Eur. J. Biochem. *169*, 449–455 (1987)

Isolation of a cDNA for adrenodoxin reductase (ferredoxin–$NADP^+$ reductase)

Implications for mitochondrial cytochrome *P*-450 systems

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(Received June 22, 1987) – EJB 87 0709

Using specific antibodies against adrenodoxin reductase (AR), we screened λ gt11 cDNA expression libraries constructed from bovine adrenal cortex mRNA, and isolated several putative clones coding for this enzyme. Concurrently we determined the amino acid sequences of fragments from it. A deoxyinosine-containing oligonucleotide probe, generated for one of the sequences, reacted specifically with one of the cloned cDNAs of about 1600 base pairs. The codon sequence of this cDNA matched the peptide sequences, further confirming its identity as a copy of AR mRNA. RNA blot analysis indicates that in the adrenal cortex and corpus luteum there is only one major mRNA (≈ 2000 bp) for AR. The levels of this mRNA are at least 40-fold lower in the liver and kidney which are also known to contain in homologue of AR. As compared to adrenodoxin and cytochrome *P*-450_{scc} mRNAs, AR mRNA levels in the adrenal cortex appear to be about 10-fold lower. Southern blot analysis of bovine and human genomic DNAs reveals that in both of these species there is only one gene for AR. These results indicate that only a single reductase serves the different mitochondrial *P*-450 systems in steroidogenic tissues.

The mitochondrial cytochrome *P*-450 systems catalyze a number of physiologically important reactions: (a) the conversion of cholesterol to pregnenolone, which is the first and rate-limiting step in the biosynthesis of steroid hormones in all steroidogenic tissues [1–7]; (b) 11β-hydroxylation of steroids for the biosynthesis of glucocorticoids and mineralocorticoids in the adrenal cortex [4, 5, 8]; (c) 26-hydroxylation required for bile acid biosynthesis in the liver [9–12]; (d) 1α-hydroxylation required for vitamin D biosynthesis in the kidney [13–15].

Each of these cytochrome *P*-450 systems is composed of three enzymes which constitute an electron transfer chain located on the matrix side of the inner mitochondrial membrane [16, 17]. A ferredoxin–$NADP^+$ reductase is the first enzyme in this electron shuttle system, and its function is to transfer electrons from NADPH to a specific ferredoxin, which in turn transfers them one at a time to the cytochrome *P*-450 [2, 6, 18]: NADPH → ferredoxin reductase → ferredoxin → cytochrome *P*-450. The substrate specificity of this system is determined by the cytochrome *P*-450 component, which is known to be different for the different reactions noted above. Although the first two proteins of the systems from different tissues show certain biochemical and immunological similarities, there is evidence that these may also differ from one tissue to another [12, 13]. Hence, ferredoxin from the adrenal cortex is called as adrenodoxin and that from the liver as hepatoredoxin.

In the adrenal cortex, corpus luteum, and testis the synthesis of all three enzymes of this system is dependent on trophic hormone stimulation of the tissue [19–23]. The hormonal induction of these enzymes is especially dramatic in the corpus luteum, wherein the concentrations of these enzymes can increase over 50-fold compared to the levels found in the ovary [21, 24]. Yet, despite the large fluctuations in the levels of these enzymes in the corpus luteum, their relative molar ratios remain nearly constant, indicating tight coordinate regulation of all three enzymes [24].

After the recent isolation of cloned cDNAs for adrenodoxin, cytochrome *P*-450_{scc} and cytochrome *P*-$450_{11\beta}$ [25–29], adrenodoxin reductase (AR) remained as the only enzyme of these mitochondrial *P*-450 systems the cDNA of which had not yet been cloned. Thus, because of our interest in understanding the mechanisms of induction and coordinate regulation of these enzymes, we undertook the cloning of AR mRNA. As no protein sequence was available for adrenodoxin reductase, we generated antibodies against AR and screened adrenal cortex cDNA expression libraries using these antibodies. Concomitantly we also determined the amino sequences of tryptic fragments of AR to confirm the identity of the antibody selected cDNAs.

MATERIALS AND METHODS

Adrenodoxin reductase was purified from bovine adrenal cortex to the same purity levels as in our previous reports [6, 7, 30]. For amino acid sequence determinations purified AR was digested with trypsin, and HPLC-purified fragments were sequenced by manual Edman degradation as previously described [31]. The amino terminal of the protein proved to be unblocked and its sequence was determined directly. Oligodeoxynucleotides matching the sequence of a peptide fragment of AR were synthesized by Dr Boaz Amit (Biotechnology General, Rehovot, Israel).

