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Sequence elements essential for the rapid turnover of *Crithidia fasciculata* ornithine decarboxylase

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Summary. Ornithine decarboxylase (ODC) has a very fast turnover in mammalian cells, but is a stable enzyme in *T. brucei* and other trypanosomatid parasites like *Leishmania donovani*. However, *Crithidia fasciculata*, which is a phylogenetically closely related trypanosomatid to *L. donovani*, has an ODC with a rapid turnover. Interestingly, *C. fasciculata* ODC, but not *L. donovani* ODC, is rapidly degraded also in mammalian systems. In order to obtain information on what sequences are important for the rapid degradation of *C. fasciculata* ODC, we produced a variety of *C. fasciculata*/*L. donovani* ODC hybrid proteins and characterized their turnover using two different mammalian expression systems. The results obtained indicate that *C. fasciculata* ODC contains several sequence elements essential for the rapid turnover of the protein and that these regions are mainly located in the central part of the enzyme.

Keywords: Polyamines – Ornithine decarboxylase – Protein turnover – *Crithidia fasciculata* – *Leishmania donovani*

Introduction

Ornithine decarboxylase (ODC) catalyzes the first and what is often considered as the rate-limiting step in the biosynthesis of polyamines, which are cellular constituents essential for growth (Heby and Persson, 1990; Pegg et al., 1995). The enzyme is strongly regulated in mammalian cells. The regulation is dependent on a very fast turnover of the enzyme, with a half-life in the minutes–hour range (Heby and Persson, 1990; Coffino, 2001; Persson, 2006). The molecular mechanisms involved in the rapid degradation of mammalian ODC are presently being disclosed. The C-terminal part of mammalian ODC has been demonstrated to be highly important for the fast turnover of the enzyme (Ghoda et al., 1989; Rosenberg-Hasson et al., 1991; Takeuchi et al., 2007). If this part is removed or changed by mutagenesis, the ODC protein is transformed into a metabolically stable protein. Moreover, ODC from the protozoan parasite *Trypanosoma brucei*, that is the causative agent of African sleeping sickness, lacks the sequence corresponding to the C-terminal end of mammalian ODC and has a very slow turnover in the parasite as well as when expressed in mammalian cells (Ghoda et al., 1990). However, recombining *T. brucei* ODC with the C-terminus of mammalian ODC transforms this ODC into a protein with a short half-life when expressed in mammalian cells (Ghoda et al., 1990, 1992). The slow turnover of *T. brucei* ODC has been suggested as one of the reasons for the effectiveness of the drug eflornithine, an ODC inhibitor, on African sleeping sickness (Heby and Persson, 1990; Carrillo et al., 2000; Bacchi and Yarlett, 2002; Burri and Brun, 2003).

The mammalian ODC is, like most other proteins with a short half-life, degraded by the 26S proteasome (Murakami et al., 1992, 2000). However, unlike most other proteins degraded by this proteolytic system, mammalian ODC is not ubiquitinated before being degraded (Rosenberg-Hasson et al., 1989). Instead, it appears that the degradation of mammalian ODC is induced by the binding of a specific protein, antizyme, to the enzyme (Hayashi and Murakami, 1995; Hayashi et al., 1996; Murakami et al., 2000; Coffino, 2001). The synthesis of antizyme is stimulated by polyamines, which thus regulate the first step of their own synthesis. Mammalian ODC contains, like many other proteins with short half-lives, so-called PEST regions, which are regions rich in proline (P), glutamic acid (E), aspartic acid (D), serine (S) and threonine (T) (Rogers et al., 1986). Mammalian

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ODC has two PEST regions, of which one is located within the C-terminus of the protein (Ghoda et al., 1989). It is possible that this part of the protein is involved in the recruitment of the 26S proteasome. It is believed that the binding of antizyme to ODC causes a structural change exposing the C-terminal end of the protein (Li and Coffino, 1993).

