Variants with Temperature-sensitive Folding Defects Occurs after Import into Mitochondria*  

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Most disease-causing missense mutations in short-chain acyl-CoA dehydrogenase (SCAD) and medium-chain acyl-CoA dehydrogenase are thought to compromise the mitochondrial folding and/or stability of the mutant proteins. To address this question, we studied the biogenesis of SCAD proteins in COS-7 cells transfected with cDNA corresponding to two SCAD missense mutations, R22W (identified in a patient with SCAD deficiency) or R22C (homologous to a disease-associated R28C mutation in medium-chain acyl-CoA dehydrogenase deficiency). After cultivation at 37 °C the steady-state amounts of SCAD antigen and activity in extracts from cells transfected with mutant SCAD cDNAs were negligible compared with those of cells transfected with SCAD wild type cDNA, documenting the deleterious effect of the two mutations. Analysis of metabolically labeled and immunoprecipitated SCAD wild type and mutant proteins showed that the two mutant proteins were synthesized as the 44-kDa precursor form, imported into mitochondria and processed to the mature 41.7-kDa form in a normal fashion. However, the intramitochondrial level of mature mutant SCAD proteins decreased rapidly to very low levels, indicating a rapid degradation of the mutant proteins at 37 °C. A rapid initial elimination phase was also observed following cultivation at 26 °C; however, significantly higher amounts of metabolically labeled and immunoprecipitated mature mutant SCAD proteins remained detectable. This corresponds well with the appreciable steady-state levels of SCAD mutant enzyme activity observed at 26 °C. In addition, confocal laser scanning microscopy of immunostained cells showed that the SCAD mutant proteins were localized intramitochondrially. Together, these results show that newly synthesized SCAD R22W and R22C mutant proteins are imported and processed in the mitochondrial matrix, but that a fraction of the proteins is rapidly eliminated by a temperature-dependent degradation mechanism. Thermal stability profiles of wild type and mutant enzymes revealed no difference between the two mutants and the wild type protein. Furthermore, the turnover of the SCAD mutant enzymes in intact cells was comparable to that of the wild type, indicating that the rapid degradation of the mutant SCAD proteins is not due to lability of the correctly folded tetrameric structure but rather to elimination of partly folded or misfolded proteins along the folding pathway.

A number of mutations have been identified in human inborn errors of both short-chain acyl-CoA dehydrogenase (SCAD)1 (EC 1.3.99.2) and medium-chain acyl-CoA dehydrogenase (MCAD) (EC 1.3.99.3) (1–5). SCAD and MCAD are two of four homologous acyl-CoA dehydrogenases that catalyze the initial α,β-dehydrogenation step in mitochondrial β-oxidation of straight-chain fatty acids (6–8). Subunits of both enzymes are nuclear encoded and synthesized in the cytosol as precursor proteins, whereupon they are imported into mitochondria, proteolytically processed, folded, and assembled into the biologically active homotetrameric form in the matrix, with one molecule of flavin adenine dinucleotide per subunit (9). Although the turnover of SCAD and MCAD proteins in cultivated skin fibroblasts (10, 11) and the involvement of chaperones in folding (12, 13) have been studied, the degradation mechanism of these proteins is not known at present.

In one of the patients with SCAD deficiency two distinct mutant alleles of the SCAD gene were identified, which shows that this patient is a compound heterozygote (1). Both mutant alleles were found to contain a C to T transition, one at position 136 and the other at position 319 of the cDNA sequence. The 136 C → T transition, leading to replacement of arginine-22 in the mature protein with tryptophan (R22W), is of particular interest, as a disease-causing mutation at the homologous position in MCAD (R28C) has been described (14). Analysis of cultivated skin fibroblast cells from the patient harboring the two SCAD mutant alleles has shown that the synthesized SCAD R22W mutant protein is unstable (10).