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Abbreviations. AR, adrenodoxin reductase; cytochrome *P*-450_{scc}, cytochrome *P*-450 specific for cholesterol-side-chain cleavage.

Enzymes. Adrenodoxin reductase, ferredoxin–$NADP^+$ reductase (EC 1.18.1.2); cytochrome *P*-450_{scc} (EC 1.14.15.6).

The tissues used for RNA isolation were obtained from cows within 20 min after slaughter, and frozen in liquid nitrogen. The tissues were then ground to powder in liquid nitrogen, and polysomal RNA was isolated by Mg^{2+} precipitation [32]. Poly(A)-rich RNA was isolated by chromatography on oligo(dT)-cellulose (type 3, Collaborative Research). From the same batch of poly(A)-rich RNA two cDNA libraries were generated: the first one was prepared with cDNA sized above 500 bp on a Bio-Gel A-5M column as previously described [33], the second one was prepared with cDNA sized above 1000 bp by Stratagene. In both cases the double-stranded DNA was ligated to *Eco*RI-cut λ gt11 using *Eco*RI linkers.

The antibodies used for screening the cDNA libraries were generated in rabbits as described recently [24]. The libraries were screened with antisera at a 1:500 dilution, using Protoblot kits and protocols (Promega Biotec) for detection of the bound antibody. The Protoblot method using second-antibody alkaline-phosphatase conjugates proved to be about 20 times more sensitive than protein-A–horseradish-peroxidase and ^{125}I-protein A immunodetection methods, as judged by reaction with Western blots of purified AR. The positive plaques were purified by rescreening with the antibody.

All three of the positive clones were examined by plaque-hybridization with the 5′-end-labelled oligodeoxynucleotide probe (using [γ-^{32}P]ATP and T4 polynucleotide kinase). The phage DNAs were adsorbed on nitrocellulose filters [34]. The filters were then placed in 6 × SSC (SSC = 0.15 M NaCl, 15 mM sodium citrate, pH 7.0), 5 × Denhardt's solution (Denhardt's solution = 0.02% polyvinylpyrrolidone, 0.02% Ficoll, 0.02% bovine serum albumin), 0.5% SDS, 100 μg/ml sonicated denatured salmon sperm DNA. After 2 h at 42 °C, the labelled oligonucleotide probe was added to the same solution and hybridization carried out for 12 h at 42 °C. The blot was then washed four times with 6 × SSC, 0.1% SDS at 42 °C for 30 min and once in the same buffer at 47 °C for 2 min, and autoradiographed.

The phage DNA from antibody-positive plaques was purified from plate lysates using a DEAE-cellulose column procedure [35]. The cDNA inserts were isolated from agarose gels after *Eco*RI digestion of the phage DNA, and ligated to *Eco*RI-cut pIBI76 plasmid (IBI). After transformation into *Escherichia coli* JM101, the white colonies growing on LB plates containing ampicillin and spread with isopropyl β-D-thiogalactopyranoside and 5-bromo-4-chloro-3-indolyl-β-D-galactoside were selected and further examined by mini-preparations of plasmid DNA [34]. For large-scale isolation of plasmid DNA, we used a recently described method [36], but the initial lysis of the bacteria was carried out in 8% sucrose, 0.5% Triton X-100, 50 mM EDTA, pH 8.0, 10 mM Tris, pH 8.0 [34, 37] instead of the buffer recommended in the procedure since the latter invariably resulted in chromosomal DNA contamination of the plasmid preparation.

For Southern blot analysis shown in Fig. 3B, the plasmid DNA in agarose gel was electro-transferred to GeneScreen (New England Nuclear) according to instructions in the GeneScreen manual. After transfer, the filter was briefly washed in fresh transfer buffer and exposed to ultraviolet light for 2 min, exactly as described [38], to fix the DNA on the filter. The blot was then hybridized with the 5′-end-labelled oligonucleotide probe as described above for plaque hybridization.

