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DEDUCED AMINO ACID SEQUENCE OF MATURE CHICKEN TESTIS FERREDOXIN

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The cDNA sequence encoding the complete mature form of the steroidogenic ferredoxin from chicken testis has been determined and the amino acid sequence deduced therefrom has been compared with the sequences of bovine, human and porcine steroidogenic ferredoxins. The chicken sequence is between 84% and 88% identical with those of the other mitochondrial iron-sulfur proteins. Thus, the amino acid structure of steroidogenic ferredoxins which transfer electrons to mitochondrial forms of cytochrome P-450 has been very highly conserved over evolutionary time.

Adrenal ferredoxin (adrenodoxin) is an iron-sulfur protein which is a component of the two steroidogenic enzyme complexes in adrenocortical mitochondrial, 11β-hydroxylase and cholesterol side chain cleavage (1,2). While the 11β-hydroxylase system is expressed only in the adrenal cortex, the cholesterol side chain cleavage complex is expressed in testicular Leydig cells (3), ovarian follicles and corpus luteum (4,5), placenta (6) and brain (7). It is generally believed that the same iron-sulfur protein is present in each of these tissues although early workers used names such as testodoxin to identify the protein in Leydig cells. Recently the primary structure of the steroidogenic ferredoxin from human adrenal and placenta have been deduced from cDNA sequencing and found to be very similar or identical (8,9). Thus it would seem that a single gene encoding this protein is expressed in different tissues. We have now determined the cDNA sequence of the steroidogenic ferredoxin from a chicken testis cDNA library. The amino acid sequence of

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mature chicken testis ferredoxin is very similar to that of bovine adrenodoxin (10) as well as those of the two human ferredoxins (8,9). The results provide further evidence for a common ferredoxin protein being present in all steroidogenic tissues and indicate that the amino acid sequence of this iron-sulfur protein has been very highly conserved over evolutionary time.

Materials and Methods

A chicken testis cDNA library in λgt11 was obtained from Dr. Michael Groudine, Fred Hutchinson Cancer Research Center, Seattle, WA. The library was plated and 180,000 plaques (30,000/15 cm plate) were screened using procedures described in Maniatis et al. (11). Screening was carried out using the Bam HI fragment of the bovine adrenodoxin cDNA clone, pCD Adx 4 (10). This insert contains the complete coding sequence of the bovine adrenoxin precursor protein and was radiolabeled with ^{32}P by nick translation prior to use in screening. Upon rescreening of positive clones, 6 clones were determined to be true positives and their sequences were determined by the dideoxynucleotide sequencing procedure (12). The inserts were removed from lambda phage by digestion with Eco RI and inserted into pUC 19. In some cases the inserts were then subcloned into the same vector using Bgl II, Xba I and Hind III sites indicated in Fig. 1. Sequencing was then carried out using universal and reverse primers as well as the two primers indicated in Fig. 1.

Results and Discussion

The composite sequence of chicken ferredoxin obtained from these clones is presented in Fig. 1. As with most mitochondrial proteins encoded on nuclear genes, the steroidogenic ferredoxin is synthesized as a higher molecular weight precursor which is proteolytically processed upon insertion into mitochondria (10,13). The arrow in Fig. 1 indicates the corresponding site of cleavage of the precursor segment from bovine adrenodoxin (10,14) and human placental ferredoxin (9,15), yielding the mature ferredoxin proteins. Consequently, Fig. 1 presents the complete mature sequence of chicken testis ferredoxin as well as a portion of the precursor sequence. The precursor sequence of bovine adrenodoxin is 58 amino acids long (10) while that of human placental ferredoxin is 60 amino acids long (9). From the clones identified in this library, we were able to determine with certainty (from more than one clone) the sequence of the C-terminal 19 amino acids of the chicken ferredoxin precursor, perhaps only one-third of the precursor sequence. Comparisons of the 19 C-terminal amino acids of the chicken, bovine and human ferredoxins

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5'  TGC AGC GCC GTG GCC GTG AGG ACG TTG CGG CCG CTC AGC
    Cys Ser Ala Val Ala Val Arg Thr Leu Arg Pro Leu Ser

    CTC TCG GCT CGG GCG GCG TGC AGC TCA GAA GAT AAA ATA
    Leu Ser Ala Arg Ala Ala Cys Ser Ser Glu Asp Lys Ile

    ACT GTT CAT TTC ATA AAC CGC GAT GGT GAT AAG CTA ACA
    Thr Val His Phe Ile Asn Arg Asp Gly Asp Lys Leu Thr

    GCC AAA GGA AAA CCT GGA GAC TCA TTG CTG GAT GTT GTT
    Ala Lys Gly Lys Pro Gly Asp Ser Leu Leu Asp Val Val
                          1
    GTT GAA AAT AAT CTA GAC ATA GAT GGT TTT GGT GCA TGT
    Val Glu Asn Asn Leu Asp Ile Asp Gly Phe Gly Ala Cys

    GAA GGG ACG TTA GCC TGT TCA ACT TGT CAT TTA ATC TTT
    Glu Gly Thr Leu Ala Cys Ser Thr Cys His Leu Ile Phe

    GAA GAC CAC ATA TTT GAG AAA CTA GAT GCA ATT ACT GAT
    Glu Asp His Ile Phe Glu Lys Leu Asp Ala Ile Thr Asp