Interestingly, ODC is a stable protein in most protozoan parasites studied so far (Persson, 2007). However, the trypanosomatid *Crithidia fasciculata* was shown to have an ODC with a very fast turnover (Ceriani et al., 1992). The phylogenetically closely related trypanosomatid, *Leishmania donovani*, on the other hand, has a stable ODC (Hanson et al., 1992; Mukhopadhyay and Madhubala, 1995). Both ODCs from *C. fasciculata* and *L. donovani* lack a sequence corresponding to the C-terminal degradation domain of mammalian ODC (Hanson et al., 1992; Svensson et al., 1997). We have shown that the turnover of *C. fasciculata* ODC and *L. donovani* ODC in CHO cells as well as in a mammalian in vitro degradation system correspond very well to what is found in the parasites (Nasizadeh et al., 2003). Thus, in the mammalian systems *C. fasciculata* ODC has a rapid turnover, whereas *L. donovani* ODC has not, indicating that *C. fasciculata* ODC contains unique degradation signals. The fast turnover of *C. fasciculata* ODC in the mammalian systems was shown to be inhibited by inhibitors of the 26S proteasome, indicating that the enzyme was degraded by the same proteolytic system as mammalian ODC (Nasizadeh et al., 2003). However, unlike mammalian ODC, *C. fasciculata* ODC is not down-regulated by polyamines indicating that antizyme is not involved in the induction of degradation (Ceriani et al., 1992; Nasizadeh et al., 2003).

Interestingly, there is a large similarity in sequence (with an almost 70% identity) between *C. fasciculata* ODC and *L. donovani* ODC, in spite of the difference in metabolic stability (Svensson et al., 1997; Nasizadeh et al., 2003). The major difference is found in the amino-terminal part of the protein (Svensson et al., 1997). Here, *C. fasciculata* ODC contains a PEST region, which is absent in *L. donovani* ODC. In an attempt to gain information on what sequences are important for the rapid degradation of *C. fasciculata* ODC, we have in the present study designed, expressed and determined the turnover of various *C. fasciculata*/*L. donovani* ODC hybrid proteins. It is demonstrated that *C. fasciculata* ODC is likely to have more than one sequence element important for its rapid turnover and that these sequences are mainly located in the central part of the enzyme.

Materials and methods

Materials

The expression vector pCl-neo, TNT T7 Quick Coupled Transcription/Translation System, nuclease treated rabbit reticulocyte lysate and creatine kinase were purchased from Promega. L-[1-^{14}C]Ornithine (57 Ci/mol) and L-[^{35}S]methionine (1000 Ci/mmol) were obtained from New England Nuclear and Amersham Pharmacia Biotech, respectively.

Restriction enzymes, DNA ligase and Taq DNA polymerase were from Roche. Pfu polymerase was obtained from Stratagene.

DNA constructs

ODC DNA from *C. fasciculata* [GenBank: Y08233] and *L. donovani* [GenBank: M81192], subcloned into the expression vector pCl-neo were used to generate a series of *C. fasciculata*/*L. donovani* ODC chimeric proteins (Fig. 1). A common PflM I site located close to the middle of the coding region of both enzymes was used to create the hybrids C and F, consisting of equal parts from the two enzymes. Hybrid C corresponds to amino acids 1–322 and 361–721 from *L. donovani* ODC and *C. fasciculata* ODC, respectively. Hybrid F corresponds to amino acids 1–360 and 323–707 from *C. fasciculata* ODC to *L. donovani* ODC, respectively. Hybrids D and E were made using a common Sma I site in the sequences corresponding to the C-terminal part of the enzymes. Hybrid D cor-