For Northern blot analysis, poly(A)-rich RNA was run on 1.2% agarose gel with 2.2 M formaldehyde and electro-transferred to GeneScreen and fixed on it by ultraviolet irradiation as noted above. The blot was then placed in 50% formamide, 5 × SSC, 5 × Denhardt's solution, 1% SDS, 100 μg/ml sonicated denatured salmon sperm DNA, 10 μg/ml poly(A). After 3 h at 42 °C, the radiolabelled probe was added to the same solution and hybridization carried out for 17 h at 42 °C. The blot was washed as previously described [39], and autoradiographed.

Plaque-hybridization analysis of the two cDNA libraries with AR, adrenodoxin and P-450$_{scc}$ cDNAs was carried out as previously described [34]. The cDNAs for bovine adrenodoxin and P-450$_{scc}$ were isolated from plasmid preparations of *E. coli* cultures [25, 26] generously provided by Dr Michael R. Waterman (University of Texas Health Science Center, Dallas, Texas). The β-actin cDNA was isolated from a human cDNA library [40].

Total genomic DNA was isolated from bovine adrenal cortex after digestion with 200 μg/ml proteinase K (Sigma) in 50 mM Tris, pH 8.0, 100 mM EDTA, 0.5% SDS, at 55 °C for 16 h, followed by phenol extraction and ethanol precipitation. For Southern blot analysis, the restriction-enzyme-cut genomic DNA samples were electrophoresed on a 1% agarose gel, electro-transferred to GeneScreen Plus (New England Nuclear), and hybridized with radiolabelled AR cDNA at 42 °C, according to the instructions in the GeneScreen Plus manual.

The cDNA probes used in hybridization analyzes were labelled by nick translation using [α-^{32}P]dATP or [α-^{32}P]dCTP (3000 Ci/mmol, New England Nuclear) [34].

The DNA sequencing of the cDNA insert was done by the Maxam and Gilbert method with some modifications [41, 42]. All other aspects of the DNA fragment labelling and isolation were as previously described [34, 43].

RESULTS

Amino acid sequences of proteolytic fragments of adrenodoxin reductase and production of an oligonucleotide probe

When we undertook the isolation of AR cDNA there was no amino acid sequence information available for this enzyme, except one report on the last three residues of the protein at the carboxy terminal [44]. Thus, to facilitate our task of identification of a cDNA and final confirmation of its identity, we determined the amino acid sequences of tryptic digest fragments of AR. From the sequences shown in Fig. 1 we chose the longest one outside the amino-terminal region as the sequence to be used in an oligonucleotide production. Although the amino-terminal fragment sequence was longer, it would not have been a good candidate for this, because our expression libraries were not expected to contain full-length copies of the cDNA. Following the recently developed method of substituting deoxyinosine [45] in positions of codon degeneracy, the oligonucleotide shown in Fig. 2 was synthesized to match the amino acid sequence of the fragment chosen. The use of deoxyinosine allowed us to generate a probe matching an eight-amino-acid-long sequence with a mixture of only four oligonucleotides instead of 4096 oligonucleotides that were theoretically possible (Fig. 2).

Identification of adrenodoxin reductase cDNAs

In the initial antibody screening of the cDNA libraries three plaques, out of nearly 120000 screened, gave strong signals with the Protoblot detection kit (see Fig. 3A for an example). These three clones were then purified and re-screened with the [^{32}P]oligonucleotide probe by plaque-

hybridization. Only one of the three clones showed hybridization with the probe but we isolated the cDNA inserts from all three recombinant phage DNAs and subcloned them into plasmid pIBI76. Henceforth the plasmid containing the cDNA insert (≈1600 bp) from the clone that showed a positive result with both the antibody and the oligonucleotide probe will be referred to as pAR as an acronym for adrenodoxin reductase plasmid.

To further confirm the identity of the pAR insert we then decided to determine the sequence of a fragment of pAR that would hybridize with the probe. For this purpose we cut pAR with several different restriction enzymes, electrophoresed the products on an agarose gel and then blotted onto GeneScreen. As seen in Fig. 3B, the smallest fragment of pAR that reacted with the [^{32}P]oligonuleotide probe was a *Bam*HI fragment of about 700 bp. After end-labelling *Bam*HI-cut pAR, this fragment was cut with *Pst*I at a site near its center and its sequence determined by the Maxam and Gilbert method. This revealed a DNA sequence that matched the oligonucleotide (Fig. 2). Although the probe was made to match only the first eight amino acids of peptide 5, the cDNA sequence downstream from the probe sequence contained codons that matched exactly the remaining four amino acids in peptide 5 (Fig. 2). The sequence of this *Bam*HI fragment of cDNA also revealed codons (data not shown here) that matched eight other peptides from the list in Fig. 1. Thus, these results established unequivocally the identity of the pAR insert as a cDNA of AR mRNA.

