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Genomic Sequence and Expression of a Cloned Human Carbonyl Reductase Gene with Daunorubicin Reductase Activity

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SUMMARY

Carbonyl reductase (NADPH:secondary-alcohol oxidoreductase; EC 1.1.1.184), a widely distributed NADPH-dependent enzyme considered as both an aldo-keto reductase and a quinone reductase, was cloned from a human liver genomic library and transiently expressed in COS7 cells. The gene contains 3142 bases comprising three exons and two introns. The absence of a CAAT and TATA box and the presence of a GC-rich island are characteristic of many "housekeeping" genes. Transient expression of the genomic gene in COS7 cells using an expression vector containing an SV40 origin of replication resulted in a >50-fold increase in both menadione reductase activity and daunorubicin reductase activity, suggesting that both activities are derived from the same enzyme. Carbonyl reductase mRNA levels reflected enzyme activity levels in the transfected cells. Other parameters, such as pH profile, cofactor requirements, substrates, and inhibitors, were similar to those of carbonyl reductase purified by other investigators. Potential regulatory elements with consensus sequences for two GC boxes and the transcriptional activator protein AP-2 were present upstream of the transcriptional start site. Although the precise role of carbonyl reductase is unknown, the enzyme is involved in drug metabolism and in the reduction of activated carbonyl compounds. Its ability to act as a quinone reductase also implies a potential to modulate oxygen free radicals.

Carbonyl reductase (NADPH:secondary-alcohol oxidoreductase; EC 1.1.1.184) belongs to a group of NADPH-dependent cytosolic enzymes called aldo-keto reductases (1, 2). The enzyme is ubiquitous in nature, catalyzing the reduction of a large number of biologically and pharmacologically active carbonyl compounds to their corresponding alcohols, and is reported to be able to provide an enzymatic basis of quinone detoxification in humans (2–5).

The role of carbonyl reductase in drug metabolism is well documented; however, the effects of the metabolic products on cell function are not understood (6). Carbonyl reductase reduces the methyl ketone in the carbon side chain of the antitumor antibiotic daunorubicin to its corresponding alcohol. The reduction of daunorubicin by carbonyl reductase changes the pharmacological properties of this drug and may affect drug toxicity and tumor killing. Differences in anthracycline drug metabolism in liver from several animal species have been reported (7). Human and rabbit liver display enhanced daunorubicin metabolism, the bulk of which is accounted for by carbonyl reductase with a pH optimum of 6.0, whereas rat and mouse liver lack the pH 6.0 activity.

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In addition to reducing aldo-keto side groups of several drugs, carbonyl reductase has been reported to act as a quinone reductase, accounting for most of the NADPH-reducing activity in human liver (5). There are conflicting reports on the metabolism of daunorubicin by human liver carbonyl reductase, suggesting that the aldo-keto reductase activity and the quinone reductase activity might be attributable to different proteins (5, 7). In this report, we show that carbonyl reductase cloned from human liver is an aldo-keto reductase and a quinone reductase, metabolizing daunorubicin as well as other natural and synthetic quinones.

Materials and Methods

Cloning. A carbonyl reductase genomic clone was isolated from a human fetal liver library that was screened with a ^{32}P-labeled cDNA probe (8). A 7000-bp *Eco*RI fragment containing the entire carbonyl reductase gene was subcloned into Bluescript vectors (Stratagene, San Diego, CA) by standard procedures (9).

DNA sequencing. Double-stranded DNA sequencing was performed by a modified Sanger (10, 11) procedure, with a Sequenase kit (United States Biochemical Corporation, Cleveland, OH), using ^{35}S-dATP (New England Nuclear, Boston, MA), and with an ABI model 373A fluorescent sequencer (Applied Biosystems Inc., Foster City, CA). Cyclic sequencing with *Thermus aquaticus* polymerase in a thermal cycler (Perkin-Elmer Cetus, Norwalk, CT) was used for genomic se-

ABBREVIATION: bp, base pairs.

quencing in the primer extension experiments. Both strands of DNA were sequenced using synthetic primers and overlapping clones.