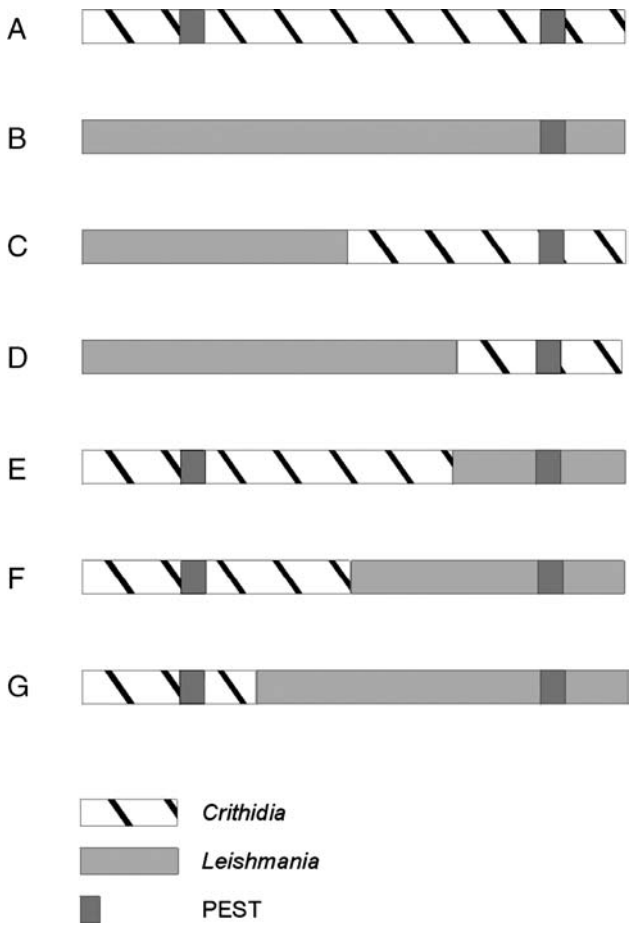

Fig. 1. Schematic structures of *C. fasciculata* ODC (**A**), *L. donovani* ODC (**B**) and the various chimeric ODCs (**C**–**G**) used. Regions containing a PEST sequence are indicated

responds to amino acids 1–490 and 461–721 from *L. donovani* ODC and *C. fasciculata* ODC, respectively. Hybrid E corresponds to amino acids 1–460 and 491–707 from *C. fasciculata* ODC to *L. donovani* ODC, respectively. In hybrid G, the N-terminal extension of *L. donovani* ODC (amino acids 1–119) was replaced with that of *C. fasciculata* ODC (amino acids 1–257) using PCR.

Cell culture and transfection

COS-7 cells were grown at 37 °C in Dulbecco's MEM containing 10% fetal calf serum, nonessential amino acids and antibiotics (50 units of penicillin and 50 μg/ml of streptomycin) in the presence of 5% CO_2. For transfection, COS cells were harvested during exponential growth and resuspended in 0.8 ml fresh growth medium at a density of 10×10^6 cells/ml. The transfection was performed by electroporation. The cells were mixed with 10–15 μg of DNA and then pulsed with 0.3 kV at 250 μF. Following a 5 min recovery period at room temperature the cells were seeded in fresh medium at a density of 27,000 cells/cm^2. Two days after the transfection the turnover of ODC was determined by following the decay of ODC activity after cycloheximide treatment (100 μg/ml).

Determination of ODC activity

Cells were sonicated in ice-cold 0.1 M Tris–HCl (pH 7.5) containing 0.1 mM EDTA and 2.5 mM dithiothreitol, followed by centrifugation at $30,000 \times g$ for 20 min. ODC activity was determined in aliquots of the supernatants by measuring the release of $^{14}CO_2$ from carboxyl-labelled ornithine in the presence of saturating levels of pyridoxal 5-phosphate (0.1 mM) and L-ornithine (0.5 mM).

Degradation of ODC in vitro

Labelled ODC was obtained by coupled in vitro transcription/translation using the TNT™ Coupled Reticulocyte Lysate System essentially as described in (Nasizadeh et al., 2003). The in vitro degradation of the labelled ODC was analysed in a rabbit reticulocyte lysate system in accordance with the method described by Bercovich et al. (1989). Aliquots were taken at various times during the degradation for analysis using SDS-polyacrylamide gel electrophoresis (8%). The radioactivity was visualised by fluorography after incubating the gels in Amplify (Amersham Pharmacia Biotech). [^{14}C]-Methylated proteins (Amersham Pharmacia Biotech) were used as molecular mass markers.

Results

ODC hybrids

C. fasciculata ODC and *L. donovani* ODC are about the same size, consisting of 721 and 707 amino acids, respectively (Hanson et al., 1992; Svensson et al., 1997). Both enzymes have a long N-terminal extension, which contains ~250 amino acids. The sequence homology in this region is lower compared to the other parts of the proteins. *C. fasciculata* ODC contains two PEST regions, one in the N-terminal extension and one in the C-terminal part of the enzyme. *L. donovani* ODC, on the other hand, only contains one PEST region. It is lacking the one corresponding to that found in the N-terminal extension of *C. fasciculata* ODC, but has a PEST region in its C-terminal part. However, the PEST region of *L. donovani* ODC is much weaker (according to the algorithm described by Rogers et al. (1986)) than the one found in the C-terminal part of *C. fasciculata* ODC.

PEST regions have been strongly correlated with proteins having a fast turnover, although the correlation is not compulsory (Rogers et al., 1986). Since the metabolically stable *L. donovani* ODC is lacking one PEST region compared to the unstable *C. fasciculata* ODC, it is conceivable that this particular PEST region is of importance for the unstable property of *C. fasciculata* ODC.

