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Biochimica et Biophysica Acta 1188 (1994) 391–397

BBA Biochimica et Biophysica Acta

Developmental regulation of tissue-specific isoforms of subunit VIa of beef cytochrome *c* oxidase

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Received 11 July 1994

Abstract

The switching of the subunit VIa isoforms of cytochrome *c* oxidase has been followed in heart tissue during bovine development both by transcript levels and in terms of the incorporation of L- (liver) and H- (heart) polypeptides into mitochondria. In early fetuses, e.g., 60-days development, there are high levels of VIaL transcript and high levels of the VIaL polypeptide incorporated into mitochondria. In late fetuses (after 200 days), the levels of VIaL transcript are still high, with less but still significant amounts of VIaL polypeptide present in comparison to adult heart in which the amount of this isoform is negligible. As the proportion of VIaL transcript is reduced, the proportion of VIaH transcript increases along with the amount of the VIaH isoform in mitochondria. These data indicate isoform switching during late fetal development. The presence of COLBP (cytochrome oxidase liver isoform binding protein) (Preiss, T. and Lightowlers, R.N. (1993) J. Biol. Chem. 268, 10659–10667) was examined at different developmental stages. COLBP binding activity was observed in hearts of late fetuses but not found in adult heart tissue, providing a correlation between the presence of this factor and the presence of the VIaL polypeptide in mitochondria.

Keywords: Enzyme isoform; Cytochrome *c* oxidase; Subunit VIa; Mitochondrion; Tissue development; (Bovine)

1. Introduction

Cytochrome *c* oxidase, the terminal enzyme of the electron transfer chain, catalyzes the transfer of electrons from cytochrome *c* to molecular oxygen. Mammalian cytochrome *c* oxidase contains a total of 13 different subunits, three of which are mitochondrially encoded (subunits I, II and III), the remainder are nuclear in origin. Subunit I contains the prosthetic groups heme *a* and a_3 and Cu_B, while subunit II contains the cytochrome *c* binding site and is the apoprotein for Cu_A [1,2]. A complex containing only subunits I and II can be isolated from bacteria, which is active in electron transfer and shows low but significant proton pumping activity [3]. These subunits, therefore, are the catalytic core of the enzyme. Subunit III appears to have a role in the assembly of subunits I and II [4], and also acts to facilitate and possibly regulate proton translocation [5].

The role(s) of the 10 nuclear encoded subunits is (are) only now becoming apparent. Subunits IV, Va, Vb, VIc, and VIIa (mammalian nomenclature) are all necessary for stabilizing an assembled complex based on studies of mutants in yeast that are null for these subunits (e.g., [6–10]). Nuclear encoded subunits may also act as receptors for various messengers thought to regulate cytochrome *c* oxidase activity, such as nucleotides, fatty acids, and thyroid hormone [11].

A strong argument for the regulatory role of subunits VIa, VIIa and VIII in mammalian cytochrome *c* oxidase is their presence as isoforms, an L form ubiquitous to all tissues and an H form found exclusively in heart and skeletal muscle [12–14]. Several recent studies have shown that subunits VIa, VIIa and VIII are not only tissue-specific but may also be regulated developmentally [15,16]. Here we describe experiments on subunit VIa isoform switching during bovine development (which has a gestation period of 270 days), a

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SSDI 0005-2728(94)00150-2

study made possible by the availability of nucleotide probes to examine transcript levels, as well as antibodies that distinguish the L and H isoform polypeptides.

Preiss and Lightowlers [17] recently implicated a bovine cytosolic RNA binding protein, the cytochrome *c* oxidase L-form transcript binding protein, or COLBP, in the post-transcriptional regulation of the L isoforms of subunit VIIa and VIII. Their evidence suggests that this factor binds to the 3′ untranslated region (3′UTR) of the subunit VIIa and VIIIL transcripts. We present evidence for post-transcriptional regulation of subunit VIaL and for the binding of COLBP or a similar factor to the transcript of this subunit in adult and in fetal tissues.

