Unequal Synthesis and Differential Degradation of Propionyl CoA Carboxylase Subunits in Cells from Normal and Propionic Acidemia Patients

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Summary

We have characterized further the molecular basis of human inherited propionyl CoA carboxylase deficiency by measuring steady state levels of the mRNAs coding for the enzyme's two protein subunits (α and β) and by estimating initial synthesis and steady state levels of the protein subunits in skin fibroblasts from controls and affected patients. We studied cell lines from both major complementation groups (pccA and pccBC) corresponding, respectively, to defects in the carboxylase's α and β subunits. Analysis of pccA lines revealed the absence of α chain mRNA in three and an abnormally small α-mRNA in a fourth. Despite the presence of normal β-mRNA in each of these pccA lines, there was complete absence of both α and β protein subunits under steady state conditions, even though new synthesis and mitochondrial import of β precursors was normal. Results in nine pccBC lines revealed normal α mRNA in each, while the amounts of β-mRNA were distinctly reduced in every case. Correspondingly, α protein subunits were present in normal amounts at steady-state, but β subunits were uniformly decreased. In addition, in six of the nine β deficient cell lines, partially degraded β-subunits were observed. To help interpret these results, synthesis and stability of carboxylase subunits were studied in intact HeLa cells using a pulse-chase protocol. Whereas α chains were stable over the four hour interval studied, β chains—initially synthesized in large excess over α chains—were degraded rapidly reaching equivalence with α chains after two hours. From these results we conclude that: β chain subunits are normally synthesized and imported into mitochondria in excess of α chains, but only that portion assembled with α-subunits escapes degradation; in pccA patients, the primary defect in α chain synthesis leads secondarily to degradation of normally synthesized β chains; and, in some pccBC patients, mutant β chains are intrinsically unstable. Finally, we posit that the differential rates of synthesis of α and β chains account for the prior reported finding that individuals heterozygous for pccBC mutations have normal carboxylase activity in their cells.

Introduction

Propionic acidemia is a recessively inherited disorder of organic acid metabolism caused by deficiency of propionyl CoA carboxylase (PCC; E.C.6.4.1.3) activity (Hsia et al. 1971). PCC, a biotin-dependent mitochondrial enzyme, functions in the catabolic pathway for odd-chain fatty acids, isoleucine, threonine, methionine, and valine (Rosenberg 1983). The native enzyme is probably a dodecamer (Haase et al. 1984; Goodall et al. 1983) composed of six biotin-containing α subunits (70,000–72,000 kDa) and six β subunits (54,000–56,000 kDa) (Kalousek et al. 1980). The α and β subunits are each synthesized initially as larger precursors on cytoplasmic polysomes. These precursors, containing NH₂-terminal leader sequences, are subsequently transported across both mitochondrial membranes, cleaved to their mature size, and assembled (Kraus et al. 1981, 1983).

Studies by Gravel et al. (1977) with heterokaryons
of cultured skin fibroblasts from patients with propionic acidemia revealed the existence of two major complementation groups, *pccA* and *pccBC*. *pccBC* is a complex group consisting of three subgroups (*pccB*, *pccC*, and *pccBC*). Mutants in the *pccA* group failed to complement each other but complemented mutants of all other groups. In the *pccBC* group, *pccB* and *pccC* mutants complemented each other in addition to *pccA* mutants, but they did not complement *pccBC* mutants (Gravel et al. 1977; Saunders et al. 1979). A full-length cDNA for the β subunit has been cloned in our laboratory from rat liver (Kraus et al. 1986a) and has been used to map the PCCB gene to the region q13.3–q22 of human chromosome 3 (Kraus et al. 1986b). Recently, Lamhonwah et al. (1986) reported the isolation of partial cDNA clones encoding the α and β chains of human PCC and mapped the corresponding genes, *PCCA* and *PCCB*, to chromosomes 13 and 3, respectively. Using cDNA clones, they established that *pccA* mutants have primary defects of the *PCCA* gene leading to the absence of α-mRNA in most of the *pccA* patients and that the *pccBC* group corresponds to mutations in the PCCB gene (Lamhonwah and Gravel 1987). They also proposed that normal β chains may be unstable in the absence of α chains (Lamhonwah et al. 1983; Lamhonwah and Gravel 1987). Several years ago, Wolf and Rosenberg (1978) observed that the cells from parents of *pccBC* group patients (i.e., obligate heterozygotes) have a normal level of carboxylase activity, while those from parents of the *pccA* group have activities about 50% of normal. They speculated that normal cells synthesize or retain twice as many β as α subunits and that cells from parents of β PCC-deficient patients have balanced their α and β chain synthesis.