To investigate further the deleterious effect of the R22W mutation we have studied this SCAD mutant protein in a COS-7 cell-derived expression system at 37 °C. Using such a system we have shown that the SCAD R22W enzyme activity is undetectable at steady state (40), whereas MCAD R28C displayed activity levels from 50 to 100% of MCAD wild type (WT) (14, 15). Furthermore, the folding properties of the MCAD mutant were studied in Escherichia coli. By manipulating the amount of available chaperones and growth temperature of E. coli cells overexpressing this mutant, the yield of active enzyme

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could be modulated between undetectable and 100% of the MCAD WT level, thus indicating that the deleterious effect of the mutation is predominantly related to the folding process (12).

Sequence alignment of human precursor pSCAD and pM-CAD shows that these proteins have highly similar sequences with 37% identical residues (16). Moreover, the two enzyme proteins display almost superimposable three-dimensional structures (17, 18) and basically identical catalytic mechanisms (19). Following mitochondrial import, unfolded MCAD (and by inference possibly also unfolded SCAD) initially complexes with mitochondrial hsp70 and subsequently with hsp60, whereby it attains an assembly-competent conformation (13). In view of the structural similarity of MCAD and SCAD, it is surprising that the expression of the SCAD R22W protein is much lower than the MCAD R28C protein. An explanation for this may be that the folding of the SCAD R22W protein is very severely compromised, resulting in almost complete proteolytic elimination. We therefore speculate that the SCAD mutant may serve as probe for the processes in the protein quality control machinery in which chaperones promote folding of folding-competent chains and proteases eliminate aberrant polypeptide chains (20, 21).

As a first approach to define the protein quality control system involved we have studied the synthesis, intracellular processing, localization, residual enzyme activity, thermal stability profiles, and site of degradation of the protein variants SCAD R22W and SCAD R22C in transfected COS-7 cells cultivated at different temperatures.

**EXPERIMENTAL PROCEDURES**

**Plasmids—** Mutagenesis of the SCAD WT cDNA was performed according to the megaprimer polymerase chain reaction mutagenesis procedure described by Sarkar and Sommer (22). As template for the SCAD mutagenesis plasmid pCpreWT (vector comprising the coding region of human SCAD WT cDNA) (23) was used. Amplified product was purified and ligated into the pcRII vector (Invitrogen) followed by transformation of competent E. coli cells. Clones were checked for the absence of polymerase chain reaction errors. SCAD WT, R22W, and R22C plasmid DNA fragments were isolated from vectors and ligated into the expression plasmid pM6 (an adenovirus-associated virus-based vector) between the cytomegalovirus promoter and the SV40 processing signal. Supercopied pM6 plasmids were purified by using a Maxi preparation kit (Qiagen).

**Expression of SCAD Proteins in COS-7 Cells and Analysis of Extracts—** COS-7 cells (24) were cultivated in 75-cm² flasks, 10-cm² slide flasks (Nunc, Roskilde, Denmark) at 5% (v/v) CO₂ in RPMI 1640 medium (In Vitro, Copenhagen, Denmark) containing 10% (v/v) fetal calf serum (Life Technologies, Inc.). COS-7 cells were transfected with SCAD WT, R22C, or R22W cDNA constructs using the calcium phosphate co-precipitation method (25, 26). As a negative control for SCAD expression we used the vector without SCAD cDNA insert. All cells were cultivated at 37 °C, 33 °C, or 26 °C and examined between 24 and 48 h post-transfection. In some experiments cells were fixed in freshly prepared 4% (w/v) paraformaldehyde (Merck) for 5 min at 4 °C followed by one wash in phosphate-buffered saline. Disruption of cells and Northern and Western blotting were performed as described elsewhere (26, 27). As primary antibody a polyclonal rabbit anti-rat SCAD antibody was used (28). The protein concentration was determined by the Bradford assay (Bio-Rad). The activity of SCAD enzymes was determined by the ferricyanide-based colorimetric assay described previously (29). When rhodamine 6G chloride (Sigma) was added, it was used to add the medium at a final concentration of 2.1 µM 30 min before the medium was replaced with the medium containing label (31, 32). The cells were then incubated for 15 min in the labeling medium containing the same concentration of rhodamine 6G. Cells in the chase experiments were labeled as described above. After a 15-min pulse, the labeling medium was removed and cells were rinsed twice in phosphate-buffered saline. Then 1 ml of medium containing no label was added to each dish, and the cells were harvested after a 15-, 30-, 60-, or 180-min chase period, respectively.