In order to identify regions of *C. fasciculata* ODC that may be essential for its rapid turnover, we designed a series of chimeric proteins between *C. fasciculata* and *L. donovani* ODCs. Figure 1 shows the various hybrid proteins made. A and B are schematic drawings of *C. fasciculata* ODC and *L. donovani* ODC, respectively. The PEST regions are marked with a dark box. C and D correspond to ODC chimeras, in which increasing parts of the N-terminal region of *C. fasciculata* ODC have been replaced by corresponding parts of *L. donovani* ODC. E–G correspond to ODC chimeras, in which increasing parts of the C-terminal region of *C. fasciculata* ODC have been replaced by corresponding parts of *L. donovani* ODC.

Unfortunately, no ODC knockout models of *C. fasciculata* are yet available. However, it has earlier been shown that *C. fasciculata* ODC is rapidly degraded also in mammalian systems, whereas *L. donovani* ODC is not, demonstrating that the degradation signals are recognised by mammalian systems (Svensson et al., 1997; Nasizadeh et al., 2003). Thus, two different mammalian expression systems were used to determine the turnover of the various hybrid proteins. The two systems used were COS cells and a rabbit reticulocyte lysate in vitro degradation system.

Turnover in COS cells

C. fasciculata and *L. donovani* ODCs as well as the various chimeric ODC proteins were expressed in COS cells. All of the proteins, except hybrid G, gave rise to very high ODC activity levels in the COS cells. The half-lives of the proteins were determined by following the decay in enzyme activity after addition of cycloheximide. As shown in Fig. 2, there was a marked difference in the turnover of *C. fasciculata* ODC compared to that of *L. donovani* ODC in COS cells. The estimated half-lives of the enzymes were about 1.4 and 5.7 h, respectively. Thus the difference in turnover corresponds very well to what is found in the parasites.