Adrenodoxin reductase mRNA sizes and tissue specificity of expression

To estimate the number and size of mRNAs that code for AR, we reacted a Northern blot of poly(A)-rich RNA from several tissues known to contain AR with nick-translated pAR cDNA insert. The results of this hybridization analysis revealed that there is one major form of AR mRNA in the adrenal cortex and corpus luteum which is approximately 2000 bp long (Fig. 4). In the liver and kidney, which also contain AR, a hybridization band could not be detected even when the amount of the poly(A)-rich RNA was increased tenfold (results not shown). Based on the results of these blots we estimate that if there are indeed mRNAs homologous or identical to AR mRNA, their levels are at least 40-fold lower than those in the adrenal cortex.

In addition to the major band of mRNA there was an additional faint band of hybridization at a position corre-

1.	STQEQTPQICVVGSGPAG
2.	GQASGK
3.	EAARPR
4.	LLGH
5.	TATEKPGVEEAA
6.	LDAEEVS
7.	ALLDSR
8.	AHVDIYEK
9.	SPQQVLPSPDG
10.	FFR
11.	SRPIDPS
12.	NVINTFTQTA

Fig. 1. *Amino acid sequences of fragments of adrenodoxin reductase.* The peptide fragments were isolated and sequenced as described in Methods. Peptide 1 is the sequence of the amino terminal of AR

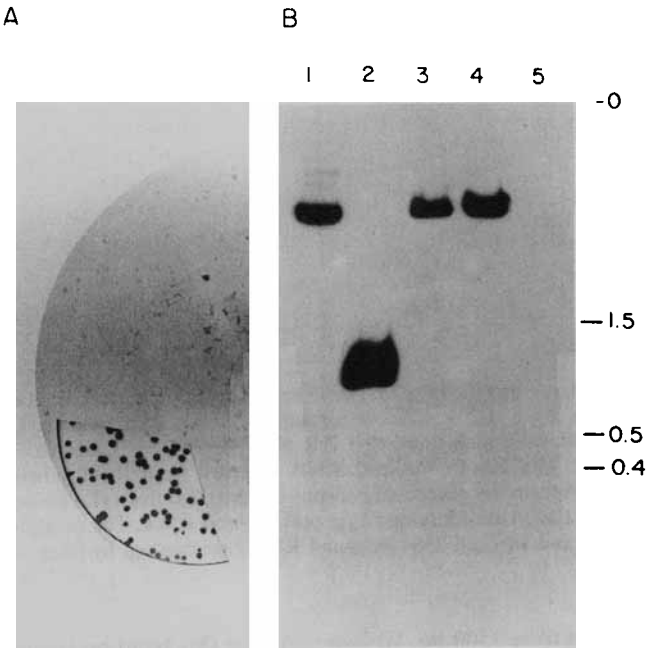

Fig. 3. *Identification of (A) an AR cDNA using antisera against AR and (B) AR cDNA that hybridizes with the radiolabelled synthetic oligodeoxynucleotide (see Fig. 2).* (A) The large nitrocellulose filter was placed on a 15-cm-diameter petri dish with about 13500 plaques for 16 h. It was then reacted antisera as described in Methods. The one darkly stained spot on the filter is the AR cDNA isolated in this study. The small filter with many spots shows the same clone after plaque-purification. (B) The autoradiogram shown is of a 1% agarose gel blot prepared as described in Methods. The lanes contained the following DNA samples: 1–4, 0.5 µg pAR cut with *Hin*dIII, *Bam*HI, *Pst*I and *Sal*I; 5, another cDNA which also reacted with the antibody but was smaller in size. The strongly labelled *Bam*HI fragment was taken for sequence analysis. The positions of origin and molecular mass standards (in kb) are marked by lines

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Peptide 5:          Thr Ala Thr Glu Lys Pro Gly Val Glu Glu Ala Ala

Possible codons:  5' ACA GCA ACA GAA AAA CCA GGA GUA GAA GAA GCA GCA 3'
                       C   C   C   G   G   C   C   C   G   G   C   C
                       G   G   G           G   G   G           G   G
                       U   U   U           U   U   U           U   U