**Immunoprecipitation of Radiolabeled SCAD Proteins—** The frozen cells were resuspended, lysed, and immunoprecipitated as described previously (30). 10 µg of anti-rat SCAD antibody (29) was added to each sample. Aliquots from immunoprecipitated SCAD protein samples were subjected to SDS-PAGE. The gels were subjected to fluorography using Amplify (Amersham Pharmacia Biotech), dried, and fluorographed as recommended by the manufacturer. Protein bands were quantitated using a PhosphorImager (Molecular Dynamics).

**Influence of Temperature on Yield of Active SCAD Mutant Enzymes—** We have previously shown that the SCAD R22W protein and enzyme activity in transfected COS-7 cells are undetectable at steady state (15). This is in contrast to the behavior of the MCAD R28C protein with the positionally homologous mutation (14, 15). To examine the extent to which this difference is caused by the different replacements at the homologous position, the SCAD R22C mutation was also constructed, and plasmids harboring the various SCAD cDNAs (WT, R22C, and R22W) were transfected into COS-7 cells. Transcription and processing of SCAD mRNA did not differ significantly as judged by the amount of SCAD mRNA (Northern blot analysis; data not shown).

Because lower temperature in itself has a positive effect on folding of MCAD mutant proteins (12), the effect of cultivation temperature (26 °C, 33 °C, and 37 °C) on the biogenesis of active SCAD mutant proteins was tested. For the WT enzyme, the activity in the samples was highest at 33 °C (Fig. 1, top). The activity at 26 °C and 33 °C was about 68% and 121% of the activity at 37 °C, respectively. In all three samples immunoreactive protein with a size corresponding to that of mature SCAD WT protein was observed (Fig. 1, bottom) by immunoblotting with rabbit anti-rat SCAD antibodies. At 37 °C, no significant SCAD activity above background could be detected from COS-7 cells transfected with SCAD R22W or R22C cDNAs, showing that at this temperature no significant amount of active mutant enzyme is accumulated. This notion was further supported by the fact that only slightly higher amount of immunoreactive protein than detected with the background level were observed in these cells at 37 °C. However, the residual activity of the two SCAD mutant variants became detectable at reduced cultivation temperatures. For the R22C variant the activity was increased up to approximately 12% (33 °C) and 20% (26 °C) of that of WT activity (Fig. 1). The activity of the R22W variant was increased up to approximately...
2% (33 °C) and 6% (26 °C). Concomitantly, increased levels of immunoreactive SCAD mutant proteins became detectable at these temperatures. This shows that the SCAD mutations affect protein biogenesis and that the mutant enzymes can be partially rescued by reducing the cultivation temperature.

Thermal Stability Profiles of SCAD Variant Enzymes—Reduced cultivation temperature might have an influence on the kinetic stability of the synthesized SCAD proteins. As a measure of this parameter thermal stability profiles of residual active mutant enzymes produced at the permissive temperature (26 °C) were determined in extracts from transfected COS-7 cells. Aliquots from cell extracts were incubated at different temperatures ranging from 4 °C to 60 °C for 10 min, and the SCAD enzyme activity was measured in duplicate with the colorimetric assay using the ferricenium ion as electron acceptor (range indicated by bars). The residual enzyme activities measured are plotted versus the incubation temperature (percentage of activity measured at 32 °C). For comparison, the profile obtained with extracts from cells overexpressing SCAD G185S enzyme is shown. A, SCAD WT and SCAD R22C; B, SCAD WT and SCAD R22W; C, SCAD WT and SCAD G185S. These results are representative of two separate experiments.

Turnover Analysis of SCAD Variant Enzymes in Protein Synthesis-arrested COS-7 Cells—Next we investigated whether the
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unaltered resistance to thermal inactivation of SCAD variant enzymes observed correlates with an unchanged kinetic stability of the variant enzymes in intact transfected COS-7 cells cultivated at 26 °C. For this purpose SCAD activity was measured at different time points in cells where the protein synthesis was arrested with cycloheximide. As shown in Fig. 3, the SCAD activity, normalized on a percentage scale, from cells expressing WT, R22C, or R22W mutant enzymes remains unchanged up to 24 h after addition of cycloheximide. This illustrates that for that proportion of the mutant tetramers which acquires the native conformation the kinetic stability in the cells cultivated at 26 °C is indistinguishable from that of WT.