Expression. The expression vector pBSV was constructed to allow amplification in COS7 cells that constitutively express the large T antigen. Large T antigen causes amplification of vectors with an SV40 origin of replication (12). The SV40 origin of replication was isolated from the vector pSV2gpt as a *Xho*I-*Hind*III fragment and cloned into the *Xho*I-*Hind*III site of the Bluescript vector (Stratagene). Genomic carbonyl reductase fragments were cloned into unique multicloning sites of the expression plasmid.

Transfection. Transfections were performed in COS7 cells (CRL 1651; American Type Culture Collection) grown in Dulbecco's minimal essential medium with 10% fetal calf serum. Transfection was performed with Lipofectin (Bethesda Research Labs, Bethesda, MD) according to the supplied protocol. Cells grown in 35-mm dishes were transfected with 3 μg of DNA. The DNA complex remained on the cells for 12 hr before addition of an equal volume of medium with 20% fetal calf serum. In some experiments, co-transfection with 1 μg of a β-galactosidase plasmid was used as a control to measure transfection efficiency (13). Cells were harvested after 72 hr.

Northern analysis. Total RNA was isolated with RNAZol (Cinna/Biotecx Laboratories, Friendswood, TX), denatured in formaldehyde/formamide, and separated in a 1.3% agarose-formaldehyde gel (14). The RNA was blotted to Genatran/45 nylon membranes (Plasco, Inc., Woburn, MA) and hybridized with a synthetic oligonucleotide spanning the first intron. Band intensity was measured with an AMBIS radioanalytical imaging system (AMBIS, San Diego, CA).

Enzyme assays. Cells were washed twice with phosphate-buffered saline, collected in 0.5 ml of 2 mM Tris buffer, pH 7.5, sonicated three times for 10 sec with a Branson 350 cell disruptor at a setting of 6, and centrifuged for 15 min at 14,000 × *g*. The supernatant was collected and assayed for activity. Carbonyl reductase activity (quinone reductase) was measured in 100 mM potassium phosphate buffer, pH 6.0, 200 μM NADPH, 1.5 mM cytochrome *c*, 200 μM menadione, with a Hitachi U-3110 spectrophotometer. Menadione was used as the quinone substrate for carbonyl reductase (5). Cytochrome *c* was used to reoxidize the menadiol formed. The reduction of cytochrome *c* was measured at 550 nm ($\epsilon = 18.5\ mM^{-1}\ cm^{-1}$) (15). Daunorubicin reductase was assayed using thin layer chromatography, according to the method of Ahmed (16). The reactions were performed at 37° in 0.5 ml of 100 mM sodium phosphate buffer, pH 6.0, containing 0.5 mM NADPH, 0.6 mM daunorubicin, and enzyme extract. Other conditions for detection of product and quantitation are described in the legends to Figs. 4 and 5. Inhibition of carbonyl reductase was tested with 20 μM rutin, a flavonoid known to inhibit the enzyme (4), and with 10 μM dicumarol, a potent inhibitor of DT-diaphorase (15). Inhibitors were added to the enzyme reaction mixture 5 min before the reaction was started with the addition of NADPH.

Primer extension. The transcriptional start site was located by hybridizing the complementary primer AACACCTGCGTGGAGAA-CAGACCTGGCTC (starting position, nucleotide 59) (Fig. 1) to 2 μg of poly(A) RNA extracted from the carbonyl reductase-expressing clones, and the primer was extended with murine reverse transcriptase (Superscript, Bethesda Research Labs), according to the procedure of Calzone *et al.* (17). Annealing temperatures were 30°, 35°, and 40° for 12 hr. One hundred units of murine reverse transcriptase were added to each tube and then incubated at 45° for 1 hr. Samples were treated with DNase-free RNase for 30 min at 37°, extracted with phenol, and analyzed on a 6% sequencing gel.