2. Materials and methods

2.1. Tissue samples

Adult and fetal tissue samples were obtained at the abattoir as soon as possible after slaughter. The tissues were frozen in liquid N_2 at the abattoir and transported on dry ice to the −80°C freezer.

2.2. RNA isolation

Frozen tissue was ground to powder in liquid N_2. RNA was isolated by a modification of the cold guanidinium-thiocyanate Cs/Cl method [18]. After ultracentrifugation, the RNA pellet was resolubilized in 50 mM Tris HCl (pH 7.4), 10 mM EDTA (TE buffer). RNA was treated with 0.1 mg proteinase K/ml at 37°C for 30 min, extracted twice with phenol/chloroform, precipitated twice with ethanol [19], dried under vacuum, and dissolved in diethyl-pyrocarbonate-treated water. Quantitative analysis was performed by spectrophotometric scanning of 2 μl samples from 210–310 nm. Preparations were stored at −80°C.

The integrity of the RNA preparations was visually checked by electrophoresis through a 1.25% agarose in 0.5 × 45 mM Tris Borate 1 mM EDTA containing 0.5 mg ethidium bromide (EtBr)/ml.

2.3. Northern analysis

For Northern blotting, RNA was denatured with glyoxal (250 μl deionized glyoxal, 30 μl 0.5 M sodium phosphate buffer (pH 6.5) and 750 μl dimethyl sulfoxide). 2.7 μl glyoxal per 10 μg RNA was added, the samples were then vortexed, and incubated at 50°C for 1 h. After incubation, 1/5 volume sample buffer (30% Ficoll 400, 0.2% bromophenol blue and 0.2% xylene cyanol in 20 mM sodium phosphate buffer (pH 6.5)) was added. Denatured RNA samples were then subjected to gel electrophoresis through 1.25% agarose gels in 10 mM sodium phosphate buffer (pH 6.5) and transferred to Gene Screen Plus filter in 20 × SSC with a vacuum blotting unit (2016 Vacugene, Bromma, Sweden) at 0.04 bar for 2 h. Filters were then stained with methylene blue (0.03% methylene blue, 0.3 M sodium acetate (pH 5.2)) for 30 s, rinsed with distilled water 2 × 5 min and photocopied. This provided a permanent record of the integrity of the RNA, the efficiency of transfer, and the equality of loading. The filters were then either air dried or used wet in hybridizations. Probes derived from cDNA were labeled with [α-^{32}P]dCTP using the Amersham multiprime (VIaH) or megaprime (VIaL) labeling kits to a specific activity of $(6–8) \cdot 10^9$ cpm/μg. For hybridization, a modification of the procedure developed by Church and Gilbert [20] was used. Blots were prehybridized in 0.25 mM sodium phosphate buffer (pH 7.2), 1 mM EDTA, 7% SDS, 1% bovine serum albumin (BSA). After hybridization to the DNA probes in the same solution overnight, filters were washed twice for 5 min consecutively in: (i) 100 mM sodium phosphate buffer (pH 7.2), 5% SDS, (ii) 50 mM sodium phosphate buffer (pH 7.2), 1% SDS, (iii) 20 mM sodium phosphate buffer (pH 7.2), 1% SDS. All steps were performed at 65°C in a rotation hybridization incubator. Filters were wrapped in Saran wrap and exposed to Kodak XAR-5 film with intensifying screen at −80°C.