Intramitochondrial Degradation of SCAD Variant Proteins—To compare further the intracellular fate of the SCAD WT and mutant proteins pulse-labeling experiments of transfected COS-7 cells cultivated at 37 °C followed by immunoprecipitation were performed. Cells were labeled for 15 min in the absence or presence of rhodamine 6G, an inhibitor of the mitochondrial uptake of precursor polypeptides (10). Aliquots of immunoprecipitated SCAD proteins were subjected to SDS-PAGE and fluorography as shown in Fig. 4. When pulse-labeled in the absence of rhodamine 6G, a band with a molecular mass of 41.7 kDa was observed, representing mature SCAD protein (Fig. 4, lanes 1–3). Scanning of the gels of two separate experiments shows that the signal of the fluorographic band for the mature SCAD protein varies up to 24 h after addition of cycloheximide. This illustrates that for that proportion of the mutant tetramers which acquires the native conformation the kinetic stability in the cells cultivated at 26 °C is indistinguishable from that of WT. The lack of detection of the two mutant SCAD proteins at steady state at 37 °C, observed in Fig. 1, therefore cannot be due to cytoplasmic degradation or defective transport into mitochondria. As the kinetic stability of the correctly folded enzyme variants is indistinguishable from that of WT (see above), we hypothesize that the disappearance is caused by increased intramitochondrial degradation of partly folded or misfolded intermediates.

In order to test this hypothesis, pulse-chase and immunoprecipitation experiments in transfected COS-7 cells cultivated at two different temperatures (37 °C and 26 °C) were performed. In all cases the initial counts were normalized to 100% (Fig. 5). In cells expressing WT protein at 37 °C the intensity of the fluorographic band for mature SCAD decreased only slightly with time, and after 3 h the signal was approximately 80% of the signal immediately after the pulse labeling (Fig. 5A). However, in cells transfected with either SCAD R22C or R22W cDNAs, the bands corresponding to the two mutant proteins were clearly diminished at 15 min to approximately 40%. After 1 h only approximately 20% of the SCAD R22C and R22W proteins were left, and after 3 h the R22W and R22C bands were almost unchanged compared with those detected at 1 h (Fig. 5A).

At 26 °C the turnover of SCAD WT was similar to that observed at 37 °C (Fig. 5, A and B). If the failure to detect the two SCAD mutant proteins at steady state (Fig. 1) was due to
degradation of unstable intermediates, improved folding conditions should increase the cellular level of R22C and R22W SCAD mutants. In fact, as shown in Fig. 5B, we found that reduced cultivation temperature increased the intramitochondrial level of SCAD R22C and R22W compared with the level detected at physiological temperature. The intensity of both mutant proteins was approximately 70% after 15 min of chase. However, after 30 min the intensity of the R22W band was decreased to a lower level than that of R22C. After 1 h the rapid elimination phase of the two mutant bands had almost stopped, and at 3 h the intensities of the R22C and R22W bands were approximately 45% and 10%, respectively. This indicates that the mutations strongly affect the kinetic stability of the mutant proteins at some stage in the folding pathway, and that this effect is to some extent compensated by reduced cultivation temperature.

**Confocal Laser Scanning Microscopic Analysis of SCAD Proteins in Transfected COS-7 Cells**—To pinpoint the intracellular localization of the SCAD R22C and R22W mutant proteins, double-labeling with the anti-rat SCAD antibody and a monoclonal antibody directed against human hsp60 followed by confocal laser scanning microscopy of transfected COS-7 cells was performed (Fig. 6). Analysis of cells cultivated at 26 °C revealed in all cases a typical mitochondrial staining pattern, and no other subcellular structure was labeled. No mitochondrial staining was observed in cells transfected with vector without SCAD cDNA insert (data not shown). Furthermore, our results showed complete co-localization between SCAD and hsp60 immunoreactivity, demonstrating that mutant SCAD and hsp60 produced at 26 °C co-localize in mitochondria. These data further corroborate our hypothesis that SCAD R22C and R22W mutant proteins are imported and processed in the mitochondrial matrix.

