Human cysteine dioxygenase type I (CDO-I; EC 1.13.11.20): 5' flanking region and intron–exon structure of the gene

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Abstract

Aim—The elucidation of the structure of the 5' flanking region and the exonic organisation of the human cysteine dioxygenase type I gene.

Methods—Material for sequence studies was generated by polymerase chain reaction (PCR) methods using human genomic DNA, a cosmid clone containing the entire gene, and a commercial gene walking kit.

Results and Conclusions—The gene was found to be ~ 12 kb in length and made up of five exons (418, 78, 155, 170, and 731 base pairs, respectively). The immediate 5' flanking region did not contain the canonical TATAA box. One DNA source (Clontech promoter finder kit) contained a liver specific nuclear factor response element approximately 550 base pairs from the transcription start site, whereas a second source did not. This and other cis acting factors in the 5' flanking region were identified by computer analysis. The physiological significance of such elements requires detailed experimental evaluation.

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High levels of cysteine, due to reduced levels of cysteine dioxygenase (CDO), have been claimed to be present in the brain of a patient with Hallervorden-Spatz disease (HSD), a rare neurodegenerative condition.¹ Cysteine is thought to be an excitatory amino acid, acting via the NMDA receptor,² and an excess of it may cause excitotoxicity and thus contribute to the neuronal loss in HSD. The oxidation of cysteine by CDO is a one step reaction involving addition of molecular oxygen.³ CDO activity is expressed not only in the brain but also in the liver (the major site of expression)⁴ and the kidney,⁵ and data from expressed sequence tags suggest that it is expressed in numerous other tissues (GenBank numbers AA097105, AA166228, AA266612, AA016488, AA345878, AA347812, AA368632). A considerable amount of information is present in the literature concerning the properties of the entity or entities responsible for CDO activity, some of which is conflicting. These data were reviewed by Ho *et al.*⁶ Whether the differences reported represent real differences or are merely methodological arte-

facts is unclear. Only the enzyme mediating hepatic cytosolic activity (CDO-I) has been characterised in detail. Rat CDO-I is a single 200 residue polypeptide (molecular weight 23 kDa) binding one Fe²⁺ per molecule. Its primary sequence has been derived from cDNA.⁷ We have reported the complete sequence of the mRNA encoding human CDO-I,⁸ and shown that the human enzyme is encoded on chromosome 5q22–23, and the murine enzyme on chromosome 18, a region with considerable homology with human chromosome 5.⁹ Here, following a study using a combination of polymerase chain reaction (PCR) techniques and DNA base sequencing, we report: that the gene is in excess of 12 kb and contains five exons; the base sequence of ~ 70% of the gene and its adjacent 5' flanking region; and the results of computer analyses of 608 base pair of the 5' flanking region.

Material and methods

TEMPLATES FOR PCR AMPLIFICATIONS

Three sources of DNA to act as templates for PCR amplification were used: (a) genomic DNA (male caucasian) extracted from white blood cells by the method of Sykes¹⁰; (b) a clone from a cosmid library, identified as described previously⁸; and (c) a “promoter finder” library (Clontech, Cambridge Bioscience, Cambridge, UK).

PCR AMPLIFICATIONS

Using oligomeric primers (18–21 mers) synthesised by Alta Bioscience (Birmingham, UK) and designed with reference to the CDO-I cDNA reported previously,⁸ a series of PCR amplifications was carried out using genomic DNA as the template and a relatively short range amplification protocol to map exon–intron boundaries approximately. The amplification protocol was: denaturation at 99°C for five minutes, equilibrium at 72°C for five minutes, followed by addition of Taq polymerase, and 30 cycles of 95°C for 30 seconds, 55°C for 30 seconds, and 72°C for one minute.

Amplicons that were the same size as that anticipated if cDNA was the template, were considered as entirely exonic. Amplicons that were larger were considered as containing intronic material. If no amplicon was produced, it was considered likely that either the primer lay across an exon–intron boundary or the intron between the primers was of such a size as to prevent amplification with this protocol.

