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Propionicacidemia: Absence of Alpha-Chain mRNA in Fibroblasts from Patients of the *pccA* Complementation Group

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SUMMARY

Propionicacidemia is an autosomal recessive metabolic disease resulting from a deficiency of propionyl-CoA carboxylase (PCC) activity. The enzyme has the structure $\alpha_4\beta_4$, with the α chain containing a covalently bound biotin prosthetic group. Patients have been placed into two major complementation groups, *pccA* and *pccBC*, that may correspond to the genes encoding the α and β chains of PCC. The *pccBC* group is further divided into two subgroups, *pccB* and *pccC*, apparently owing to intragenic complementation. We previously reported combined α- and β-chain deficiency in *pccA* mutants and absence of β chain in *pccC* and *pccBC* mutants after isotope-tracer labeling and immunoprecipitation of cultured-fibroblast extracts. Using cDNA clones coding for the α and β chains as probes, we found absence of α mRNA in four of six *pccA* strains and presence of β mRNA in all *pccA* mutants studied. We also found presence of both α and β mRNAs in three *pccBC*, two *pccB*, and three *pccC* mutants. From these data, we confirm the gene assignments of the complementation groups (*PCCA* gene = *pccA* complementation group; *PCCB* gene = *pccBC* and subgroups) and support the view that *pccA* patients synthesize a normal β chain that is rapidly degraded in the absence of complexing with α chains.

INTRODUCTION

Propionyl-CoA carboxylase (PCC; E.C.6.4.1.3) is a mitochondrial enzyme that functions in the degradation of branched-chain amino acids, fatty acids with

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odd-numbered chain lengths, and other metabolites (Rosenberg 1983). The enzyme is a tetramer (relative molecular weight [M_r] = 560,000) composed of an equal number of α (M_r = 75,000) and β (M_r = 60,000) subunits to give the structure $\alpha_4\beta_4$ (Gravel et al. 1980). The α subunit contains a covalently bound biotin ligand that is directly involved in carboxyl transfer.

Inherited PCC deficiency produces the disorder propionicacidemia. Affected patients may present in the first days of life with lethargy, dehydration, and protein intolerance. The disease is characterized by episodic ketoacidosis, hyperammonemia, and hyperglycinemia. Death may occur in the first months or years of life, often owing to crises precipitated by infection. Some patients have a much milder course, with later onset and survival into childhood, although such children may also develop ketoacidosis (Hsia et al. 1968, 1971; Ando et al. 1971; Mahoney et al. 1971; Wolf et al. 1981). Affected patients can be successfully managed on a protein-restricted diet even when severely affected (Brandt et al. 1974). There are rare instances of affected children who are asymptomatic despite a severe PCC deficiency (Paulsen and Hsia 1974; Wolf et al. 1979). The disease has been shown to be inherited in an autosomal recessive manner, although some parents with affected children have shown normal levels of PCC activity whereas others had the expected 50% activity (Wolf and Rosenberg 1978).

The extensive clinical heterogeneity associated with propionicacidemia led us to examine complementation between patient cell lines to determine whether the disorder was genetically heterogeneous (Gravel et al. 1977; Saunders et al. 1979). Patients could be grouped into one of two major complementation groups, called *pccA* and *pccBC,* with the latter consisting of two subgroups, *pccB* and *pccC,* that showed intragenic complementation. We have proposed that *pccA* and *pccBC* correspond to the genes, recently named *PCCA* and *PCCB* (Lamhonwah et al. 1986), coding for the α and β subunits of PCC, respectively (Lam-Hon-Wah et al. 1983); but the basis of the intragenic complementation between the *pccB* and *pccC* groups is unknown.

Recently, we isolated cDNA clones encoding the α and β chains of PCC and mapped the corresponding genes, *PCCA* and *PCCB,* to chromosomes 13 and 3, respectively (Lamhonwah et al. 1986). In the present study, we used these clones to examine the status of mRNA and DNA in cell lines of patients of both complementation groups. The results support the previous gene assignments and provide an explanation for the pleiotropic deficiency of α and β chains in several *pccA* patients.