**DISCUSSION**

The data presented indicate strongly that substitution of the conserved arginine-22 in SCAD with either tryptophan or cysteine compromises biogenesis and/or turnover of these proteins, as evidenced by the negligible amounts of mutant proteins detectable in transfected cells cultivated at 37 °C. The aim of the present investigation was to elucidate the cellular mechanisms involved in the handling of these missense mutant SCAD proteins. SCAD and MCAD are mitochondrial matrix proteins and participate in the fatty acid β-oxidation spiral. The biogenesis and turnover of the nuclear encoded mitochondrial SCAD and MCAD matrix proteins involves a series of steps, all of which may be influenced by mutations. However, missense mutations in genes involved in the fatty acid oxidation very rarely compromise the transcription of the gene and the maturation of the RNA transcript (14, 16, 33). Because the
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136 C→T transition leading to the R22W substitution is localized distally to the exon-intron boundaries (34), this mutation would not be expected to affect maturation of the precursor mRNA. We therefore reasoned that the primary pathogenic defect in the two SCAD mutant proteins investigated here resides in their biogenesis. To elucidate the mechanisms involved we have transfected COS-7 cells with cDNA containing the two SCAD missense mutations. Northern blot analysis of the transfected COS-7 cells cultivated at 37 °C revealed no difference in the levels of SCAD mRNA from the mutant and WT SCAD cDNAs and showed that the defective mechanism is post-transcriptional. The post-transcriptional processes are: 1) translation of the mRNA to the 44-kDa pSCAD; 2) targeting to and transport through the mitochondrial membrane and maturation of pSCAD to the 41.7-kDa mature SCAD; 3) folding and assembly of the mature SCAD to the tetrameric functional SCAD; and 4) degradation of the tetrameric SCAD protein.

Our investigations have pinpointed the defective site in the biogenesis pathway of the R22W and R22C mutant proteins to folding of the polypeptide chains. The evidence is based on the following experimental findings. First, both SCAD R22W and R22C mutant proteins are synthesized as the 44-kDa precursor form, imported into mitochondria, and processed to the mature 41.7-kDa form in a normal fashion. Second, the elimination of the imported mutant proteins is biphasic (Fig. 5). At 37 °C the initial phase is very fast and proceeds nearly to complete elimination before reaching the second phase, which seems to parallel the kinetics of the WT protein. At 26 °C the initial phase is slower and the second phase is reached at a higher level, from which the elimination parallels the WT. The elimination rate in the initial phase is thus temperature-dependent and the amounts of rescued mutant SCAD proteins are also dependent on the cultivation temperature. The temperature effect is possibly a combination of both slowing productive folding and misfolding pathways as well as the rate of degradation. Third, following a lowering of the cultivation temperature the steady-state SCAD activity from the R22W and R22C mutant proteins increases from negligible up to approximately 6% and 20% of WT activity, respectively. Furthermore, confocal laser scanning microscopy of immunostained cells showed that the mutant SCAD proteins are localized intramitochondrially. Thus, there is a correlation between the increase in the protein amounts left for the second phase in the pulse-chase experiments and the amount of rescued intramitochondrial enzyme in the steady-state experiments. We therefore conclude that the majority of the SCAD protein detected in the second phase represents active tetrameric SCAD and that the steep slope in the initial phase of the pulse-chase experiment is due to impaired folding of the SCAD mutant proteins and a rapid elimination of folding intermediates at some stage in the folding pathway. This interpretation of the results is supported by the normal kinetic stability of the tetrameric functional SCAD mutant proteins at low cultivation temperature, which indicates that the degradation rate of the tetramers is not affected. In a similar fashion, it has been shown that temperature-sensitive folding mutations in, for example, the phage P22 tail spike and coat proteins, which show stability identical to that of WT, can be rescued by lowering the cultivation temperature (35, 36).