2.4. Preparation of mitochondria

Bovine tissue was frozen for RNA isolation. The same tissue was used for isolation of mitochondria; thus, samples were first crushed under liquid N_2. The samples were then incubated in an equal volume of incubation buffer (0.3 M sucrose, 1 mM $CaCl_2$, 5 mM MOPS, 5 mM KH_2PO_4, 0.1% BSA, to pH 7.4 with KOH) and 10 mg/ml collagenase for 1 h on ice. The incubation was stopped with 2 mM EDTA and a spin for 3 min, at 4°C and 10000 × *g* (Beckman JA-20 rotor). The pellet was then resuspended in 5 × vol preparation buffer (0.3 M sucrose, 1 mM EDTA, 5 mM MOPS, 5 mM KH_2PO_4 0.1% BSA, to pH 7.4 with KOH) and 10 mM/ml phenylmethylsulfonyl fluoride (PMSF). The sample was minced with a Ystral Y/20 mechanical homogenizer (3 × 3 s bursts at maximum setting), and homogenized with a glass Teflon motorized Potter-Eveljhem homogenizer (6 passes at 100–300 rpm). Next the homogenate was spun for 10 min at 4°C at 1500 × *g* (Beckman JA-20 3.5 K). The supernatant was collected and spun at 10000 × *g* for 10 min at 4°C (Beckman JA-20 9 K). The pellet was washed with 0.25 M sucrose to yield intact mitochondria. The mitochondria were resuspended in preparation buffer and PMSF, snap frozen with liquid N_2, and stored at −80°C. The concentration of cytochrome oxidase was determined spectrophotometrically. The mitochondria

were diluted with 4 volumes of 100 mM potassium phosphate buffer (pH 7.2), 25 mM NaCl, 2% (w/v) lauryl maltoside, incubated for 30 min at 4°C, and undissolved material was removed by centrifugation. Concentrations of heme aa_3 were determined by air-oxidized versus sodium dithionite-reduced difference spectra at room temperature, recorded on a Beckman DH-7 spectrophotometer, using the extinction coefficient at 605 nm of 24 $mM^{-1} cm^{-1}$ [21].

2.5. Western analysis

Samples of mitochondria were dissociated for 30 min in 50 mM Tris-HCl (pH 6.8), 4% SDS, 2% β-mercaptoethanol, 12% glycerol, 0.01% Bromophenol blue (dissociation buffer) at 37°C. Dissociated samples with similar amounts of cytochrome oxidase, based on air-oxidized minus dithionite-reduced spectra, were loaded on SDS-polyacrylamide mini-gels (as described in [14]), and blotted onto poly(vinylidine difluoride) (PVDF) (immobilon-p; millipore) at 100 V for 90 min with a Mini-Trans blot cell (Bio-Rad) cooled with ice. Protein binding sites on the blots were blocked with 10% (w/v) nonfat dry milk (Carnation) in PBS. The blots were then incubated overnight with monoclonal antibodies against subunits IV and VIaH, or a polyclonal antibody against VIaL. The blots were then washed 3 times, incubated with secondary antibody, either alkaline phosphatase conjugated gα m or gα r (Bio-Rad) for 2 h, washed again 3 times and developed using nitrobluetetrazolium and 5-bromo-4-chloro-3-indolylphosphate in alkaline phosphatase buffer (100 mM Tris-HCl (pH 9.5), 5 mM $MgCl_2$, 100 mM NaCl). All washes and incubations were conducted in PBS/0.3% (v/v) Tween-20.

2.6. 3'UTR isolation and sequencing

To isolate the 3'UTR of cytochrome oxidase subunit VIaL, the 3'RACE kit (Gibco BRL) was used with the oligonucleotide 5'TA GGAATTC GCT GAA TGT CTT CAT GAA GTC GC3' as the gene-specific primer. The *Eco*RI site was added to facilitate subsequent cloning and sequencing. The polymerase chain reactions were performed in 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 2.5 mM $MgCl_2$, 0.1 mg/ml BSA and 10 mM dNTP with a COY Tempcycler model 50 (COY Laboratory Products). cDNA (0.01 μg), prepared from total liver bovine RNA (isolated as above) using reverse transcription protocol supplied with 3'RACE kit, the VIaL primer, the Universal amplification primer (UAP 3'RACE kit), and 2.5 units of *Taq* DNA polymerase (Perkin-Elmer Cetus) in a final volume of 50 μl were subjected to 30 cycles of denaturation at 94°C for 1 min, annealing at 58°C for 45 s, and elongation at 72°C for 1.3 min.