We report here results of experiments designed to (1) determine the relative amounts of α- and β-mRNA in normal cells, (2) confirm the report that the majority of *pccA* patients lack α-mRNA (Lamhonwah and Gravel 1987), (3) examine in more detail the finding that *pccA* patients, as well as those from the *pccC* and *pccBC* groups, have no detectable β subunit (Lamhonwah et al. 1983), and, finally, (4) uncover the molecular basis for the observation that heterozygotes of the *pccBC* group have normal PCC activity.

**Material and Methods**

**Cell Lines and Cell Culture**

The cell lines used were cultured skin fibroblasts from controls or from 14 patients with propionic acidemia. These mutant cell lines had previously been assigned to specific complementation groups. Conditions for culturing and harvesting cells were as described elsewhere (Skovby et al. 1982; Fenton et al. 1987).

**RNA Isolation and Northern Blot Analysis**

Polyadenylated RNA was prepared from human skin fibroblasts as described by Skovby et al. (1984). Blot hybridization analysis was performed after electrophoresis of the RNAs through a 1% agarose gel containing formaldehyde (Maniatis et al. 1982, pp. 202–203). The BamHI-Xhol fragment (1.3 kb) from a human αPCC cDNA (pPCC9-5) and the BamHI fragment (1.2 kb) from a human βPCC cDNA (pPCC41A2) (Lamhonwah et al. 1986) were used as α and β probes, respectively. DNA fragments were labeled with [32P]dCTP (Amersham) by the random oligomer method (Feinberg and Vogelstein 1983). After total radioactivities of α and β probes were adjusted to be equal, they were used simultaneously. To control for RNA loading, the blots were probed with a cDNA coding for the α subunit of electron-transfer flavoprotein. This cDNA detects a single 1.6-kb mRNA species (Fincocchiaro et al. 1988). Densitometric tracings were performed using several different film exposures of the same blot to ensure that the readings were in the linear range and that the ratio of the bands in each lane remained constant.

**DNA Isolation and Southern Blot Analysis**

High-molecular-weight DNA was isolated from cultured fibroblasts according to a method described elsewhere (Greever et al. 1981). DNA samples were digested with restriction endonuclease, electrophoresed, blotted, and hybridized according to a method reported elsewhere (Rozen et al. 1985). The BamHI fragment (2.0 kb) from the human α-PCC cDNA and the BamHI fragment (1.2 kb) from the human β-PCC cDNA (Lamhonwah et al. 1986) were used as α and β chain probes, respectively.

**Protein (Western) Blot Analysis**

Frozen cells were solubilized for 30 min on ice in 0.24% Lubrol WX® (Sigma)/30 mM potassium phosphate, pH 6.0, and were centrifuged for 5 min. Anti-PCC antiserum was added to the immunoprecipitation buffer (150 mM NaCl/10 mM EDTA pH 7.4/0.5% Triton X100/0.1% SDS) containing cell extracts (250–500 µg protein), and the mixture was incubated overnight at 4°C. The antigen-antibody complexes were recovered with Staphylococcus aureus cells (BRL), then washed and eluted according to a method described...
Radiolabeling, Immunoprecipitation, and Gel Electrophoresis

Confluent monolayers were labeled with \( L-[^{35}S] \) methionine (100 \( \mu \)Ci/dish) for 30 min essentially as described (Ikeda et al. 1985), with the exception that the labeling medium contained 75% Puck's saline F. When dinitrophenol (DNP; Sigma) was used, it was added (final concentration \( 4 \) mM) 5 min before adding \( L-[^{35}S] \) methionine, and the dishes were incubated at 37°C for 30 min. For pulse/chase experiments, monolayers were labeled as above, washed with PBS, and incubated with Eagle's minimal essential medium supplemented with 10% FBS (MEM-E) and 5 mM \( L \)-methionine. Immunoprecipitation, gel electrophoresis, and fluorography were carried out as described elsewhere (Fenton et al. 1984).