Oligonucleotides: 3' TGI CGI TGI CTC TTC GGI CCI CA  5'
                               T   T

Actual cDNA:      5' ACA GCC ACG GAG AAG CCA GGG GTG GAG GAG GCT GCC 3'
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Fig. 2. *The oligodeoxynucleotides synthesized to match the amino acid sequence of AR fragments 5 (Fig. 1) and the DNA sequence found in the isolated AR cDNA.* The letter I in the sequence represents deoxyinosine. The two T that appear under the oligonucleotide sequence, mark the two positions where two nucleotides were added together during the synthesis reaction resulting in the formation of a mixture of four oligonucleotides at the end of the synthesis

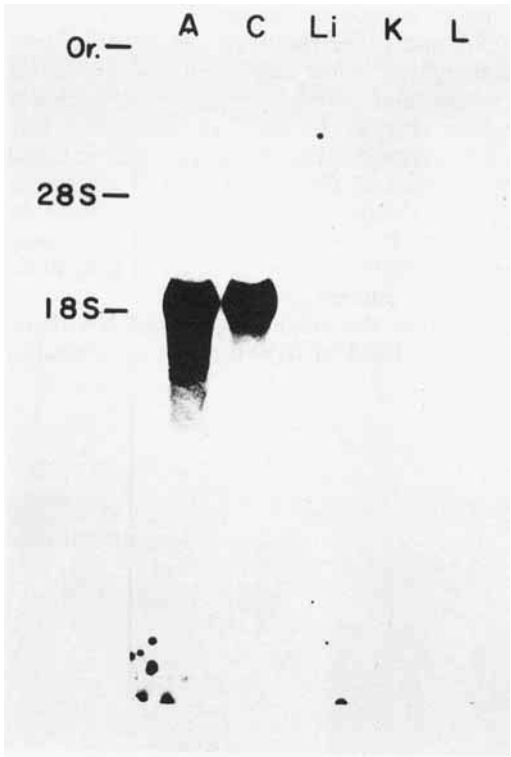

Fig. 4. *Tissue specificity of AR gene expression.* The autoradiogram shown is of a 1.2% agarose/formaldehyde gel blot which was hybridized with nick-translated AR cDNA insert as described in Methods. The lanes contained RNA samples from the following tissues: (A) adrenal cortex, (C) corpus luteum, (Li) liver, (K) kidney, (L) lung. Each lane contained 2 μg poly(A)-rich RNA. The positions of origin and 18S and 28S ribosomal RNAs are marked by lines

sponding to ≈ 3500 bp. We estimate that this band represents an RNA the amount of which is at least 20-fold lower than the major band. This band may represent a minor form of AR mRNA. The possibility that it represents an unprocessed form is rather unlikely as the RNA used for the Northern blot was polysomal poly(A)-rich RNA and not total RNA.

Comparison of the relative abundance of adrenodoxin reductase, adrenodoxin and cytochrome P-*450$_{scc}$ cDNAs in the cDNA libraries*

With the availability of cDNAs for AR, adrenodoxin and cytochrome *P*-450$_{scc}$, we wished to determine the relative abundance of these cDNAs in our libraries. In addition we also screened the libraries with a β-actin cDNA as a control and as a reference for a cDNA of a relatively abundant protein. Plaque-hybridization screening of the two cDNA libraries with the radiolabelled cDNAs indicated that AR cDNA is much rarer than the cDNAs of the other two proteins of the mitochondrial *P*-450 system (Fig. 5, Table 1). The screening of the > 1000-bp cDNA library with the pAR insert revealed one positive/≈ 7000 plaques. As expected, this ratio was higher than that observed with the antibody (at most 1 in 40000), because theoretically only 1 out of 6 cDNAs should be linked in the correct orientation and reading frame with the β-galactosidase gene in λ gt11, to produce an mRNA that could be translated into an immunogenic AR product. Interestingly, while the number of the adrenodoxin cDNAs in the > 500-bp cDNA library was about 20-fold higher than that in the > 1000-bp cDNA library, AR, *P*-450$_{scc}$ and actin cDNAs were more abundant in the > 1000-bp cDNA library (Table 1). This is understandable in view of the fact that two out of the three species of adrenodoxin mRNA are relatively small (1400 and 950 bp) [25] in comparison to those of AR, *P*-450$_{scc}$ and actin. Hence, these are much less likely to be represented in a cDNA population with a 1000-bp cut off.