Results

Genomic clone. The carbonyl reductase clone was sequenced, and the data were compared with a previously cloned cDNA (8). The carbonyl reductase genomic clone contains three exons, of 289 bp, 106 bp, and 439 bp, and two introns, of 545 bp and 1388 bp (Fig. 1). The splice site for the first intron conforms to a consensus sequence in six of nine bases; the second intron splice site conforms to a complete consensus sequence. The 5′ noncoding region extends 93 bp upstream of the first exon, and the 3′ noncoding region extends 282 bp downstream of the translational stop codon, giving a total transcript length of 3142 bases. The gene does not have a TATA or CAAT box but does have a GC island (18) of approximately 300 base pairs that extends into the first exon. Potential regulatory elements are also shown in Fig. 1. There are two consensus GC boxes (Fig. 1, *double underline, line 2* at positions −62 and −73), which are recognized by the transcription factor SP1 (19). A consensus sequence for the transcriptional activator protein AP-2 (Fig. 1, *double underline, line 1* at position −163) is on the complementary strand upstream of the GC boxes (20).

Transcriptional start site. The transcription start site was determined by primer extension and is located 93 bp upstream of the translational start codon in COS7 cells expressing the cloned gene (Fig. 2, *arrow A*). A second minor transcript shown in Fig. 2 (*arrow B*) was also detected. However, this transcript is also present in the control and decreases >10-fold in transfectants expressing a >50-fold increase in carbonyl reductase transcripts (data not shown). This suggests that the carbonyl reductase transcripts are competing with nonspecific endogenous transcripts for the primer.

Carbonyl reductase expression. A 7000-bp *Eco*RI genomic fragment containing the entire carbonyl reductase gene plus an additional 3000–3500 bp upstream of the carbonyl reductase gene was cloned into the pBSV vector (see Materials and Methods) and transfected into COS7 cells. Carbonyl reductase activity measured with menadione as substrate was 30-fold greater than in the control COS7 cells (Fig. 3A, clone 26). Several other carbonyl reductase clones of different lengths and orientations were transfected into COS7 cells. Clone 4 is a *Nhe*I-*Eco*RI fragment, and clone 5 is a *Nhe*I-*Eco*RI fragment in the opposite orientation of clone 4. The *Nhe*I site is located 147 bp upstream of the transcriptional start site. Digestion with *Nhe*I removes >3000 bp of the 5′-flanking sequences from the *Eco*RI 7000-bp fragment. Clone 2 is a *Nhe*I-*Hind*III fragment that was filled in and blunt-end ligated into a filled-in *Xba*I-HindIII-digested pBSV vector. The *Hind*III restriction cut removes approximately 600 bp at the 3′ end of the *Eco*RI fragment, leaving an extra 37 bases downstream of the polyadenylation site. Expression of the *Nhe*I-*Eco*RI fragment (clones 4 and 5) in both orientations suggests that promotor activity is located within 147 bases of the transcriptional start site. This does not rule out other regulatory sequences futher upstream, which may be tissue specific or active only in human cells. Each clone displayed different levels of activity, which correlated with levels of carbonyl reductase mRNA within the cells (Fig. 3B). Carbonyl reductase mRNA was measured by hybridizing total RNA isolated from the transfected clones in a Northern blot with a ^{32}P-labeled oligonucleotide primer (20-mer) that spanned the first intron, thereby being specific for properly spliced carbonyl reductase message.

Sudan 1, an azo dye that induces enzymes involved in cancer and xenobiotic protection, was previously shown to induce carbonyl reductase in two mammalian cell lines (8). The azo dye was added at 10 μM to the transfectants, 24 hr before harvesting for enzymatic assays. The effects of sudan 1 treatment on carbonyl reductase activity or mRNA transcription in