This interpretation of our results is compatible with the emerging view of the biogenesis pathway and quality control concept for mitochondrial proteins (20). Consistent with this view it has been shown that the homologous MCAD protein, upon import into the mitochondria, first forms an intermediary complex with the mitochondrial hsp70 chaperone before it is handed over to the hsp60 chaperonin which, in cooperation with the co-chaperonin hsp10, assists folding to the native structure (13). Work on the bacterial hsp60/10 homologues GroEL and GroES (for review, see Refs. 37 and 38) has suggested that hsp60/10-assisted folding occurs through iterative rounds of binding in the cavity of the chaperonin ring complex and ATP-driven release of fully or partly folded polypeptide chains. After release from the chaperonin, polypeptide chains committed to the native state do not rebind to the chaperonin, whereas partly folded or misfolded chains may bind again and try a second round. During each round a certain percentage of the protein is fully folded, implying that another fraction, which is not folded to the final structure, may be susceptible to proteolytic attacks. It can be inferred from this model that mutations which impair folding will decrease the fraction of the protein that is fully folded after each round. This may in turn result in a larger fraction of partly folded or misfolded chains that at any time is complexed with the chaperonins, as has been observed for the MCAD K304E mutant (13). Moreover, co-overexpression of the GroESL chaperonins and a series of missense mutant SCAD (G185S) and MCAD (K304E and R28C) proteins in E. coli has shown that increasing the pool of chaperonins increases the fraction of these proteins that acquire the native structure (5, 12, 23, 27). Our observation that

**FIG. 6. Co-localization of SCAD mutant and hsp60 proteins in transfected COS-7 cells visualized by confocal laser scanning microscopy.** Cells were maintained (26 °C) and transfected in slide flasks, fixed, and permeabilized. The localization of overexpressed SCAD proteins was visualized using the rabbit anti-rat SCAD antibody followed by fluorescein-conjugated porcine anti-rabbit antibodies. Endogenously expressed hsp60 was stained with mouse anti-human hsp60 antibody followed by rhodamine-conjugated goat anti-mouse antibody. **Left (A, C, and E),** SCAD labeling in a focal section of COS-7 cells transfected with SCAD WT, R22C, or R22W cDNA, where fluorescein-labeled antibodies indirectly immunostain SCAD. **Right (B, D, and F),** labeling of endogenously expressed hsp60 in same focal sections as shown on the left. Rhodamine fluorescence represents antibodies visualizing hsp60 in mitochondria. The results are representative of two separate experiments. A and B, cells transfected with SCAD WT cDNA; C and D, cells transfected with SCAD R22C cDNA; E and F, cells transfected with SCAD R22W cDNA.
the elimination rate of the SCAD R22W and R22C mutant proteins is slowed by lowering the temperature and that the rescued fraction of mutant proteins is increased support the concept of a mitochondrial protein quality control system, as has been proposed by Langer and Neupert (20).

Because only negligible amounts of the SCAD mutant proteins “escape” the quality control system at physiological temperature and because they can only be partially rescued by cultivating the cells at 26 °C, we believe that the two SCAD mutations investigated here can be considered as severe folding mutations. Conversely, we have characterized a SCAD R147W mutation in a number of patients with SCAD deficiency that may be considered as a mild folding mutation (39). This mutant protein, which actually represents a polymorphic variant with an allele frequency of 8% in the general population, displays a steady-state SCAD activity in transfected COS-7 cells of 45% of WT at 37 °C. At 26 °C the activity can be rescued to 85% of WT activity. This illustrates that the cellular handling of missense mutations may be quite different for different mutations.

Taken together, we have demonstrated that the two SCAD mutant variants studied here are subjected to rapid intramitochondrial degradation of partly folded or misfolded intermediates and that they can be partially rescued in intact cells by lowering the cultivation temperature. Furthermore, the study has indicated that a matrix-located mitochondrial protein quality control system is involved in the cellular handling of certain SCAD mutant proteins. Further work is in progress to elucidate the components of the proteolytic system involved in the degradation of misfolded mitochondrial proteins.

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REFERENCES