Hybridization of adenodoxin reductase cDNA to bovine and human genomic DNA

To estimate the number of genes with a sequence homologous to AR cDNA, we carried out Southern blot analysis of bovine and human genomic DNA using AR cDNA as a probe (Fig. 6). This revealed that in both bovine and human genomic DNA that is cut with three different restriction enzymes, at most two major fragments (ranging over 4000 – 8000 bp) show strong hybridization with the AR cDNA. A single major band of hybridization was observed for *Eco*RI-cut or *Hin*dIII-cut bovine genomic DNA, and *Eco*RI-cut human genomic DNA. The lengths of these *Eco*RI fragments were between 4500 – 6000 bp. When two bands of hybridization were observed, one of these was invariably significantly weaker. This was probably indicative of a cut within one continuous sequence resulting in the formation of two fragments, one of which contained the majority of the coding sequence of the gene, thus showing a greater degree of hybridization with the radiolabelled cDNA. Longer exposure of the blot showed the presence of additional bands which may belong to other homologous genomic sequences.

DISCUSSION

By screening an adrenal cortex cDNA expression library using polyclonal antisera against AR, we have isolated a nearly full-length cDNA for AR. The final verification of this cDNA was facilitated by the use of a deoxyinosine-containing oligonucleotide probe which allowed us to identify easily a relatively small fragment of cDNA for sequencing (Fig. 3B). The match between the predicted codon sequence of this fragment and the sequences of the tryptic digest fragments of AR confirms unequivocally the identity of this cDNA as a copy of AR mRNA. The estimated size of the mRNA (Fig. 4) is also consistent with the molecular mass of the precursor form of AR (≈ 53 kDa) [46]. Using the AR cDNA as a probe, our analyses of genomic DNAs and selected tissue mRNAs provide the following information about AR and its role in the mitochondrial cytochrome *P*-450 systems.

Adrenodoxin reductase is encoded by a single gene in both bovine and human genomes

Southern blot analysis of bovine and human genomic DNAs indicates that in both of these species there is only one major genomic sequence homologous with the AR cDNA (Fig. 6). The sizes of some of the genomic DNA fragments that show hybridization with the AR cDNA probe are less than 6000 bp. As the size of the mature mRNA for this enzyme is 2000 bp, it is most likely that these genomic DNA fragments represent only one gene which includes introns. Southern blot analysis of genomic DNA isolated from bovine liver showed similar results (data not shown), indicating that this gene does not undergo a rearrangement in the adrenal cortex where its level of expression is highest. The finding of a single gene establishes that the two different mitochondrial cytochrome