Analysis of the products on a 1% (w/v) agarose/ethidium bromide gel revealed a band of approximately 500 bp. The band was isolated from the gel by a modified freeze squeeze method. The band was cut out of the gel, equilibrated with 300 mM sodium acetate, 1 mM EDTA for 15 min, and cut into pieces which were then put into an 0.5 ml Eppendorf tube that was punctured in the bottom and contained a glass wool plug. The 0.5 ml Eppendorf was snap frozen in liquid nitrogen, and microcentrifuged for 10 min in a decapped 1.5 ml Eppendorf. To the DNA collected 1/100 vol, 1 M $MgCl_2$ and 10% (v/v) acetic acid was added. The mixture was precipitated with 2 volumes ethanol at −80°C for 15 min and used for cloning.

The 500 bp fragment isolated from PCR was digested with *Eco*RI, phosphorylated with T4 DNA kinase, ligated with pBluescript digested with *Eco*RI and *Sma*I, and cloned in *E. coli* XL1-blue according to standard procedures [20]. Recombinant plasmid DNA (pVIaL UTR) isolated by the magic miniprep purification kit (Promega) was subjected to DNA sequencing in both directions (M13 universal primer and T3 primer) using the Sequenase® Version 2.0 DNA sequencing kit and protocol (US. Biochemical Corp.), and [α-^{35}S]dATP (DuPont).

2.7. Preparation of tissue homogenates

Tissue homogenates were prepared as in [17] with the exception that the tissue was frozen and was therefore crushed in liquid N_2 before resuspension in homogenization buffer.

2.8. In vitro transcription

The pVIaL UTR (cloning and sequencing) was linearized with *Bam*HI, gel purified, and used in T7 RNA polymerase in vitro transcription assays. pVIaH transcripts were made by linearization with *Bam*HI and in vitro transcription with SP6 polymerase. The standard transcription buffer was identical to the stratagene protocol. For each 20 μl reaction, 1 μg of substrate, 100 μCi of [α-^{32}P]UTP (800 Ci $mmol^{-1}$), 20 units of RNA polymerase, and a 500 mM concentration of the other NTPs were incubated for 1 h at 37°C. The reaction was stopped with 30 μl TE buffer and phenol/chloroform/isoamyl alcohol extraction. After three consecutive 0.75 M ammonium acetate/ethanol precipitations to remove unincorporated nucleotides, the specific activity was estimated and the RNA was resuspended in TE buffer to approximately 100000 cpm/μl. An aliquot was electrophoresed through a 5% polyacrylamide (20:1 acrylamide/bisacrylamide, 7 M urea) gel, to confirm the efficiency of full length transcript production. SP6 transcription gave higher yields

of labeled transcript when spermidine was omitted from the transcription buffer.

2.9. Assay of RNA–protein complexes by gel retardation studies

Binding assays were performed essentially as in [17]. Unless stated, all reactions consisted of approximately 0.1 ng labeled transcript (100000 cpm) incubated with 100 or 150 μg of tissue homogenate at room temperature for 15 min. Nonspecific binding was prevented by the addition of 20 units of RNase T1 (Sigma) for 5 min followed by 50 μg of heparin (Sigma) for an additional 5 min. Samples were placed on ice after addition of 98% glycerol, 1% bromophenol blue. Samples were electrophoresed through a 4% 0.4 mm polyacrylamide nondenaturing gel (79:1 acrylamide/bisacrylamide) with 10% glycerol, at 15 V/cm for 3 h in 45 mM Tris borate, 1 mM EDTA at 4°C. After gel electrophoresis, gels were dried for 10 min at 80°C and RNA–protein complexes visualized by autoradiography using intensifying screens at −80°C.