Potentially large introns were investigated by a long range PCR strategy¹¹ using cosmid DNA

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-608					CGACGGCC
-600	CGGGCTGGTA	AAAAATATGT	GTGTGTGTAT	ACTTAAATAA	ATATATATAT
-550	ATAAAGTCTT	GGACTTATTT	TCACCTAGCC	TGGGGCATAT	CGTGGATAGT
-500	TTCAATCGAC	AAAACAGGCA	CTCTCTGGCC	TAGGAGTGGA	ATCCATCCTC
-450	AATTTTTCGA	GGCTCCTCAC	AATTCTCTAT	TTGGAAGAAG	TGTCCCTCTC
-400	CTCCCTTTT	CTTTTCCTCC	TTTACTCAGC	GTCAGTCCCC	GCAGCCATCT
-350	CCTCCGACCC	TTTTTGTCTA	CGTCCCAGCG	TCGCGAACCA	CAGCGGCGGA
-300	GGTGGAGCGG	GGAGAGGCGT	TAGGCCGGGC	GGCTAAACCG	CGCCGTAAAA
-250	GTGGGGGAGA	GATTGCGCGG	AGCCCACGCG	ATCCCTGGGA	CGCCGGAGAC
-200	AACGGGGCTC	TTGGGAAGGC	GCGGAGCCCG	GGGAAGCCGG	GGATGTGCGC
-150	GTGAGCCGTG	CCCGCAGGGT	CTCCCCGCTT	CCGCCACCTT	TCTTGGGTGG
-100	CTCTCCGCCT	CGTCCTCCCT	CCGAGGGCCG	TTGGTACATT	CCTAGTGACT
-50	CCAAGCGCTT	AAAAGGGGCC	CGGGAGGGTG	AACCCACAG	ATCTGAACCT-1

Figure 2 Sequence of the 5' flanking region. The two underlined bases (−561 and −562) generate the HFH2/HNF3B site not seen in some templates.

transcription start site; and (b) the presence of AP1, c and v Myb, MZF1, NF B, Ikaross2, NKX2.5, and HFH2/HNF-3B elements. In addition, the MatInspector package identified potential AHRARNT, BARBIE, CREB, and Delta Efl elements, all of which may warrant further experimental exploration.

Using the sequence data obtained from the promoter finder template we devised primers to amplify the same region using the cosmid as template. One important difference was seen between the two sequences—the cosmid template had two bases (AA) absent (promoter finder, cttaaatAAatatat; cosmid, cttaatatatat). This deletion destroyed the HFH2/HNF-3B response element. Immediately 3' to this site are repeated AT units, which are absent in the rat sequence. These repeat units may also form a polymorphic site. The sequence of the 5' flanking region is shown in fig 2.

Discussion

The gene encoding human CDO at position 5q21-23 is ~ 12 kb in size and contains five exons. The human gene has a very similar exon-intron organisation to that of the rat gene, described by Tsuboyama *et al.*¹³ It lacks a canonical TATAA box in its 5' flanking region adjacent to the putative transcription start site although it, like the rat gene, possesses a related element (TTAAA).

Previous workers have shown that upregulation of the enzymic activity occurs in liver following in vivo administration of cysteine in the rat.¹⁴ How such regulation is effected is unclear as yet. It could indicate the presence of an as yet undescribed nuclear receptor for cysteine (so called orphan receptors for which no endogenous ligands are well recognised) or cysteine may alter the redox state within the cell and influence transcription via AP1 and NF-Kappa B, which respond to several factors including redox state.¹⁵ Both AP1 and NF-Kappa B were identified here. The presence of a hepatocyte specific response element in the human gene was thought initially to account for the fact that the liver expresses high levels of CDO activity. However, the absence of this element in a second template, and in sequence

data from Japanese workers (GenBank D85778S1) suggest that this may be a polymorphic site. Whether relatively high liver expression occurs independently of the presence or absence of the HFH2/HNF-3B site remains to be determined. Because all the regulatory elements have been identified by computer search only, further work is required to elucidate the physiological importance of these sites and to identify the factors interacting with them in the liver and other tissues.

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