Results

Northern Blot Analyses of RNA from Propionic Acidemia Patients

Normal human fibroblasts contain a single mRNA species of 2.8 kb coding for the \( \alpha \) chain of PCC and a major species of 2.0 kb coding for the \( \beta \) chain of PCC (fig. 1; Kraus et al. 1986a; Lamhonwah et al. 1986). Three \( pccA \) cell lines examined (lines 427 and 117 in fig. 1; line 1749 not shown) had no detectable \( \alpha \)-mRNA; a fourth \( pccA \) line (line 540) had an aberrant \( \alpha \)-mRNA which was both slightly smaller than normal in size and reduced in quantity. When twice as much poly A+ mRNA was loaded, a small amount of \( \alpha \)-mRNA was detected in cell line 117 after four-times-longer autoradiographic exposure (not shown). \( \beta \)-mRNA in all \( pccA \) patients was indistinguishable, in both size and amount, from that in controls.

In contrast, both \( \alpha \)- and \( \beta \)-mRNAs were easily detectable in cell lines from the \( pccBC, pccB, \) and \( pccC \) groups, and the mRNA sizes were indistinguishable from those in controls (fig. 1). We attempted to estimate the relative amounts of \( \beta \)-mRNA in this group. The intensity of the \( \alpha \) (2.8-kb) and \( \beta \) (2.0-kb) bands were measured by densitometry, and the \( \beta \)-mRNA:\( \alpha \)-mRNA ratio was determined (table 1). The results showed an excess of \( \beta \) transcript over \( \alpha \) transcript in poly A+ mRNA from normal fibroblasts (\( \beta: \alpha = 2.41 \pm 0.47 \)). This ratio was markedly reduced in the \( pccBC \) group (0.53–1.1; mean 0.78 \pm 0.21), indicating that the amount of \( \beta \)-mRNA in the \( pccBC \) group is reduced relative to that of \( \alpha \). On the other hand, the amount of \( \alpha \)-mRNA was not affected when compared with an unrelated mRNA coding for the \( \alpha \) subunit of electron-transfer flavoprotein (not shown).

![Figure 1](image-url)  
**Figure 1**  
Northern blot analysis of mRNA from propionic acidemia patients. Poly A+ RNA (8 \( \mu \)g/lane) was electrophoresed in a formaldehyde/agarose gel, transferred to nitrocellulose paper, and hybridized with both \( \alpha \)PCC and \( \beta \)PCC probes of nearly equal size. The total radioactivities of these probes were adjusted to be equal, and they were used simultaneously. \( \alpha \)-mRNA = position of the \( \alpha \) transcript; \( \beta \)-mRNA = position of the \( \beta \) transcript; N = normal; A = \( pccA \); B = \( pccB \); C = \( pccC \); BC = \( pccBC \).
Table 1

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Ratio</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (n=6)</td>
<td>1.61–2.97</td>
<td>2.41 ± .47</td>
</tr>
<tr>
<td>68 (B)</td>
<td>.66</td>
<td></td>
</tr>
<tr>
<td>148 (C)</td>
<td>1.1</td>
<td></td>
</tr>
<tr>
<td>269 (C)</td>
<td>.89</td>
<td></td>
</tr>
<tr>
<td>467 (C)</td>
<td>.86</td>
<td></td>
</tr>
<tr>
<td>519 (BC)</td>
<td>.53</td>
<td></td>
</tr>
</tbody>
</table>

Note.—Intensities of the 2.8-kb α-mRNA band and of the 2.0-kb β-mRNA band were measured by densitometry, and the β-mRNA:α-mRNA ratios determined. All values are the average of duplicate determinations.

Southern Blot Analysis

Genomic DNA from all cell lines was examined to determine whether any gross alterations in gene structure could be identified. No differences from the normal gene pattern were identified following digestion with BamHI and Rsal (data not shown).

Steady-State Levels of PCC Subunits in Normal and Propionic Acidemia Patients

To analyze the PCC polypeptides under steady-state conditions, we carried out Western blot analysis using rabbit anti-native human PCC antiserum. Figure 2 shows a typical set of results. Both α and β subunits were readily detected in normal cell lines (figs. 2a and 2b, lanes N) and in the two parents of pccB patient 269 (fig. 2b, lanes 284 and 285). Neither α nor β subunits were detected in any of the five pccA patients tested (results for three are shown in fig. 2a). Whereas α subunits in the nine patients of the pccBC group were unremarkable (fig. 2b), the results obtained for the β subunits in this group were more complex. No β subunit was detected in one pccB cell line (no. 68); two lines (nos. 1751 [pccB] and 148 [pccC]) had decreased amounts of normal sized β subunit; and the remaining six patients (nos. 467 [pccC], 269 [pccC], 519 [pccBC], all shown in fig. 2b, and 1752 [pccC], 534 [pccBC], and 1747 [pccBC], none shown) had β subunits smaller than normal size and greatly reduced in quantity. These smaller β subunits, migrating just above the background band of the heavy chain of IgG, were reproducibly detectable in these six lines: It is interesting that the parents of patient 269 (284 and 285) and that other parents (not shown) had no detectable levels of the