MATERIAL AND METHODS

Source and Growth of Fibroblast Strains

The fibroblast strains used in the present study were subcultures of skin biopsies from propionicacidemia patients. These cell lines had previously been assigned to specific complementation groups (Gravel et al. 1977; Saunders et al. 1979), and the synthesis of their α and β chains of PCC had been characterized [Lam-Hon-Wah et al. 1983]). They were routinely grown in α–minimal essen-

tial medium supplemented with 10% fetal calf serum without antibiotics (Dulbecco and Vogt 1954). No evidence of mycoplasma contamination was detected during the study. This was determined by direct culture and uridine phosphorylase assay.

Northern Blot Analysis

Isolation of total cellular RNA and conditions of electrophoresis and blotting were according to a method described elsewhere (O'Dowd et al. 1985). A *Sph*I-*Bam*HI fragment from pPCC9-5 was used as the α-chain probe and an internal fragment, *Nsi*I-*Xba*I, or a *Bam*HI fragment from pPCC41A2 was used as the β-chain probe (Lamhonwah et al. 1986).

Analysis of Genomic DNA

Genomic DNA from cultured human fibroblasts was prepared (Willard et al. 1983) and examined by blotting on nitrocellulose according to a method described elsewhere (Southern 1975). A ^{32}P-dCTP-labeled *Bam*HI fragment from pPCC9-5 was used as the α-chain probe, and a *Bam*HI fragment from pPCC41A2 was used as the β-chain probe in the hybridization buffer, according to a method described elsewhere (O'Dowd et al. 1985; Lamhonwah et al. 1986).

RESULTS

Northern Blot Analysis

Total RNA from fibroblast cell lines was examined by means of Northern blot analysis, using probes corresponding to the α and β chains of PCC (fig. 1). As shown elsewhere (Lamhonwah et al. 1986), normal human fibroblasts contain a single RNA species of 2.9 kb coding for the α chain of PCC (fig. 1, upper panel, lanes N) and two species—a major one at 2.0 kb and a minor one at 4.5 kb—coding for the β chain of PCC (fig. 1, lower panel, lanes N). Nonspecific hybridization of the β-chain cDNA probe with ribosomal RNA was ruled out in experiments with poly-A$^+$ RNA (Lamhonwah et al. 1986).

RNA from fibroblast cell lines from patients from each complementation group was examined for α-chain mRNA by using the *Bam*HI insert of pPCC9-5 as probe (fig. 1, upper panel lanes; A = *pccA*, B = *pccB*, BC = *pccBC*, and C = *pccC*). Mutants of *pccBC* and its subgroups (two cell lines each) all contained the 2.9-kb band in levels indistinguishable from normal (fig. 1, lanes B, BC, and C). However, when six different *pccA* mutants were examined for α-chain mRNA, none was detected in four of them and normal levels of RNA were found in two others (fig. 1, lanes A). None of these six lines expressed α or β chains (Lam-Hon-Wah et al. 1983).

Similarly, β-chain mRNA was examined in the same mutant cell lines by using a *Nsi*I-*Xba*I fragment of pPCC41A2 as probe (fig. 1, lower panel). In