3. Results

There have been several reports on transcript levels of various cytochrome *c* oxidase subunits both in different tissues and during developmental changes in rat [22], beef [15,23] and humans [24]. Also, there have been protein chemical analyses of incorporation of isoforms of the tissue-specific subunits in different tissues and at different developmental stages (e.g., [13–15]). However, with the exception of our earlier studies on subunit VIII of the beef heart enzyme [23], transcript levels and steady state expression of subunits have not been correlated directly. The availability of cDNA probes and antibodies that can differentiate the two forms of subunit VIa now make it possible to examine the relationship between mRNA levels and incorporation of this subunit into beef cytochrome *c* oxidase. Here we have focused on the developmental changes in heart tissue.

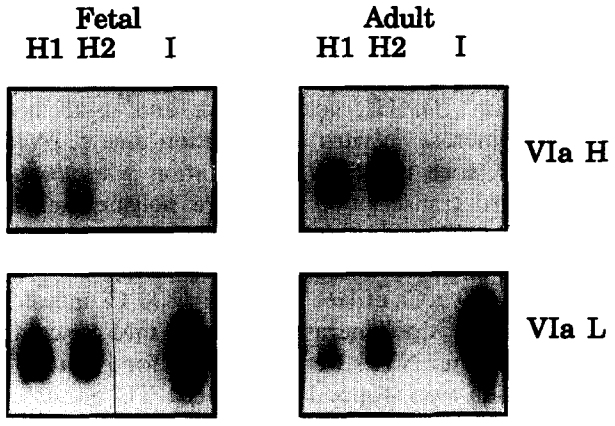

Fig. 1. Northern analysis of VIaH and L isoforms from bovine fetal and adult heart and intestinal tissues. Each lane contains 10 μg total RNA. H1 and H2 are two different heart samples. I is intestine. Left panels are from a 220-day fetus. Right panels are from an adult cow. Top panels have been probed with a VIa H cDNA. Bottom panels have been probed with a VIaL cDNA. Transcripts are 370 and 580 nucleotides, respectively. Equality of loading and efficiency of transfer were monitored by methylene blue staining (see Section 2). Analysis reveals developmental regulation of VIaH and L in bovine heart tissue.

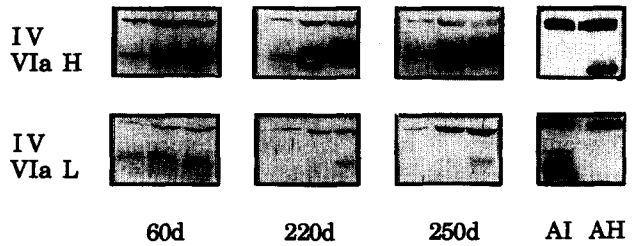

Fig. 2. Western analysis of subunit VIaH and L isoforms. Mitochondria isolated from bovine fetal and adult heart and intestinal tissue were run on SDS polyacrylamide gels (see Section 2). Alkaline phosphatase detection with a monoclonal antibody to VIaH and a polyclonal antibody to VIaL was used to detect subunits VIaH and L respectively. A monoclonal antibody against subunit IV was used to standardize for equal loading, transfer, and antibody development. The analysis reveals developmental regulation of the VIaH and L polypeptides, and post-transcriptional regulation of VIaL in adult heart tissue. 60d, 60-day-old fetus; 220d, 220-day-old fetus; 250d, 250-day-old fetus, (the three lanes represent 0.15, 0.75, 1.25 mg of cytochrome oxidase determined by heme spectra (aa_3)); AI, adult intestine (1.0 μg aa_3); AH, adult heart (1.0 μg aa_3).

Northern analyses of total RNA extracted from heart tissue of fetal and adult animals were conducted using probes to the subunit VIaH and L transcripts. As a control, samples of RNA extracted from intestine (a tissue which expresses exclusively VIaL) were used. The ratio of VIaL to VIaH transcript was found to be high in all fetuses examined (ranging in age from 60 to 250 days) compared with that in adult heart. Fig. 1 shows the data for a fetus of 220 days gestation in comparison with that for two adult hearts. Note that the levels of the VIaL transcript are still high in this late fetus, and also, that VIaL transcript is present in adult heart although in much less amount of the total RNA for this subunit than in the two fetuses.