    GAA GAA ATG GAC ATG CTA GAC CTG GCA TAT GGT CTC ACA
    Glu Glu Met Asp Met Leu Asp Leu Ala Tyr Gly Leu Thr
                                      2
    GAA ACA TCA CGT TTA GGC TGC CAG ATC TGC TTG AAG AAG
    Glu Thr Ser Arg Leu Gly Cys Gln Ile Cys Leu Lys Lys

    TCA ATG GAC AAT ATG ACT GTT CGA GTA CCA GAA GCT GTT
    Ser Met Asp Asn Met Thr Val Arg Val Pro Glu Ala Val

    GCT GAT GCT AGA CAA TCA GTA GAT TTG AGT AAA AAC TCC
    Ala Asp Ala Arg Gln Ser Val Asp Leu Ser Lys Asn Ser
                                        3
    TAA aagaaaatatcgtaggagttttaaagaagcttaattatttttataac
    END

    ttatttttaaatgtgtaattaaatatggaaactt(a)n   3'
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Fig. 1 - Composite DNA sequence and deduced amino acid sequence of ferredoxin from a chicken testis cDNA library. The arrow indicates the putative cleavage site for the mitochondrial precursor sequence based on results obtained for the bovine and human proteins. Therefore, the sequence downstream from the arrow is the complete mature coding sequence of chicken ferredoxin as well as the 3'-untranslated sequence (the underlined sequence being a putative poly A addition site). Only the C-terminal portion of the precursor sequence is shown upstream from the arrow because the cDNA library used in this study was not found to contain a full-length ferredoxin clone. The two overlined sequences represent synthetic oligonucleotides utilized in sequence analysis and restriction sites used for subcloning are indicated; 1 = Xba I, 2 = Bgl 11, 3 = Hind III.

(Fig. 2A) show the following sequence identities: chicken/human 7/19, chicken/bovine 6/19, and bovine/human 12/19. It is noted that the positions of sequence identity (particularly those between chicken and bovine or human) tend to be clustered toward the precursor cleavage site (C-terminal end) and include two arginines. A preponderance of basic amino acids are the one common sequence feature of mitochondrial precursors (16).

On the other hand, when the sequence of mature chicken ferredoxin from Fig. 1 is compared with those of the mature bovine, human, or porcine ferredoxins, a much higher degree of identity is observed. As illustrated in

A)

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Chicken: CSAVAVRTLRPLSLSARAA CSS
Human:   GPGGSAEAS-S--V----R S--
Bovine:  GLGGGAVAT-T--V-G--Q S--
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B)

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                      20                  40                  60
C: CSSEDKITVHFINRDGDKLTAKGKPGDSLLDVVVENNLDIDGFGACEGTLACSTCHLIFEDHIFEKLDAI
B: S---------------ET--T---I---------Q------------------------Q------E--
H: S---------------ET--T---V-----------------------------------Y------
P: S---------------KT--TQ--V--------I--------------------------E--

          80                  100                 120
C: TDEEMDMLDLAYGLTETSRLGCQICLKKSMDNMTVRVPEAVADARQSVDLSKNS
B: ----N----------DR---------T-A---------D--S---E-I-MGM--SKIE
H: ----N----------DR---------T------------T-------I-VG-T-
P: ----N----------DR---------T-A---------------E-
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Fig. 2 - (A) Comparison of the amino acid sequence of the C-terminal 19 amino acids of chicken, human and bovine adrenodoxin precursor segments. The arrow indicates the site of cleavage of both the human (15) and bovine (14) precursors segments. Dashes indicate amino acid identity with the chicken sequence. (B) Comparison of amino acid sequences of chicken (C), bovine (B), human (H), and porcine (P) ferredoxins. The dashes indicate amino acid identity with the chicken sequence. The porcine sequence is only 117 amino acids long, but it should be remembered that bovine adrenodoxin was found to be 14 amino acids shorter by protein sequencing than by cDNA sequencing (10). The human sequence is that reported by Mittal et al. (9).

Fig. 2B, the following percentages of identity are found: chicken/bovine = 104/124 (84%); chicken/human = 109/124 = (88%); chicken/porcine = 103/117 (88%). The porcine adrenodoxin sequence was determined by protein sequencing (17). Several of the differences in sequence between the various species are clustered near the C-terminus. The obvious conclusion from this comparison is that the mature sequence of the steroidogenic ferredoxin is highly conserved, much more so than the precursor sequence (9).

Underlined in Fig. 1 is the poly A addition site of chicken ferredoxin determined by sequence analysis. Bovine (18) and human (8) adrenodoxin utilize at least 3 such sites and the one identified in Fig. 1 corresponds to the site in the bovine and human genes which leads to the production of the shortest ferredoxin mRNA. As expected, no significant sequence homology is observed between the chicken, bovine and human 3'-untranslated sequences.

In conclusion, it is evident that the mature sequence of steroidogenic ferredoxin is highly conserved between species. Evolutionary divergence between birds and mammals occurred during the Jurassic period which began more

than 180 million years ago (19). Obviously the sequence of this ferredoxin is highly efficient for its function of electron transport from a flavoprotein reductase to P-450$_{11\beta}$ or to P-450$_{scc}$ localized in the mitochondrial membrane and consequently has been highly conserved over a long period of evolutionary time.

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