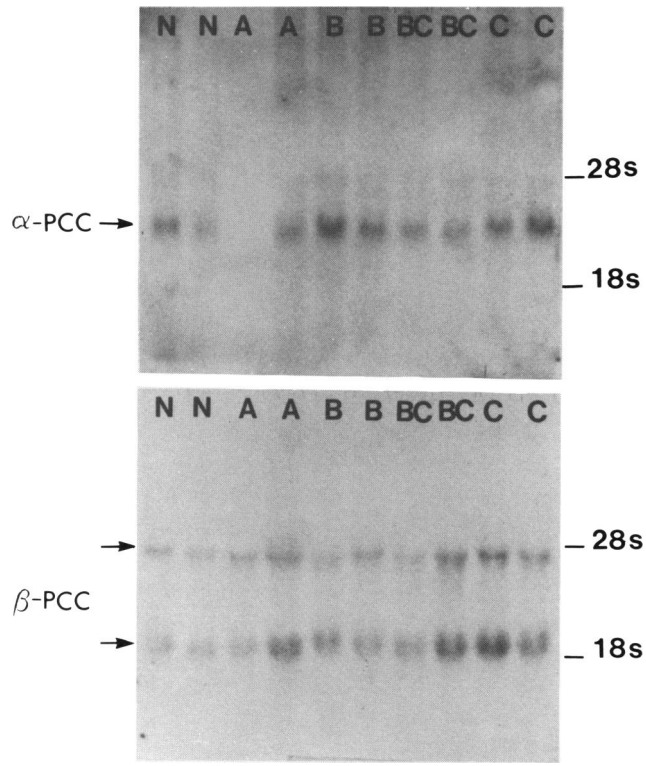

FIG. 1.—Northern blot analysis of total cellular RNA from human fibroblasts of normal individuals and patients with propionicacidemia of each complementation group. Twenty micrograms of total cellular RNA from each fibroblast cell line was denatured and separated by means of electrophoresis in a 6% formaldehyde/1% agarose gel and blotted onto nitrocellulose filter (O'Dowd et al. 1985). The upper panel shows blot hybridization with the *Sph*I-*Bam*HI fragment from pPCC9-5 as the α-chain cDNA probe, and the lower panel shows blot hybridization with the *Nsi*I-*Xba*I fragment from pPCC41A2 as the β-chain cDNA probe. Lanes N = normal cell line; and lanes A, B, BC, and C = mutant cell lines of complementation groups *pccA*, *pccB*, *pccBC*, and *pccC*, respectively. The positions of the ribosomal size markers are indicated on the right.

every case, the levels and mobility of both β-chain mRNA species were indistinguishable from normal levels.

Southern Blot Analysis

Genomic DNA from cell lines of each complementation group was examined to determine whether any gross alterations could be identified that could have resulted from mutation. We have elsewhere (Lamhonwah et al. 1986) identified a *Hin*dIII polymorphism associated with the *PCCA* (α-chain) locus and an *Eco*RI polymorphism associated with the *PCCB* (β-chain) locus. Although the different allelic forms of these polymorphisms could be detected in mutant cell lines, no difference from normal controls could be otherwise identified when

genomic DNA cut with *Eco*RI, *Hin*dIII, *Hinf*I, *Pvu*II, *Pst*I, *Sac*I, *Stu*I, *Taq*I, or *Xba*I was used (data not shown).

DISCUSSION

Propionicacidemia is defined genetically by mutations associated with either of the two major complementation groups, *pccA* and *pccBC*. The previous identification of independent genes, *PCCA* and *PCCB*, coding for the α and β chains of PCC, respectively, correlates well with the occurrence of the two complementation groups. However, the absence of detectable α and β chains in most *pccA* mutants left uncertain the genetic basis of these mutations (Lam-Hon-Wah et al. 1983). Our current finding that four of the *pccA* mutants lack α- but have β-chain mRNA clearly establishes that these mutants have primary defects at the *PCCA* locus (fig. 1, table 1) and that the β-chain defect is a secondary manifestation. Although no gross alteration of the *PCCA* gene could

TABLE 1

MOLECULAR ANALYSIS OF NORMAL AND MUTANT FIBROBLASTS FROM PROPIONICACIDEMIA PATIENTS

COMPLEMENTATION GROUP[a] AND STRAIN NO. (Patient Initials)	PCC POLYPEPTIDES		PCC mRNAs	
	α	β	α	β
Normal:				
412	+*	ND	+	+
595	+	ND	+	+
1158	+	ND	+	+
1206	+	+	+	+
1286	+	+	+	+
pccA:				
444 (A.G.)	+	+	ND	ND
447 (R.H.)	0	0	0	+
499 (J.R.)	0	0	+	+
503 (P.M.)	0	ND	0	+
533 (B.B.)	0	0	0	+
534 (M.A.)	0	ND	+	+
996 (M.B.)	0	ND	0	+
pccB:				
537 (V.S.)	+	+	+	+
445 (N.B.)	+	ND	+	+
pccC:				
448 (P.C.)	+	0	+	+
450 (N.M.)	+	0	+	+
539 (M.S.)	+	0	+	+
pccBC:				
453 (C.E.)	+	0	+	+
500 (L.S.)	+	0	+	+
854 (B.F.)	+	0	+	+

NOTE.—A plus sign (+) denotes presence of polypeptide chains or mRNAs; a zero (0) denotes absence. ND = not done.