Table 2

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Group</th>
<th>α</th>
<th>β</th>
</tr>
</thead>
<tbody>
<tr>
<td>1159</td>
<td>N</td>
<td>+ + + + +</td>
<td>+ + + + +</td>
</tr>
<tr>
<td>117 (447)</td>
<td>A</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>427</td>
<td>A</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>540</td>
<td>A</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>1748 (499)</td>
<td>A</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>1749 (533)</td>
<td>A</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>68 (445)</td>
<td>B</td>
<td>+ + + + +</td>
<td>–</td>
</tr>
<tr>
<td>1751 (537)</td>
<td>B</td>
<td>+ + + + +</td>
<td>+</td>
</tr>
<tr>
<td>148 (448)</td>
<td>C</td>
<td>+ + + + +</td>
<td>+</td>
</tr>
<tr>
<td>269</td>
<td>C</td>
<td>+ + + + +</td>
<td>+ c</td>
</tr>
<tr>
<td>467 (539)</td>
<td>C</td>
<td>+ + + + +</td>
<td>+ c</td>
</tr>
<tr>
<td>519 (536)</td>
<td>BC</td>
<td>+ + + + +</td>
<td>+ c</td>
</tr>
<tr>
<td>534 (500)</td>
<td>BC</td>
<td>+ + + + +</td>
<td>+ c</td>
</tr>
<tr>
<td>1747 (433)</td>
<td>BC</td>
<td>+ + + + +</td>
<td>+ c</td>
</tr>
</tbody>
</table>

a Estimated by the intensity of the fluorographic image for a given line relative to that of the concurrently exposed control line. + + + + + = Same as control; + + = moderately intense but less than half that of the control; + = detectable but much less than half that of the control; – = undetectable.

b Yale accession number. Numbers in parentheses are those used by Lamhonwah et al. (1983).

c Subunit is smaller than that in control.
smaller β subunits (see Discussion). Table 2 summarizes the data from these Western blots.

**Biogenesis of β Subunits in Normal and PCC-deficient Fibroblasts**

To determine whether normal β subunits are initially synthesized in the pccA mutants and whether smaller β subunits are produced in some of the pccBC-group patients, we pulse-labeled fibroblasts from these groups with L-[35S]methionine in the presence of DNP, a mitochondrial poison. In normal cells, the β subunit was synthesized as a precursor in the presence of DNP and could then be processed to mature β subunit during a chase with cold methionine in the absence of DNP (fig. 3a, lanes N). The results of experiments with pccA line 427, which had no detectable β subunit at steady state, and with pccC line 467, which had a β subunit of reduced molecular weight, were indistinguishable from those in normal cells when examined at 0- and 10-min intervals. When a longer chase period (60 min) was used, however, the β subunit in the pccA patient disappeared (fig. 3b, lane 427, 60 min), while it remained detectable in both the normal and the pccC patient.

![Figure 3](image-url)

**Figure 3** Analysis of newly synthesized β subunit in fibroblasts from normal and PCC-deficient patients. a), Four 10-cm dishes of fibroblasts were labeled with L-[35S]methionine in the presence (+) of 4 mM DNP. The chase was carried out, with two dishes, in MEM-E containing 5 mM methionine, for 10 min in the absence (−) of DNP. b), Four 10-cm dishes of fibroblasts were labeled with L-[35S]methionine in the absence of DNP, and two were subsequently chased with MEM-E containing 5 mM methionine, for 60 min. Harvest, immunoprecipitation, electrophoresis, and fluorography were carried out according to a method described elsewhere (Ikeda et al. 1985) Preβ = migration position of the normal β subunit precursor; β = position of the normal mature β subunit; X = background band. All other abbreviations are as in fig. 1.