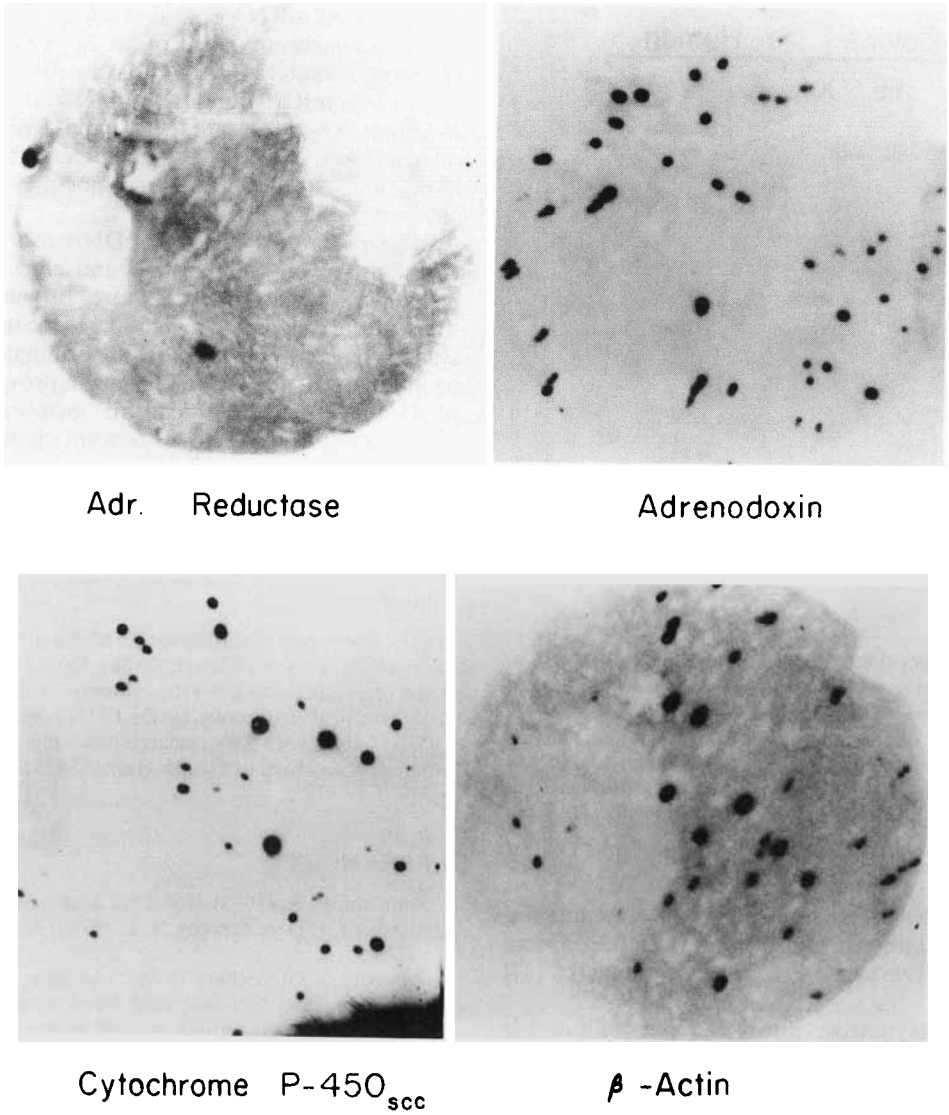

Fig. 5. *Plaque-hybridization screening of adrenal cortex cDNA libraries in λ gt11 using radiolabelled cDNAs for AR, adrenodoxin, cytochrome* P-*450$_{scc}$, and β-actin.* The autoradiograms shown are of nitrocellulose filters used in plaque-hybridization analysis. Each filter was placed on a plate with 13500 plaques, and after fixation of the DNA it was hybridized with the radiolabelled cDNA for the enzyme or protein indicated at the bottom of each. The results shown for AR, *P*-450$_{scc}$, and β-actin are from the library with cDNA > 1000 bp, and for adrenodoxin from the library with cDNA > 500 bp. After purification and rescreening of randomly selected positive plaques, at least 80–90% of these were observed to be positive

Table 1. *Relative abundance of adrenodoxin reductase, adrenodoxin, cytochrome* P-*450$_{scc}$ and β-actin cDNAs in two cDNA libraries constructed from cDNA populations > 500 bp or > 1000 bp prepared from bovine adrenal cortex poly(A)-rich RNA*
The values are based on results of screening ≈ 40000 plaques by plaque-hybridization analysis using nick-translated cDNAs

Component	mRNA size	Frequency/10000 plaques	
		cDNA > 500 bp	cDNA > 1000 bp
	kb		
Adrenodoxin reductase	2.0	0.8	1.5
Adrenodoxin	0.9, 1.4, 1.7	27.4	1.3
Cytochrome *P*-450$_{scc}$	2.1	1.5	14.1
β-Actin	1.8	2.3	23.8

P-450 in the adrenal cortex and the single mitochondrial *P*-450$_{scc}$ in the corpus luteum and testis must be dependent on the same reductase for electron transfer. Until this determination of gene number, the possibility that there could be distinct isozymes for each *P*-450 could not be eliminated based on protein chemistry alone.

AR gene is specifically expressed in steroidogenic tissues

The tissue specificity of the RNAs recognized by the pAR insert unambiguously demonstrates that the highest levels of expression of the AR gene are observed solely in steroidogenic tissues (Fig. 4). We could not detect a homologous mRNA in the liver and kidney where there are different mitochondrial cytochromes *P*-450. Yet, we estimate that if there is a homologous mRNA in these two tissues, its levels are at least 40-fold