[a] Identification of complementation groups are as in Gravel et al. (1977) and Saunders et al. (1979). A single mRNA band of 2.9 kb coding for the alpha polypeptide and two mRNA bands—a major band of 2 kb and a minor band of 4.5 kb coding for the beta polypeptide—are observed. No DNA alteration is seen when using the probes described in the text, except for the polymorphism discussed by Lamhonwah et al. (1986).

be identified in these mutants, no other explanation of the pleiotropic polypeptide defect could be consistent with a single mRNA deficiency.

We have determined elsewhere that the *pccBC* group corresponds to mutations in the *PCCB* gene coding for the β chain (Lam-Hon-Wah et al. 1983). Isotope-tracer studies had demonstrated specific absence of the β chain but presence of the α chain in mutants of both the *pccBC* group and the *pccC* subgroup (Lam-Hon-Wah et al. 1983). Although our current experiments did not provide any evidence of an alteration in either the *PCCB* gene or its mRNAs, the data are still consistent with the mutants of both *pccBC* and its subgroups having defects of the *PCCB* gene.

We have proposed elsewhere a model to account for the unexpected absence of both PCC subunits in *pccA* patients (Lam-Hon-Wah et al. 1983). We suggested that free β chains are highly unstable and rapidly cleared from cells and that the specific association of the β chain with the α chain to form native PCC protects the β chain from degradation. This model predicted the synthesis of β-chain mRNA in *pccA* mutants as demonstrated in the present study. The finding implies that the severity of the disease in these patients is partly due to the instability of free β chains in the absence of α-chain synthesis. The ensuing degradation of the β chain leaves the patient's cells devoid of either subunit, as demonstrated by isotope-tracer experiments in fibroblast cultures in which neither α nor β chains can be detected (Lam-Hon-Wah et al. 1983). An exception among *pccA* patients is patient A.G. (Lam-Hon-Wah et al. 1983), whose fibroblasts were found to contain both α and β chains. She likely has a mutation that produces a stable but inactive chain.

Much remains to be understood about the genetic basis of propionicacidemia. Unexplained by the current data are (1) the intragenic complementation detected between cell lines of patients from the *pccB* and *pccC* complementation groups and (2) the unexpectedly high PCC specific activity found in most *pccC*-heterozygous parents (Wolf and Rosenberg 1978). The suggestion by Wolf and Rosenberg (1978) that heterozygotes had excess synthesis of one polypeptide over the other, with preferential association of the normal polypeptides from the normal alleles, is not supported by the present data. Neither the levels of mRNA reported in the present study nor the levels of the polypeptide chains as reported elsewhere (Lam-Hon-Wah et al. 1983) suggest that either chain is synthesized or maintained in considerable excess vis-à-vis the other. These experiments, however, would not have detected small differences in relative synthesis or steady-state levels of the two subunits.

The absence of a detectable difference in mRNA levels between *pccB* and *pccC* mutants is not surprising. The occurrence of intragenic complementation presupposes the successful synthesis of a polypeptide product by each of the affected alleles. Our data support the expectation that *pccC* mutants synthesize a defective β chain that must turn over very rapidly (Lam-Hon-Wah et al. 1983). An understanding of the basis of the intragenic complementation will require either recovery of the *pccC* polypeptide product or direct examination of the mutant alleles. It is tempting to speculate that the heterozygote finding

and the intragenic complementation, both of which have the mutant *pccC* allele in common, may be rooted in a common mechanism.

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