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-184  gcctcggtcttcggcctgcgggttctgcaaagtcaggctagctggctctccgcctgctccgcaccccggcgaggt
-109  tccggtggggagggggtagggatggttcagccccgcccccgctagggcggggcctgcgcctgcgcgctcagcggccg
 -34  ggcgtgtaacccacgggtgcgcgcccacgaccgcCagactcgagcagtctctggaacacgctgcggggctcccgg
  42  gcctgagccaggtctgttctccacgcaggtgttccgcgcgccccgttcagccATGTCGTCCGGCATCCATGTAGC
 117  GCTGGTGACTGGAGGCAACAAGGGCATCGGCTTGGCCATCGTGCGCGACCTGTGCCGGCTGTTCTCGGGGGACGT
 192  GGTGCTCACGGCGCGGGACGTGACGCGGGGCCAGGCGGCCGTACAGCAGCTGCAGGCGGAGGGCCTGAGCCCGCG
 267  CTTCCACCAGCTGGACATCGACGATCTGCAGAGCATCCGCGCCCTGCGCGACTTCCTGCGCAAGGAGTACGGGGG
 342  CCTGGACGTGCTGGTCAACAACGCGGGCATCGCCTTCAAGGgtatggggaggggacgtggcctccccgaagaaga
 417  accgatgcactggggctcctggcgtctgcggggtccataacgcctccctagggaggagagggaggagctaggaga
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 792  gtaaccagatacagacttactttaggcagagggcactaagttttgttttttttttttttttttttagtatcat
 867  tgtatagaattttaccccatgggtacaatgtattaactatgttctctttctctcctaaaagTTGCTGATCCCACA
 942  CCCTTTCATATTCAAGCTGAAGTGACGATGAAAACAAATTTCTTTGGTACCCGAGATGTGTGCACAGAATTACTC
1017  CCTCTAATAAAACCCCAAGgtgagtctgatgggaaacagcgcaccttctctttggggcttgatttggcccctgct
1092  tgcctgggatttctcctgcaggctcctgcttcctctccatgctgcacgtgcactgacctctgtgctttttctcct
1167  gccagctgatatgtgccattttgcctcaggactttgtcctcacttctcccactcccatggccattcgtcctccct
1242  gtcgcactggtctttgcacacctggctgactctcatccttcaggtcttagctcaaacgtctcctgagagatgctc
1317  tttatttgctccattcccctccgcgtgggagtccatcctgtacccttctctgctctttcagagagatcttattc
1392  aggctggtggaggctttactaccaccagttcattaggcccttctaatacctgtcacctgtctgaaacactcctta
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1992  cctaagtaatgggacaggagatgaacccacccattaaataacacagcaattaagcagccacttttagaaaaattt
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2292  ctcttagaactcataccagtaactgtctctcatatgaaaatttttctgctccaaaatccctgcaaatttggcattc
2367  acctctctacgggattgttgcacacctttctacataatgctttgtggtgtatcttagGGAGAGTGGTGAACGTAT
2442  CTAGCATCATGAGCGTCAGAGCCCTTAAAAGCTGCAGCCCAGAGCTGCAGCAGAAGTTCCGCAGTGAGACCATCA
2517  CTGAGGAGGAGCTGGTGGGGCTCATGAACAAGTTTGTGGAGGATACAAAGAAGGGAGTGCACCAGAAGGAGGGCT
2592  GGCCCAGCAGCGCATACGGGGTGACGAAGATTGGCGTCACCGTTCTGTCCAGGATCCACGCCAGGAAACTGAGTG
2667  AGCAGAGGAAAGGGGACAAGATCCTCCTGAATGCCTGCTGCCCAGGGTGGGTGAGAACTGACATGGCGGGACCCA
2742  AGGCCACCAAGAGCCCAGAAGAAGGTGCAGAGACCCCTGTGTACTTGGCCCTTTTGCCCCCAGATGCTGAGGGTC
2817  CCCATGGACAATTTGTTTCAGAGAAGAGAGTTGAACAGTGGTGAgctgggctcacagctccatccatgggcccca
2892  ttttgtaccttgtcctgagttggtccaaagggcatttacaatgtcataaatatccttatataagaaaaaaaatga
2967  tctcttatcaattagcactcactaatgtactactaattgagcaacctacgcactcagttgactacgtaaatctgt
3042  caggtcttttgtgatttcctctgatgcaggagaggaaaaattgtaattgatgaaaataatgaatgaaaatcaaca
3117  gatgaataaatggttctttataagtg
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Fig. 1. Sequence of the genomic human carbonyl reductase gene. Position 1 (*C, bold underline, line 3*) is the transcriptional start site. The exons coding for the protein are shown in *capital letters*. The protein sequence has been reported previously (8, 24). Two introns of 545 bp and 1388 bp are shown between the exons. The putative polyadenylation signal is *underlined*, 16 bp before the polyadenylation site. Potential consensus regulatory sites are shown upstream of the transcriptional start site. GC boxes, *double underline, line 2* at positions −62 and −73; AP-2 consensus sequence, *double underline, line 1* at position −163.

this system were minimal (Fig. 3, S clones). Lack of induction may result from the interaction of SV40 large T antigen with transcriptional activator proteins or from other changes within the cellular environment of the expression system (see Discussion).