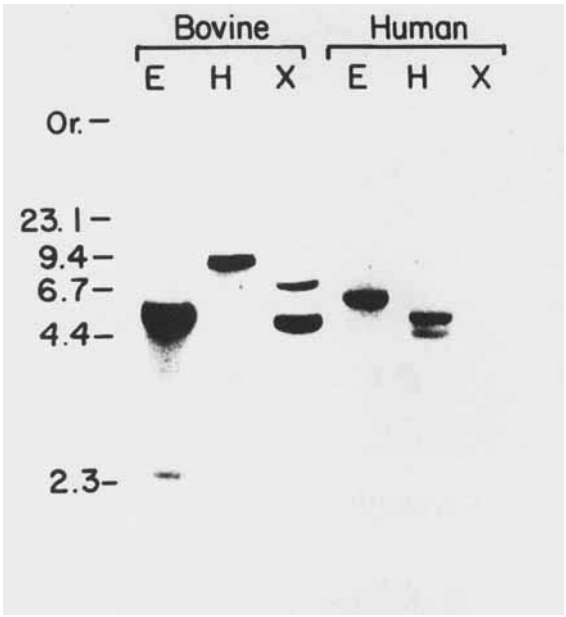

Fig. 6. *Hybridization of AR cDNA to bovine and human genomic DNA.* The autoradiogram shown is of a Southern blot of bovine and human genomic DNA. Each lane of the 1% agarose gel contained 10 μg genomic DNA cut with a restriction enzyme that did not cut the AR cDNA. The enzymes used were (E) *Eco*RI, (H) *Hin*dIII, and (X) *Xmn*I. After electrophoresis, the DNAs were electro-transferred to GeneScreen Plus and hybridized with radiolabelled AR cDNA as described in Methods

lower than those in the adrenal cortex and corpus luteum. This would correlate with the concentrations of the enzyme in liver and kidney relative to the steroidogenic tissues (cf. [47] and [24]).

The present results narrow down the possibilities for understanding the nature of the mitochondrial *P*-450 system ferredoxin reductase in non-steroidogenic tissues. Since there is only one gene for adrenodoxin reductase, the functional homologoue of this enzyme in the liver and kidney can not be a closely related isozyme; it is either the product of the same gene or a gene that is so significantly different from adrenodoxin reductase that it does not show hybridization with its cDNA under the conditions we utilized. Yet, even if the latter possibility is true, the immunological and biochemical similarities between the ferredoxin reductases from different tissues [9, 47, 48] reflect some homology between the sequences of these proteins.

Adrenodoxin reductase mRNA is manyfold less abundant than adrenodoxin and P-*450$_{scc}$ mRNAs*

Plaque-hybridization screening of the cDNA libraries indicates that the level of AR mRNA in the bovine adrenal cortex is about 10-fold lower than that of cytochrome *P*-450$_{scc}$ (Table 1). Our previous measurements of the relative levels of these enzymes in the adrenal cortex of cattle indicated that AR levels are four times lower than those of cytochrome *P*-450$_{scc}$ [24]. At present we can not provide an estimate of the mRNA levels of adrenodoxin as compared to the other two enzyme because the smaller size of adrenodoxin mRNA biases differently its representation in sized cDNA libraries (Table 1). Nonetheless, the frequency of adrenodoxin cDNAs in the >500-bp cDNA library (Table 1) provides some evidence that its mRNA is also much more abundant than that of AR, consistent with previous measurements of their apoprotein levels [24, 49]. This correlation between the relative levels of the mRNAs and the apoproteins of the mitochondrial *P*-450 system enzymes in the adrenal cortex indicates that the relatively low levels of AR are a direct reflection of its mRNA levels, and is not a result of a possible lower translational efficiency of its mRNA.

The availability of an AR cDNA provides us with a most useful probe to study further and elucidate the regulatory mehanisms and structural features of genes that are responsible for the coordinate regulation of the mitochondrial *P*-450 system enzymes. Furthermore, determination of the complete coding sequences of the cDNAs will provide us with the first structural information for this membrane-bound enzyme which interestingly shows certain characteristics of both membrane-bound and soluble proteins. As the pAR cDNA insert does not contain the amino terminus sequence of the protein (peptide 1, Fig. 1), we are currently screening our libraries further to identify additional clones that will permit us to determine the complete sequence of adrenodoxin reductase.

We express our gratitude to Dr Michael Waterman (University of Texas Health Science Center, Dallas, Texas) for generously providing the adrenodoxin and *P*-450$_{scc}$ clones. The human genomic DNA sample was provided kindly by Dr E. Canaani (Weizmann Institute of Science, Rehovot). This research was supported by in part by the US National Institute of Health grant AM33830 to I. H.

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