Fig. 2 compares the steady state levels of the VIaH and VIaL type polypeptides in mitochondria isolated from several fetuses as well as adult heart and intestine, probed by using a monoclonal antibody-specific for the H form and a polyclonal antibody that detects only the L form. A mAb against subunit IV, a subunit which is not tissue-specific, was used as a control to ensure equal loading of mitochondrial samples. In the 60-day fetus the proportion of VIaL to VIaH is high, consistent with the ratios of the transcript levels. In

adult heart, the amount of VIaL is essentially zero while inspection of the transcript levels in Fig. 1, Panel B shows significant levels of transcript. In the fetuses at 220 and 250 days, the discrepancy between the high transcript levels and low amounts of the L isoform in mitochondria is most obvious.

3.1. Cloning of the subunit VIaL 3′ untranslated region

Recent studies on bovine subunit VIII have provided evidence that an RNA binding protein, COLBP, may be involved in determining the tissue-specific distribution of this polypeptide [17]. Therefore, we have tested the possibility that the same or a similar protein is involved in the developmentally-regulated and tissue-specific distribution of subunit VIaL.

The previous studies had shown that COLBP recognized and bound to the 3′ untranslated region of bovine subunit VIIIL. Ewart et al. [15] have sequenced the coding region of bovine subunit VIaL and this information was used to prepare a primer, which was then employed in conjunction with a primer to the polyadenylation site to amplify the 3′UTR of subunit VIaL by PCR using total liver RNA as the substrate. The 3′UTR of bovine VIaL is 229 bp in length compared with a length of 33 bp for the VIaH sequence. The sequence identity of the 3′UTR of VIaL from beef, human and rat (Fig. 3A) is around 80% cf. 89% for the coding region. A comparison of the 3′UTRs of bovine VIaL, VIIaL and VIIIL reveals one region that has 70% identity for VIaL and VIIIL, 76% identity between VIaL and VIIaL, and 65% identity between VIIaL and VIIIL (Fig. 3B). This region is also highly conserved in the three species (see boxed region, Fig. 3A).

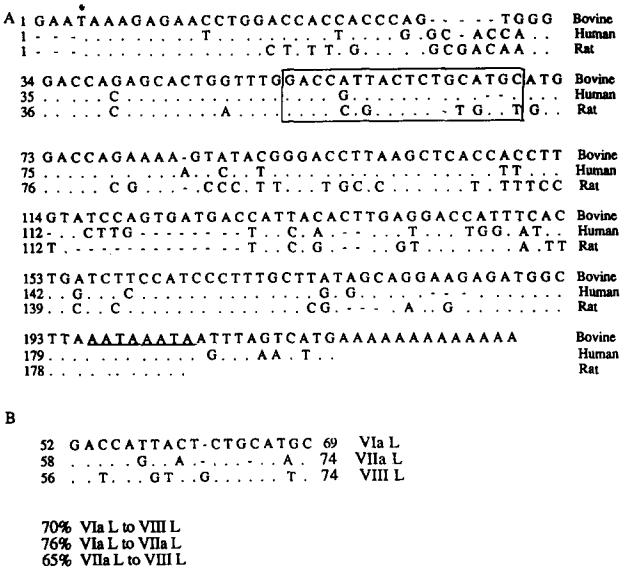

Fig. 3. (A) 3′ untranslated region of VIaL. Comparison of VIaL UTRs from beef, human and rat. Sequences were aligned using the clustal method, and exhibit about 80% identity. The boxed sequence is the region of homology from panel B. the polyadenylation signal is underlined. The stop codon is indicated by an asterisk. (•) identical nucleotide; (—) gap inserted for alignment. Updated VIaL sequence has been deposited in the EMBL data bank, Accession No. X79866. (B) Consensus regions found by aligning the 3′UTRs from bovine VIaL, VIIaL and VIIIL. (•) Indicates nucleotide; (—) indicates a space. Percent identities are listed below the sequence alignment.