We carried out a similar experiment on cell line 68 (pccB), which had no detectable β subunit under steady-state conditions. In this mutant, the precursor of the β subunit was easily detectable following the pulse-labeling but disappeared during the 10-min chase (fig. 3a, lanes 68; X is a background band). With shorter chase periods, a trace of a mature β subunit was occasionally detected (not shown).

**Synthesis and Stability of PCC Subunits in HeLa Cells**

We used HeLa cells to study synthesis and stability of normal α and β PCC subunits because of both higher levels of expression of PCC in HeLa cells and higher signal:noise ratio following immunoprecipitation of [35S]-labeled cells. Cells were pulse-labeled with L-[35S]methionine for 30 min and then chased with cold methionine for as long as 4 h. As shown in figure 4, the β subunit appeared to be synthesized in approximately fourfold excess compared with the α subunit (fig. 4, lane 0). During the chase, the amount of β subunit decreased rapidly and reached a steady-state level in about 2 h. In contrast, the amount of α subunit remained constant for as long as 4 h. The initial excess of β over α subunits may be even greater than that estimated by densitometry, because the mature α subunit contains twice as many methionine residues as does the β subunit (Kraus et al. 1986a; M. Browner, personal communication) and, therefore, yields a relatively more dense protein band after immunoprecipitation.

**Discussion**

Previous work by Lamhonwah et al. (1983) revealed both combined α and β subunit deficiencies in pccA

![Figure 4](image-url)

**Figure 4** Synthesis and stability of PCC subunit in HeLa cells. For each time point, two 10-cm dishes of HeLa cells were radiolabeled for 30 min with L-[35S]methionine in the absence of DNP and were chased with MEM-E containing 5 mM methionine for the indicated times. Harvest, immunoprecipitation, electrophoresis, and fluorography were carried out according to a method described elsewhere (Ikeda et al. 1985). aPCC = migration position of mature α subunit; βPCC = position of mature β subunit.
patients and isolated absence of the β chain in pccC and pccBC patients at steady state. Recently, Lamhonwah et al. (1986) isolated partial cDNA clones coding for the α and β chains of human PCC. Using these cDNA clones, they found no α-mRNA in four of six pccA patients. On the other hand, both α-mRNA and β-mRNA were present in pccBC, pccB, and pccC mutants (Lamhonwah and Gravel 1987). No quantitative analyses of the mRNAs were done in their experiments. From these data, Lamhonwah et al. (1983; Lamhonwah and Gravel 1987) assigned the PCC genes to the complementation groups (PCCA gene = pccA complementation group; PCCB gene = pccBC, pccB, and pccC groups) and proposed that pccA patients may synthesize a normal β chain which is rapidly degraded in the absence of α chains with which to assemble.

Our Northern blot data confirmed and expanded these findings. The virtual absence of α-mRNA in four of four pccA patients agreed with their data (fig. 1). Occasionally, tiny amounts of α-mRNA were detectable in some of the pccA patients when increased amounts of poly(A)* RNA were blotted. Analysis of genomic DNA from these patients failed to show any gross alterations in the PCC genes. Together, these data suggest that the α subunit absence characteristic of most pccA patients is not due to large deletions or insertions in the PCC gene but rather is due to instability of α-mRNA. In addition, we estimated the relative amounts of β-mRNA in normal and PCC-deficient fibroblasts. In normal cells, β-mRNA is two- to threefold more abundant than α-mRNA; β-mRNA is decreased in amount in all subgroups of β-PCC-deficient patients, leading to an approximately threefold fall in the β-mRNA:α-mRNA ratio from a mean of 2.4 to 0.78 (table 1). This confirms that pccB, pccC, and pccBC patients have defects in the PCCB gene that lead to decreased production and/or increased degradation of β-mRNA.

Our protein-blot data (table 2), showing the absence of both α and β subunits in five pccA patients, are consistent with the data from Lamhonwah et al. (1983). In contrast, the analysis of β subunits in the pccBC mutant group is quite different from theirs (fig. 2b). We detected β subunit in eight of nine βPCC-deficient cell lines, while they reported that the subunit was absent in six of seven patients. Because we and Lamhonwah et al. (1983) analyzed most of the same cell lines (table 2), we propose that this discrepancy is due to different experimental procedures. Lamhonwah et al. partially purified PCC protein from fibroblasts before immunoprecipitation; it is, therefore, possible that the mutant β subunits were lost during this procedure. Our results show that six cell lines had the same pattern, namely, one in which the β subunit was both greatly reduced in quantity and decreased in size. This finding, and evidence that these smaller β subunits may be degradation products of one or both of the mutant β chains (see below), is consistent with the notion that the mutation in most of these mutants may be identical. In fact, preliminary results indicate that one of the two mutant PCCB alleles in all three pccBC patients and in some of the pccC patients may contain the same mutation (Tahara et al. 1988).