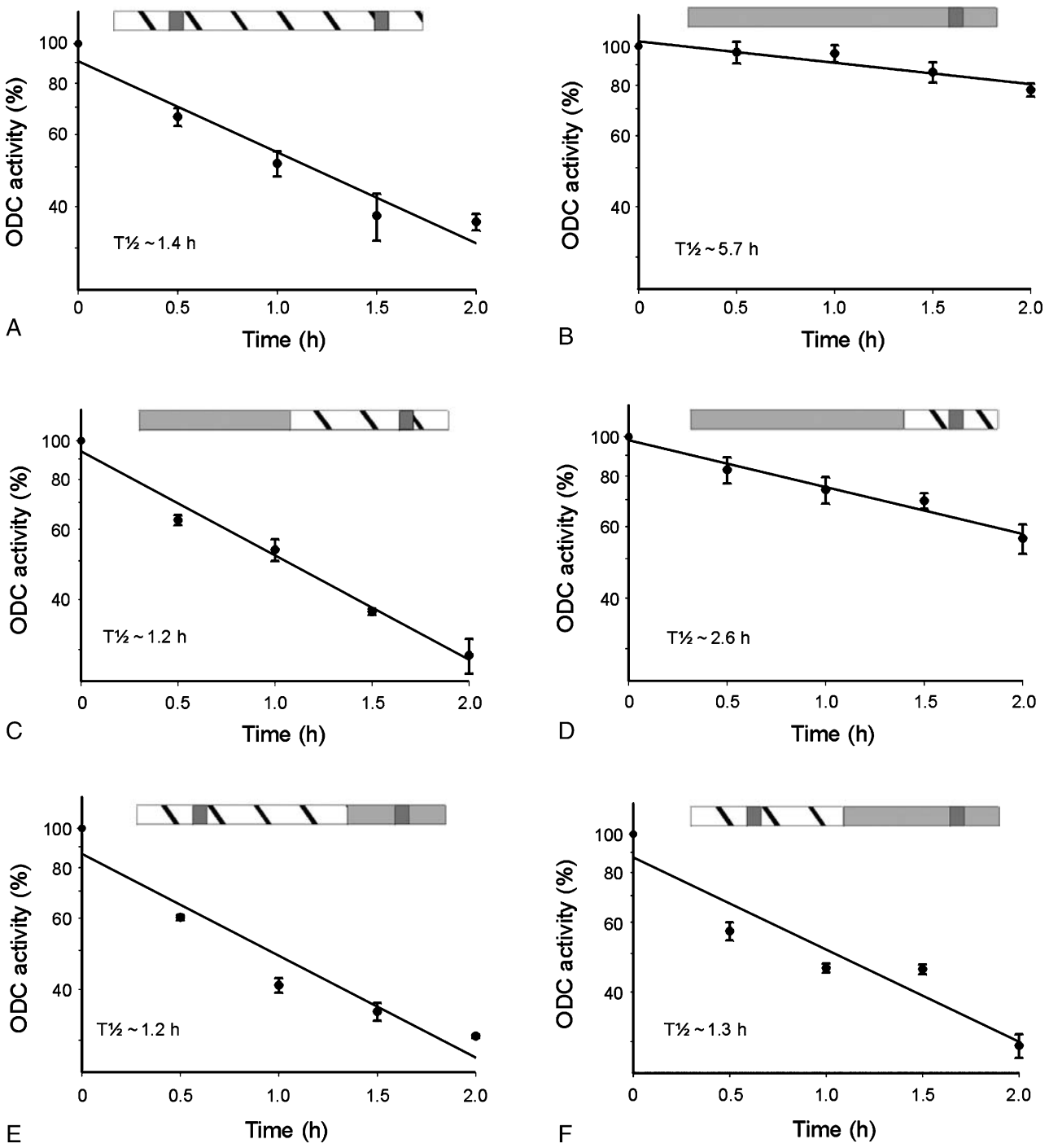

Fig. 2. Turnover of *C. fasciculata* ODC (**A**), *L. donovani* ODC (**B**) and various chimeric ODCs (**C**–**F**) in COS cells. COS cells were transfected as described in Materials and methods. Two days after transfection, the half-life of ODC activity was determined by measuring the decay after inhibition of protein synthesis using cycloheximide. **A**–**F** corresponds to the labeling in Fig. 1. Mean ± S.E.M. $n = 2$–8

Replacing the N-terminal half of *C. fasciculata* ODC, which contains the extra PEST region with that of *L. donovani* ODC did not affect the rapid turnover of the protein in the COS cells (Fig. 2C). The half-life of the hybrid protein was estimated to 1.2 h, which was close to that of the *C. fasciculata* ODC in the COS cells. However, if 2/3 of the N-terminal part of *C. fasciculata* ODC was replaced with the corresponding part of *L. donovani* ODC, there was a marked reduction in turnover rate (Fig. 2D). The half-life was increased from 1.4 to 2.6 h. Neverthe-

less, the half-life was significantly shorter than that of *L. donovani* ODC, which was 5.7 h.

The PEST region in the C-terminal part of *C. fasciculata* ODC is considered to be stronger than that of *L. donovani* ODC (Svensson et al., 1997). However, no effect on the turnover of the protein was observed by replacing the 1/3 C-terminal part of *C. fasciculata* ODC, containing this PEST region, with that of *L. donovani* ODC. The half-lives of the *C. fasciculata* ODC and the hybrid protein were 1.4 and 1.2 h, respectively. Neither was any effects seen on the turnover if 50% of the protein, corresponding to the C-terminal half of the *C. fasciculata* ODC protein, was replaced with *L. donovani* ODC sequence. The half-life of this hybrid protein was estimated to 1.3 h in COS cells. Replacing an even larger part with *L. donovani* ODC, leaving only 1/3 of the N-terminal part as *C. fasciculata* ODC (hybrid G) gave unfortunately rise to a hybrid ODC protein with low