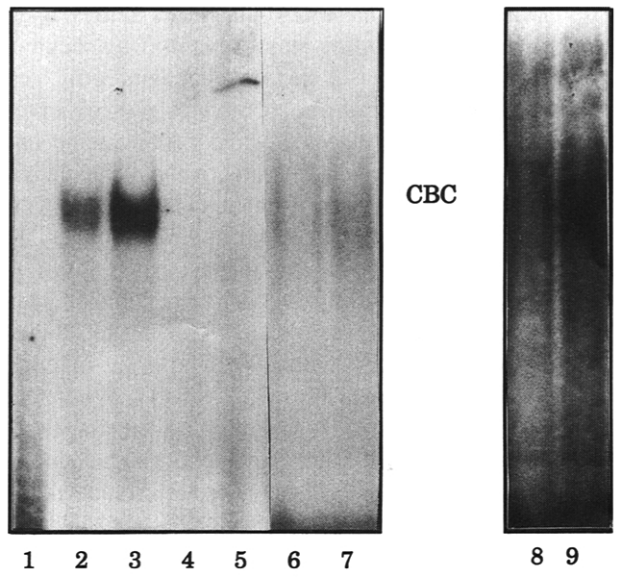

Fig. 4. Bandshift assay illustrating the association of COLBP and VIaL transcript in adult liver tissue and fetal heart tissue but not adult heart tissue. Lane 1: free transcript; lanes 2 and 3: 50 and 100 μg adult liver tissue homogenate, respectively; lanes 4 and 5: 100 and 200 μg adult heart tissue homogenate, respectively; lanes 6 and 7: 75 and 150 μg of 250 day fetal heart tissue homogenate, respectively. CBC: COLBP binding complex is indicated. Lanes 1–7 are a 1-day exposure. Lanes 8 and 9 are a 3-day exposure of the same gel. Fetal heart tissue does not contain as much VIaL protein as adult liver tissue, thus one would predict the extent of COLBP association to be less, explaining the need for a longer exposure.

3.2. Band shift assays using the 3′ untranslated region of the subunit VIaL transcript

A plasmid containing 100 bp of coding region and the entire 3′UTR along with a 30 bp linker was used for in vitro transcription studies using the T7 polymerase system. A single major transcript of the correct size, i.e., 360 bp, was produced, as confirmed by electrophoresis in a denaturing polyacrylamide gel. In vitro binding assays were conducted using this transcript after radiolabeling. The presence of a binding protein was examined with tissue homogenate from fetal and adult heart and liver. Fig. 4 shows the migration of free transcript on a 4% polyacrylamide gel (lane 1). Also shown are the results of incubating the transcript with liver tissue homogenate (lanes 2 and 3), adult heart tissue homogenate (lanes 4 and 5), and fetal heart

tissue homogenate from a 250-day fetus (lanes 6–9). When adventitious binding was prevented by incubating samples with RNase T1 and heparin, only one slowly migrating form of the transcript was observed with adult liver tissue (lanes 2 and 3 represent 50 and 100 g of tissue homogenate, respectively). The migration of this RNA transcript–protein complex is very similar to that observed with subunits VIIaL and VIIIL transcripts, suggesting that it represents binding of COLBP- or a COLBP-like protein to the subunit VIaL transcript. No complex was obtained when the labeled transcript was incubated with 100 or 200 μg of adult heart tissue homogenate (lanes 4 and 5). When the transcript was incubated with a fetal liver tissue homogenate, a slowly migrating band of the same size as that seen with adult liver tissue was observed (result not shown). As evident in lanes 6 and 7, a RNA-transcript-protein complex of similar mobility to that in liver tissue was observed in heart tissue from the 250-day fetus but the amount of this complex was low and a clear visualization of the band was only obtained after a 3-day (lanes 8 and 9) instead of the 1-day exposure of the gels used in the other lanes (e.g., 6 and 7).