Daunorubicin reductase activity. Daunorubicin reductase activity was measured using thin layer chromatography to separate the substrate, daunorubicin, from the product, daunorubicinol. All of the clones expressing menadione (quinone) reductase activity (Fig. 3) also express daunorubicin reductase activity, as shown in Fig. 4.

The cell extracts from the clones expressing carbonyl reductase activity were combined, in order to obtain an enzyme extract with a constant amount of activity to measure all of the following enzyme parameters. A kinetic study of daunorubicin reductase activity in the combined clone extracts demonstrated an activity 50–100-fold greater than the control (Fig. 5A).

The cloned carbonyl reductase was active over a broad pH range, with an optimum at pH 6.0 in 100 mM phosphate buffer. Activity was reduced by 27% at pH 7 and by 86% at pH 8 (data not shown). Carbonyl reductase used NADPH as the major cofactor; NADH was used with 7% efficiency (Fig. 5B). The enzyme was inhibited 53% and 90% by 10 μM dicumarol and 20 μM rutin, respectively (Fig. 5C). Rutin is a known inhibitor of carbonyl reductase (4). Dicumarol is often used to selectively inhibit the quinone reductase DT-diaphorase. The cross-inhibition between carbonyl reductase shown in this report suggests that caution must be used when analyzing inhibitor data. This is especially true when menadione, a substrate for both carbonyl reductase and DT-diaphorase, is used. The dicumarol inhibition shown in Fig. 5C is not due to endogenous DT-diaphorase, because COS7 cells have negligible DT-diaphorase activity (21). The pH profile, inhibitor data, and cofactor requirements for the cloned enzyme were similar to data previously reported for the purified enzyme (2, 4).

Two other quinones were tested as substrates. Plumbagin, a natural toxic quinone, was reduced at one third the rate of menadione, whereas 9,10-phenanthrenequinone, a quinone present in air pollution from diesel exhaust (22), was reduced at 3 times the rate of menadione (data not shown). Plumbagin can be metabolically activated to a mutagen with an S9 rat liver extract (23), whereas the mutagenic potential of 9,10-phenanthrenequinone is inhibited with an S9 rat liver extract (22). The metabolic role of carbonyl reductase in the mutagenic and toxic effects of these two quinone substrates remains to be determined.

Carbonyl reductase activity with menadione as substrate had an apparent K_m of 15 μM and a V_{max} of 185 nmol/min/mg (Fig. 6).

Discussion

The structure and DNA sequence of the human carbonyl reductase gene were determined, and exons corresponding to previously sequenced cDNA clones were identified (8, 24). The lack of a consensus TATA and CAAT box and the presence of a GC-rich 5′ flanking region are characteristics of other known "housekeeping" genes (25, 26). The gene was also verified by measurement of enzymatic activity, demonstrating both carbonyl reductase and daunorubicin reductase activity in COS7 cells transiently expressing the gene product.

The role of carbonyl reductase in humans is at present unknown. However, its wide distribution in nature (2), along

Fig. 2. Transcriptional start site analyses. The transcriptional start site was determined by primer extension with purified poly(A) mRNA from transfected clones. *Lanes 1*, *2*, and *3*, primer extension product from 2 μg of poly(A) RNA from the transfected clones. *Lane 4*, primer extension product from 1.4 μg of poly(A) RNA from COS7 cells transfected with a β-galactosidase plasmid, used as control. The annealing temperatures in *lanes 1*, *2*, *3*, and *4* were 30°, 35°, 40°, and 40°, respectively. Sequence data shown in *lanes ACGT* were obtained by cycle sequencing using the same primer used for primer extension. The sequence reads the strand complementary to the mRNA.

