STUDIES ON THE REGULATION OF THE HUMAN HEPATIC 5-AMINOLEVULINATE SYNTHASE GENE

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PUBLICATIONS
5-Aminolevulinate synthase (ALAS) is the first enzyme of the heme biosynthetic pathway in animals. Studies in this group have indicated that the rate of hepatic heme synthesis is controlled by feedback repression of ALAS transcription through heme, the end product of the pathway. This thesis is concerned with characterizing the promoter region of the human hepatic gene for ALAS with the aim of understanding the molecular basis of the negative regulation by heme. Briefly, the base sequence of the human hepatic cDNA was obtained and from this the predicted amino acid sequence of the precursor protein. The human hepatic ALAS gene was isolated from a human genomic cosmid library. The 5' flanking region of the gene was partially characterized and expression of the human ALAS gene promoter was examined in the human hepatoma cell line HepG2 and Chinese hamster ovary (CHO) cells. The levels of ALAS and P450IIA1 mRNA present in different human liver samples was examined by Northern analysis. Lastly the levels of erythropoietic and hepatic type ALAS in bone marrow samples from patients with sideroblastic anemia were investigated.

1. The base sequence of the cDNA for the human hepatic ALAS precursor protein was determined and from this the amino acid sequence was obtained. The amino acid sequence of human hepatic ALAS precursor protein was compared with ALAS sequences from eukaryotic and prokaryotic sources. The N-terminal signal sequences of the human, rat and chicken hepatic ALAS enzymes are highly conserved, particularly the first 42 amino acids. The processed mature mitochondrial ALAS proteins of the human, rat and chicken show strong sequence similarity except for a variable region of approximately 100 amino acids at the N-terminus. The yeast and bacterial ALAS enzymes lack the variable region and are similar to the conserved region of the higher eukaryotic enzyme.

2. The human hepatic ALAS gene was isolated from a genomic cosmid clone that had previously been isolated by Dr. M. J. Bawden. The 5' flanking region of the gene was identified and partially characterized by restriction enzyme mapping and sequence analysis. 1.3kb of the 5' flanking region of the gene was subsequently sequenced. The presence of an intron in the 5' flanking region of the gene was predicted from the sequence of the untranslated region of the cDNA. The presence of the intron was confirmed and the 3' intron-exon boundary was defined by RNAse protection analysis of human hepatic total RNA. The transcriptional start site of the gene was also determined by RNAse protection analysis of human hepatic total RNA. In contrast to the rat hepatic ALAS gene, the human gene was found to have one transcriptional start site. Computer analysis was used to identify putative regulatory consensus elements in both the intron and the 5' flanking region of the gene. The chromosomal localization of the human hepatic ALAS gene was determined by collaboration with Dr. G. Sutherland's group at the Adelaide Children's Hospital.

3. A series of chimeric promoter constructions were prepared containing increasing lengths of the 5' flanking region of the human ALAS gene fused to the human growth hormone reporter gene. Constructions were prepared which contained either all of the intron in the 5' untranslated region of the gene, or due to technical difficulties encountered when attempts were made to generate intronless constructions, the first 140bp of intronic sequence. The expression of the ALAS/hGH promoter constructions were examined by transient expression studies in the human hepatoma cell line HepG2 and CHO cells. In both cell lines the promoter constructions containing the human hepatic gene sequences up to -140bp from the transcription initiation site of the gene were sufficient to generate strong expression of hGH. However, the levels of expression generated by the promoter constructions containing all of the intron in the 5' untranslated region of the gene were considerably lower than the corresponding constructions containing only the first 140bp of the intron. The effect of heme, its precursor ALA and succinylacetone, on the expression of the human ALAS promoter constructions in both cell lines was investigated. The human hepatic ALAS promoter constructions were not responsive to treatment with any of these compounds. The expression generated by all six human hepatic ALAS promoter constructions was the same in treated and untreated cells.
The regulation of the endogenous ALAS gene in HepG2 cells was examined by Northern analysis and nuclear transcriptional run-on experiments. Northern analysis established that treatment of the cells with a final concentration of 10μM hemin or 100μM ALA strongly reduced the levels of ALAS mRNA. Treatment of the cells with a final concentration of 1mM succinyl acetone gave a two fold increase in mRNA levels. However, using nuclei isolated from HepG2 cells which had been treated with hemin, ALA, or succinyl acetone for time periods ranging from 2 to 48 hours, it was established that none of these compounds had any detectable effect on the transcriptional rate of the endogenous ALAS gene. Therefore the effects observed by Northern analysis must represent some form of post-transcriptional regulation perhaps resulting in changes in mRNA stability.

4. The levels of hepatic ALAS mRNA present in nine individual human liver samples were investigated using the human hepatic cDNA probe, and were found to vary up to four fold between individuals. Three of the patients had received dexamethasone therapy prior to organ donation, and the levels of the dexamethasone inducible cytochrome P450IIIA1 mRNA in the livers of these patients relative to the untreated patients was examined. It was established that the three dexamethasone treated patients all had elevated levels of both ALAS and P450IIIA1 mRNAs. However, the increases in the two mRNAs were not in parallel and in at least two of the patients it appeared likely that some other factor was contributing to the elevated levels of ALAS mRNA.

5. Recently it has been shown in this laboratory that a second gene for ALAS is expressed exclusively in human erythroid tissue. In collaboration with Prof. S. Bottomley, who kindly supplied the mRNA, the levels of erythroid and hepatic ALAS, α globin, β globin and glycophorin A mRNAs in the bone marrow of sideroblastic patients and healthy individuals, were determined by Northern blot analysis. Bone marrow RNA from a limited number of patients with congenital, acquired and X-linked sideroblastic anaemia was analysed. Decreased levels of erythroid ALAS mRNA were found only in the X-linked sideroblastic patients, and this represented the first indication at the molecular level that ALAS may be involved in the pathogenesis of this disorder. An interesting finding was the associated increased levels of the hepatic form of ALAS in these patients, which may represent de-repression of the gene in response to low cellular heme levels. Reduced levels of α and β globin mRNAs was also observed in these patients which may also be due to low cellular heme levels. Importantly, the levels of the erythroid specific mRNA for glycophorin A were not reduced in the X-linked patients, and this established that the observed decreases in erythroid ALAS, and α and β globin mRNA were specific.

The findings in the patients with acquired sideroblastic anaemia were less informative, and the normal levels of erythroid ALAS mRNA found in the bone marrow RNA of these patients, suggest that biochemical heterogeneity may underlie the disorder.