4. Discussion

The direct comparison of transcript levels and steady state amounts of the polypeptide VIa of cytochrome *c* oxidase in heart tissue at different developmental stages reported here, shows the following important features. Both VIaH and VIaL transcripts are present in relatively high amounts in hearts of the earliest fetuses examined (e.g., 60-day) and both isoforms of the subunit are present in high steady state levels in mitochondria. In older fetuses, the ratio of VIaL to VIaH transcript remains high but the steady state amount of the VIaL isoform now present is lower than in the earlier fetuses. In adult heart, significant amounts of the VIaL transcript are made but there is essentially none of the subunit VIaL polypeptide in mitochondria. These results confirm our previous, more preliminary data [15] based only on Northern analyses, that there is a developmental switch of VIa isoforms in bovine heart. Further, they show that most or all of this isoform switching occurs prenatally and not postnatally. It is unlikely, therefore, that switching of subunit VIa isoforms is responsible for the recovery of cytochrome *c* oxidase activity in patients with the benign form of infantile myopathy, as this recovery occurs in the first year after birth [26].

The available data suggest that the H form of the three tissue-specific subunits of cytochrome *c* oxidase is regulated transcriptionally, as the presence of subunits VIaH, VIIaH and VIIIH in the assembled enzyme correlates well with transcript levels in all tissues and in all developmental stages examined. However, this is not the case for the L isoforms of these subunits. We have shown previously that subunit VIIIL is transcribed in adult beef heart and skeletal muscle but the polypeptide itself appears to be absent from the enzyme complex. Recently, Preiss and Lightowlers [17] identified a factor, which they named COLBP, that appears to bind to the 3′UTR of the mRNA of subunit VIIIL and possibly regulates its protein synthesis. A comparison of Figs. 1 and 2 shows that the VIaL transcript is also abundant in adult beef heart while the polypeptide is not detectable in the cytochrome *c* oxidase complex. Band shift assays showed the presence of an RNA binding protein that recognizes the VIaL transcript in the 3′UTR region and which has the same mobility as COLBP. As with subunits VIIaL and VIIIL, the 3′UTR of subunit VIaL is highly conserved among different species and there is also a region of reasonably high sequence homology between the 3′UTRs of the three subunits VIaL, VIIaL and VIIIL which could be the binding site for COLBP. The 3′UTRs of each subunit are also capable of forming many stem loop structures which could provide binding sites for COLBP.

A COLBP-like binding activity for the VIaL transcript was observed in fetal and adult liver tissue and in fetal heart tissue, but not in adult heart tissue, thereby paralleling the presence of the VIaL polypeptide in the enzyme. The small amount of heart tissue obtained from the 60-day fetus, and the difficulty in obtaining such young fetuses from slaughterhouse materials, precluded RNA binding studies on early fetuses, where the levels of the VIaL polypeptide are highest. However, such binding studies on older fetuses showed that discernible levels of the subunit VIaL polypeptide in the enzyme are correlated with the presence of significant levels of COLBP binding activity. Only in adult, where the VIaL polypeptide is essentially absent, was COLBP binding activity undetectable.

Our data strongly suggest that the developmental switching of subunit VIa isoforms that occurs prenatally is regulated by the presence of the COLBP protein. We suggest that when the levels of this factor are reduced, or the protein is absent, the subunit VIaL transcript is no longer translated either because of instability of the transcript or altered ribosome binding. Consistent with this idea, Preiss et al. [27] have recently described the differential regulation of COLBP during myogenesis of human muscle cell lines.

Acknowledgements

This study was supported by the National Institute of Health Grant HL22050. T.P. is a Gottlieb Daimler

und Karl Benz Stiftung research scholar. R.N.L. is a Fellow of the Lister Institute of Preventive Medicine. Helpful discussions with Dr. J.-W. Taanman are gratefully acknowledged.

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