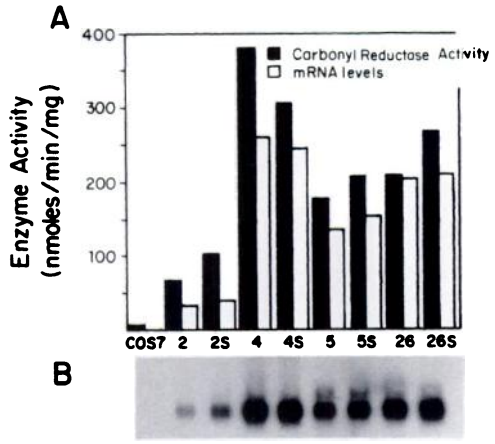

Fig. 3. Analysis of carbonyl reductase expression and mRNA levels in transfected COS7 cells. A, ■, Cell extracts were analyzed for carbonyl reductase activity using menadione as substrate. □, Properly spliced carbonyl reductase mRNA levels determined by radio-imaging analysis of the Northern blot shown in B. B, Northern analysis of total RNA isolated from transfected clones. The Northern blot was hybridized with an oligonucleotide probe spanning the first intron complementary to mRNA.

with the characteristic GC-rich island common to many housekeeping genes (18) and the abundance of potential intercellular carbonyl substrates, imply that carbonyl reductase is involved in normal cellular metabolism. The metabolism of activated carbonyl groups, including quinones, suggests a possible role in the detoxification of common cellular metabolites or other natural or xenobiotic compounds endogenous to the environ-

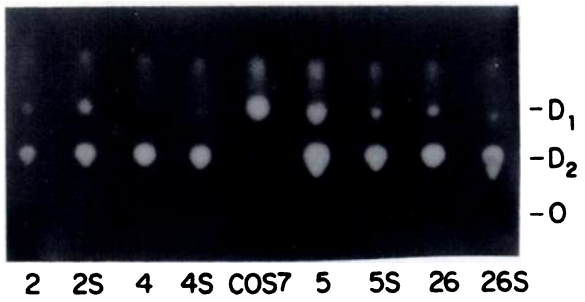

Fig. 4. Daunorubicin reductase activity. Daunorubicin reductase activity was measured on the same clones shown in Fig. 3, according to published procedures (16). Final concentration of daunorubicin was 0.6 mM. The reaction proceeded for 2 hr at 37°. Products were extracted with isopropyl alcohol and analyzed by thin layer chromatography. *O*, origin; D_2, product, daunorubicinol; D_1, substrate, daunorubicin.

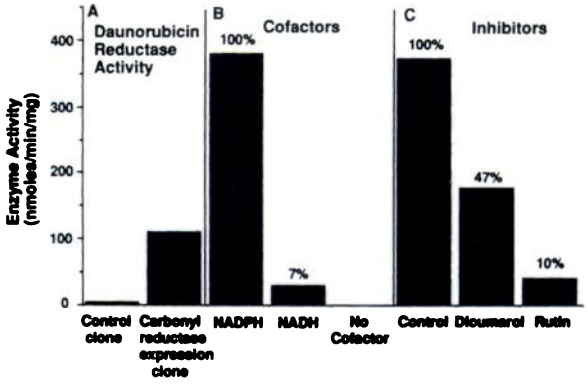

Fig. 5. Quantitation of daunorubicin reductase activity, cofactor, and inhibitor data. Samples were assayed as described in Materials and Methods. For daunorubicin reductase activity, aliquots were taken at 30-min intervals and analyzed by thin layer chromatography. Daunorubicin and daunorubicnol were separated with $CHCl_3/CH_3OH/H_2O$ (80:40:3, v/v/v) on Whatman PE SIL G silica gel plates. The spots were scraped from the plates, and products were eluted with 2 ml of 6.54 N H_2SO_4 in 95% ethanol and quantitated on a Perkin-Elmer spectrofluorimeter (excitation, 470 nm; emission, 585 nm). Daunorubicin was used as a standard. A, Daunorubicin reductase activity for the COS7 control and for the transfectants. B, Cofactor specificity of carbonyl reductase activity measured with menadione as substrate. Reactions contained 200 μM NADPH, 200 μM NADH, or no cofactor, as indicated. C, Inhibitor studies with menadione as the substrate. Dicumarol (10 μM) and rutin (20 μM) were added as described in Materials and Methods.