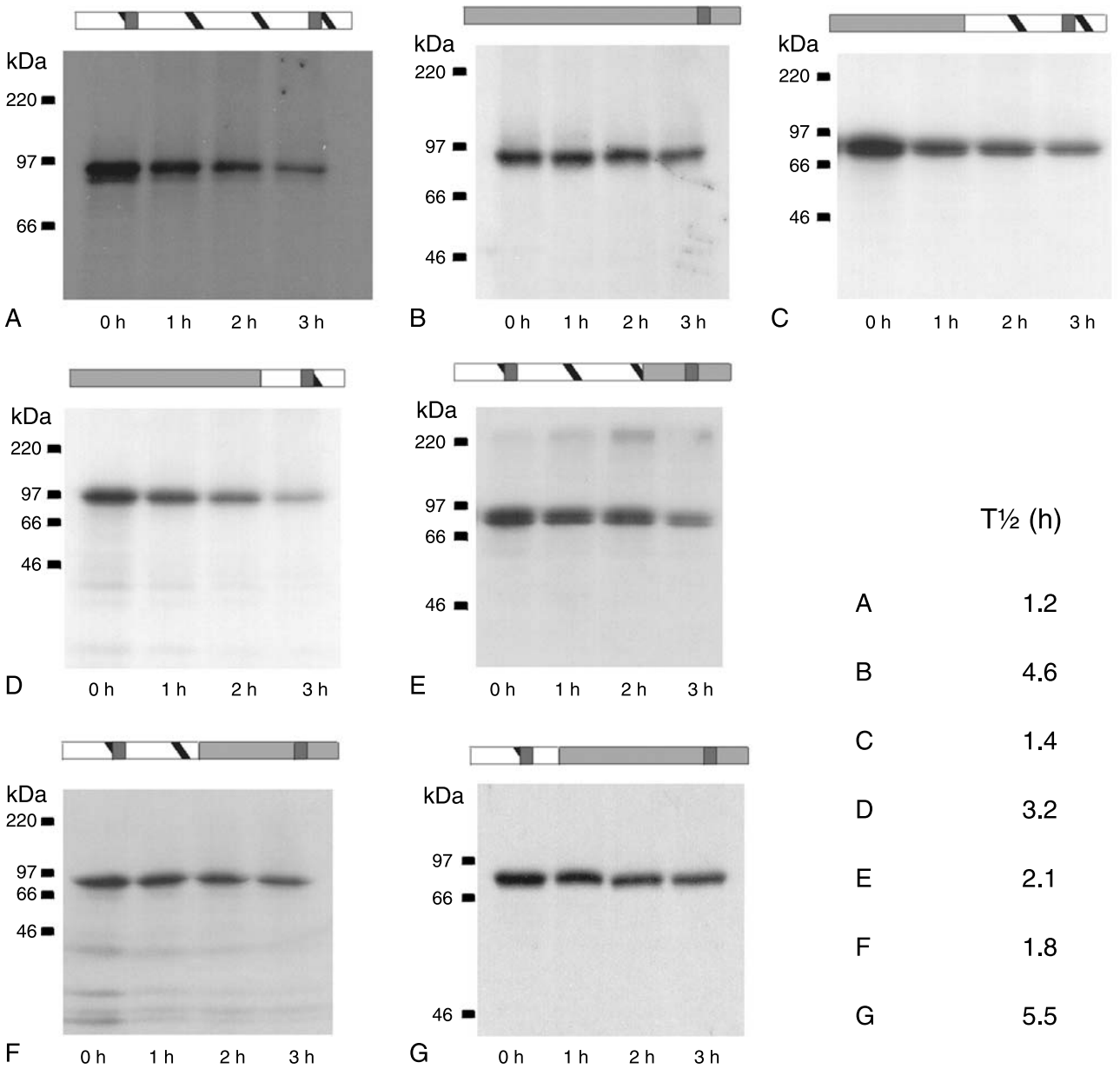

Fig. 3. Degradation of *C. fasciculata* ODC (**A**), *L. donovani* ODC (**B**) and various chimeric ODCs (**C**–**G**) in rabbit reticulocyte lysate. Radiolabelled enzyme was added to the reticulocyte lysate system and then incubated for indicated times at 37 °C. Radioactivity was visualised by fluorography after SDS-PAGE and analysed by densitometric scanning. [^{14}C]-Methylated proteins were used as molecular mass markers. **A**–**G** correspond to the labeling in Fig. 1

enzymatic activity. Thus, no half-life could be obtained in COS cells from this hybrid protein.

Degradation in vitro

Rabbit reticulocyte lysate have been shown to contain all the necessary components for the rapid degradation of mammalian ODC, which corresponds to that found in vivo (Bercovich et al., 1989). Any mutations in the mammalian ODC protein, which affect the turnover in vivo, also affect the degradation rate in the reticulocyte lysate (Rosenberg-Hasson et al., 1991). The rabbit reticulocyte lysate system does also effectively degrade *C. fasciculata* ODC (Nasizadeh et al., 2003). The degradation of *L. donovani* ODC in the rabbit reticulocyte lysate system is, however, much slower (Nasizadeh et al., 2003). The difference in degradation of the trypanosomal ODCs in the reticulocyte lysate reflects well what is found in the parasites and should be a useful supplementary tool for identifying regions of the *C. fasciculata* ODC important for the rapid turnover of the protein. Thus, we next determined the degradation of the various hybrid ODC proteins in the rabbit reticulocyte lysate system. The ODC proteins were synthesised in the presence of radioactive methionine using an in vitro transcription/translation system and then analysed for degradation in the reticulocyte lysate system (Fig. 3).