We have further extended the analysis of PCC protein biogenesis in the mutant lines by performing pulse/chase experiments to study the newly synthesized subunits in intact cells. Our results show that the β subunit was initially synthesized but rapidly degraded in a pccA line (no. 427; figs. 3a and 3b). This result confirms the hypothesis that in pccA patients the β subunit is synthesized normally but is highly unstable in the absence of the α subunit and is rapidly degraded. Similar findings were made in several cases of pyruvate dehydrogenase deficiency. In patients with no mRNA encoding the α subunit and with normal amounts of β-mRNA, absence of both subunits was observed, leading to the conclusion that the remaining uncomplexed β subunit is unstable (Wexler et al. 1988).

The size of the mature β subunit in pccC line 467 following a 60-min chase is indistinguishable from that in normal cells (fig. 3b, lane 467, 60 min). The cause of the discrepancy between the steady-state data, which showed a smaller β subunit (fig. 2b), and this cell labeling experiment is unclear. It may simply be that the degradation of this mutant β subunit proceeds relatively slowly. In contrast, the cell-labeling experiment with cell line 68 (pccB) showed a different pattern (fig. 3a, lanes 68). The precursor of the β subunit was clearly detected after pulse labeling, but only a trace of mature β subunit could be seen after a 10-min chase. These data suggest that the precursor of this β subunit is synthesized normally but is degraded very rapidly after processing in mitochondria. It is interesting that the α subunit appears to be stable in this patient in the complete absence of the β subunit (fig. 2b).

To examine further the normal biosynthesis and degradation of both α and β subunits of PCC, we used L-[35S]methionine pulse-labeled HeLa cells. The data in figure 4 show that the β subunit is initially synthesized in several-fold excess relative to the α subunit (0 min) but turns over much faster than the α subunit (cf. 120 min). Because this experiment does not distinguish free α subunits from those present in assembled holoen-
zyme, it is possible that free \( \alpha \) subunits may still be present following the chase, further increasing the intensity of the \( \alpha \) subunit band. Initial excess of \( \beta \) subunit over \( \alpha \) subunit is consistent with our finding that normal fibroblasts and normal liver contain approximately three times as much \( \beta \)-mRNA as \( \alpha \)-mRNA. A contributing factor may also be a slower rate of translation of the \( \alpha \) subunit, owing to its extremely long 5'-untranslated region of \( \geq 700 \) bp (M. Browner, personal communication). Nearly identical conclusions were made when biogenesis of spectrin in murine erythroleukemia cells was studied (Lehnert and Lodish 1988). Accumulation of equal amounts of \( \alpha \) and \( \beta \) spectrin was a result of unequal synthesis \( \left( \alpha: \beta \sim 1:3 \right) \) and unequal degradation which was approximately three times faster for \( \beta \) than it was for \( \alpha \).

The initial large excess of \( \beta \) subunit compared with \( \alpha \) subunit may also explain the finding that parents of \( \beta \)PCC-deficient patients have normal PCC activity in their fibroblasts. We have shown that mutant \( \beta \)-mRNA is decreased significantly in the cell lines from all subgroups of the \( \text{pCC} \) patients. Thus, it is reasonable to assume that cells from their parents contain significantly more normal than mutant \( \beta \)-mRNA. Because of the initial several-fold excess of the \( \beta \) subunit compared with the \( \alpha \) subunit in control cells, there are sufficient amounts of normal \( \beta \) subunits in these heterozygotes to preferentially assemble with the \( \alpha \) subunits, and the PCC activity remains within the normal range. The control levels of \( \beta \) subunits and the absence of any detectable degraded \( \beta \) subunits in cells from parents of \( \alpha \) \( \text{pCC} \) patient (fig. 2, lanes 284 and 285)—and in cells from other parents of the \( \beta \)PCC-deficient patients (not shown)—are consistent with this hypothesis.

Acknowledgments

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