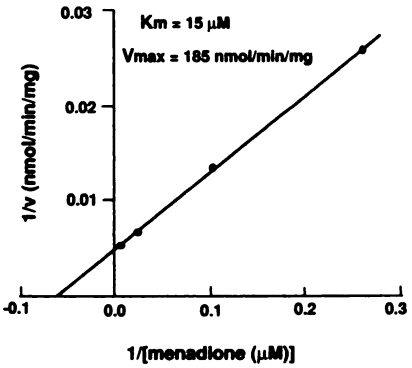

Fig. 6. Lineweaver-Burk plot of menadione reduction by COS7 cell extracts expressing the carbonyl reductase gene, at pH 6.0. Reactions conditions were as described in Materials and Methods.

ment. Wermuth *et al.* (5) reported that carbonyl reductase accounted for 50–70% of the NADPH-quinone-reducing activity in human liver and could provide the enzymatic basis for detoxification of quinones in humans.

We have shown previously that carbonyl reductase is induced approximately 2–4-fold by compounds that induce other cancer-protective enzymes (8). The identification of three consensus regulatory elements, two GC boxes that bind the transcription factor SP1 and an enhancer element that binds the transcriptional activator protein AP-2, suggests possible sites for regulation. The lack of induction with sudan 1 in this report may be due to the already high levels of carbonyl reductase generated by vector amplification in the COS7 cells. In addition, COS7 cells constitutively synthesize large amounts of SV40 large T antigen, which has been shown to inhibit the transcriptional activator protein AP-2, resulting in a decrease in mRNA transcription (20). If AP-2 is involved in transcriptional regulation, one would expect interference of transcriptional activation in COS7 cells by large T antigen. However, the mechanism and regulatory elements are not known at this time.

The role of carbonyl reductase in the formation of oxygen free radicals needs to be examined, in light of its ability to reduce quinones. One-electron reduction of quinones generates semiquinone free radicals, which can undergo reoxidation in the presence of oxygen to generate oxygen free radicals. All of the free radical species are potentially toxic and capable of causing damage to cell structures and their molecular constituents. A role exists for carbonyl reductase to protect cells against free radical damage by reducing quinones to hydroquinones, bypassing the free radical state and allowing conjugation and removal of the hydroquinone conjugate, similar to the well studied cancer-protective enzyme DT-diaphorase. This protective role has been proposed by Wermuth *et al.* (5) and remains to be proven. Chesis *et al.* (22) have suggested that the metabolism of 9,10-phenanthrenequinone by the one-electron-reducing enzyme cytochrome P-450 reductase generates oxygen free radicals that are mutagenic. The metabolism of 9,10-phenanthrenequinone by carbonyl reductase described in this report needs to be examined further, to determine whether carbonyl reductase activates or protects against the formation of mutagenic oxygen free radicals. The ability to express carbonyl reductase will allow us to determine its role in free radical protection in any cellular environment.

Initial reports of human carbonyl reductase with daunorubicin reductase activity were conflicting. Carbonyl reductase isolated by Ahmed *et al.* (7) could metabolize daunorubicin, whereas the enzyme isolated by Wermuth *et al.* (5) could not. We have shown recently (8) that human carbonyl reductase described by Ahmed *et al.* (7) is coded for by a single gene and that the cDNA is the same as the placental cDNA described by Wermuth *et al.* (24). Therefore, the two enzymes appear to be the same. The detection of daunorubicin reductase activity in this report suggests that the human enzyme contains both activities. Thus, the human enzyme appears to have two distinct catalytic functions, aliphatic aldo-keto reduction and quinone reduction.

Anthracyclines belong to a class of widely used and effective cancer chemotheraputic antibiotics. A major drawback of anthracycline anticancer treatment is the cardiotoxic side effects associated with drug therapy. Bachur (1) demonstrated that the anthracyclines daunorubicin and doxorubicin were substrates for human liver carbonyl reductase. The enzyme reduced the keto side group of the anticancer antibiotic doxorubicin, converting it to the alcohol doxorubicinol. Doxorubicinol has been reported to be up to 30 times more cardiotoxic than the parent compound doxorubicin, whereas the parent compound was a more effective tumor-killing agent (27). The expression of carbonyl reductase will allow us to examine the *in vivo* role of these drugs and their metabolites in various cells.

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