As shown in Fig. 3, the expression of the various ODCs gave rise to proteins having a molecular mass of about 90–95 kDa, which is somewhat larger than the theoretical values. The disappearance of radiolabelled *C. fasciculata* ODC after a chase with unlabelled methionine was much faster than that of labelled *L. donovani* ODC (Fig. 3A, B). The half-lives of the two proteins were determined to 1.2 and 4.6 h, respectively, which correspond very well to what was obtained in the COS cells.

As in the COS cells, the degradation of the hybrid ODC C, in which the N-terminal half of *C. fasciculata* ODC has been replaced with that of *L. donovani* ODC, was similar to the degradation of *C. fasciculata* ODC (Fig. 3C). The half-life of the ODC hybrid was estimated to 1.4 h in the reticulocyte lysate. Furthermore, the hybrid ODC D, containing $2/3$ of the N-terminal part of *L. donovani* ODC and $1/3$ of the C-terminal part of *C. fasciculata* ODC, which showed a reduced turnover in COS cells was also degraded less rapidly in the in vitro system, with a half-life of 3.2 h (Fig. 3D).

The hybrids E and F, in which up to 50% of the C-terminal part of *C. fasciculata* ODC has been replaced with corresponding sequences from *L. donovani* ODC, were degraded with rates that were only slightly slower than that of the degradation of *C. fasciculata* ODC in the reticulocyte lysate (Fig. 3E, F). The half-lives of the hybrid ODCs E and F were 2.1 and 1.8 h, respectively, compared to 1.2 h for *C. fasciculata* ODC.

Using the in vitro degradation system it was possible to analyse the degradation of the hybrid ODC G, in which all of the *C. fasciculata* ODC sequence, except for $1/3$ of the N-terminal part, has been replaced with that of *L. donovani* ODC, and that was shown to be virtually inactive in COS cells. As seen in Fig. 3G, this hybrid was degraded with a much slower rate than the other hybrids, including hybrid F. The half-life was estimated to about 5.5 h, which is in the range of the half-life of *L. donovani* ODC.

Discussion

The protein turnover was determined in two different expression systems using different parameters, enzyme activity and protein levels. The results from the two expression systems used showed a relatively good correlation. A major difference in turnover was obtained between *C. fasciculata* ODC and *L. donovani* ODC. The major sequence difference between these proteins is found in the N-terminal part of the enzyme, which also contains a strong PEST region in *C. fasciculata* ODC (Svensson et al., 1997). However, exchanging as much as 50% of the N-terminal part of *C. fasciculata* ODC, including the PEST region, with the N-terminal part of *L. donovani* ODC did not affect the half-life of the protein, indicating that the difference in turnover between *C. fasciculata* ODC and *L. donovani* ODC can not be explained by sequence differences in this part of the enzyme. Thus, the C-terminal half of *C. fasciculata* ODC must contain essential parts for the rapid turnover of the protein. This conclusion is supported by the finding that when also the first part of the C-terminal half of *C. fasciculata* ODC was replaced with *L. donovani* ODC sequence, in addition to the N-terminal half, there was a marked reduction in the turnover of the protein. This sequence corresponds to amino acids 361–461 of *C. fasciculata* ODC. Although the reduction was significant, the turnover of the hybrid protein was faster than that of *L. donovani* ODC, indicating that also the remaining part of the C-terminal region of *C. fasciculata* ODC contains sequences that may be of importance for rapid turnover of the enzyme.

The C-terminal part of *C. fasciculata* ODC contains the second PEST region of the protein (Svensson et al., 1997), which is stronger than the one of *L. donovani* ODC, and

may fulfil some important function in relation to the rapid degradation of the protein. However, replacing only the C-terminal part of *C. fasciculata* ODC (amino acids 461–721) with the corresponding sequence of *L. donovani* ODC did not induce any major change in the turnover of the protein. In the COS system, this hybrid protein was degraded with a half-life that was about the same as for *C. fasciculata* ODC, whereas in the reticulocyte lysate there was a slight reduction in the turnover rate, suggesting that this part of the protein only has a minor, if any, influence on the rapid degradation of *C. fasciculata* ODC. This assumption is supported by the finding that the rapid turnover of *C. fasciculata* ODC was hardly affected by replacing the whole C-terminal half of the protein with *L. donovani* ODC sequence. Thus, the N-terminal half of *C. fasciculata* ODC seems to contain all necessary information for a rapid turnover of the protein (when combined with the C-terminal part of *L. donovani* ODC). However, as mentioned above, there was no effect on the turnover if this part of *C. fasciculata* ODC was replaced with the corresponding part of *L. donovani* ODC, which indicates that the C-terminal half of the enzyme also contains all the information needed for a rapid turnover. Hence, it appears that *C. fasciculata* ODC contains several regions that are of importance for its rapid turnover.

