6 = 113, ex7 = 111, ex8 = 205, ex9 = 135, ex10 = 268, ex11 = 229, ex12 = 720). From ex2 until ex11 both PAPSS1 and PAPSS2b evinced same size except there was an additional 3 nt in ex2 of PAPSS2b. The ex1 of PAPSS1 contained 54 nt of 5'-UTR and 59 nt of coding sequences, whereas PAPSS2b contained 110 nt of 5'-UTR and 27 nt of coding sequences. Ex12 of PAPSS1 contains stop codon at nt 138 and a 3'-UTR length of 582 nt. Likewise ex12 of PAPSS2b contains a stop codon at nt 194 and a 3'-UTR of 379 nt. The APS kinase-ATP sulfurylase domain requires a mandatory intervening sequence region (aa ~220-268) that is located mainly in ex6. PAPSS2b promoter

contains a putative TATA box (TATACCT) at nt -18 and a GC box (GGGCGG) at -94 nt. PAPSS1 gene has a TATA-less promoter with two GC boxes at -21 and -34 nt.

DISCUSSION. PAPSS plays a pivotal role in producing the universal sulfonate donor compound PAPS that are required for all sulfotransferase reactions. PAPSS is a bifunctional enzyme, comprised of NH₂-terminal, APS kinase domain (aa 1-268) and a COOH-terminal, ATP sulfurylase domain (aa 220-623) as characterized in PAPSS1 protein[1]. The isoforms PAPSS1 and PAPSS2b are differentially expressed in various tissues[1,2]. The sizes of ex2 through ex11 of PAPSS1 and PAPSS2b are precisely placed between introns, although the chromosomal location is different. Intervening sequences (aa 220-268) located between APS kinase and ATP sulfurylase domains are coded mainly by ex6 in both genes. Cloned and expressed proteins of PAPSS1 exhibit Michaelis-Menton kinetics and PAPSS2b a sigmoidal response, with varying [ATP] vs. v/Vmax (unpublished). From gene analysis PAPSS2b is the inducible form and PAPSS1 the house keeping form, which is in agreement with the kinetics observed with reference to ATP. Understanding the proper structure/functions of these isoforms of PAPSS is crucial, since it plays a pivotal role in various biochemical processes. Failure to produce normal levels of PAPS can result in severe cartilage and bone defect [spondyloepimetaphyseal dysplasia (SEMD)][2] and perhaps even more severe defects, depending on the types of tissues that are affected.

REFERENCES

1. Venkatachalam, K.V., Akita, H., and Strott, C.A. (1998) J.Biol.Chem 273, 19311–19320.
2. ul Haque, M.F., King, L.M., Krakow, D., Cantor, R.M., Rusiniak, M.E., Swank, R.T., Superti-Furga, A., Haque, S., Abbas, H., Ahmad, W., Ahmad, M., and Cohn, D.H. (1998) Nat. Genet. 20, 157–162.