The region in the N-terminal half of *C. fasciculata* ODC that may be important for the rapid turnover of the protein appeared to be located at the C-terminal end. Replacing all of the *C. fasciculata* ODC, except for the first 257 amino acids, gave rise to a protein which in the reticulocyte lysate had a half-life similar to that of *L. donovani* ODC. However, since the hybrid protein had low enzyme activity the results could not be confirmed using COS cells. Nevertheless, replacing the N-terminal part of C-terminally truncated human ODC, which is a stable protein (Ghoda et al., 1989; Rosenberg-Hasson et al., 1991), with that of *C. fasciculata* ODC (corresponding to amino acids 1–257) did not increase the slow turnover of the protein measured in COS cells as well as in the reticulocyte lysate (results not shown), supporting the conclusion that this part of *C. fasciculata* ODC lacks sequences important for the rapid turnover of the enzyme.

C. fasciculata ODC appears to be degraded by the same proteolytic system as the mammalian ODC, namely the 26S proteasome (Nasizadeh et al., 2003). However, the targeting of the *C. fasciculata* ODC for degradation is not clear. Degradation of mammalian ODC is stimulated by binding of antizyme to the protein (Hayashi and Murakami, 1995; Hayashi et al., 1996; Murakami et al., 2000; Coffino, 2001), which is a unique mechanism. Most other proteins degraded by the 26S proteasome are targeted by poly-ubiquitination (Voges et al., 1999). Antizyme has not yet been detected in any of the trypanosomatids and it is conceivable that the rapid turnover of *C. fasciculata* ODC does not involve antizyme (Hua et al., 1995). Another possibility is that *C. fasciculata* ODC, like most other proteins, is being ubiquitinated before being degraded by the 26S proteasome. The finding in the present study that *C. fasciculata* ODC appear to contain several regions that are of importance for the rapid turnover of the enzyme, may be explained by the recent observation that ubiquitination often occurs at multiple sites (Peng et al., 2003; Hurley et al., 2006). However, it remains to be established whether *C. fasciculata* ODC is a target for ubiquitination or not.

In conclusion, it appears that *C. fasciculata* ODC contains several regions that are important for its fast turnover. The central part of the enzyme (amino acids 258–461) contains more than one region able to fully induce a rapid turnover of the protein. Also the C-terminal part of the enzyme seems to contain a region important, but not necessary, for the rapid degradation of the enzyme. The N-terminal part, containing the PEST sequence, on the other hand, does not seem to be essential for the rapid turnover of *C. fasciculata* ODC.

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References

Bacchi CJ, Yarlett N (2002) Polyamine metabolism as chemotherapeutic target in protozoan parasites. Mini Rev Med Chem 2: 553–563

Bercovich Z, Rosenberg-Hasson Y, Ciechanover A, Kahana C (1989) Degradation of ornithine decarboxylase in reticulocyte lysate is ATP-dependent but ubiquitin-independent. J Biol Chem 264: 15949–15952

Burri C, Brun R (2003) Eflornithine for the treatment of human African trypanosomiasis. Parasitol Res 90 [Suppl 1]: S49–S52

Carrillo C, Cejas S, Cortes M, Ceriani C, Huber A, Gonzalez NS, Algranati ID (2000) Sensitivity of trypanosomatid protozoa to DFMO and metabolic turnover of ornithine decarboxylase. Biochem Biophys Res Commun 279: 663–668

Ceriani C, González NS, Algranati ID (1992) Ornithine decarboxylase from *Crithidia fasciculata* is metabolically unstable and resistant to polyamine down-regulation. FEBS Lett 301: 261–264

Coffino P (2001) Regulation of cellular polyamines by antizyme. Nat Rev Mol Cell Biol 2: 188–194

Ghoda L, Phillips MA, Bass KE, Wang CC, Coffino P (1990) Trypanosome ornithine decarboxylase is stable because it lacks sequences found in the carboxyl terminus of the mouse enzyme which target the latter for intracellular degradation. J Biol Chem 265: 11823–11826

Ghoda L, Sidney D, Macrae M, Coffino P (1992) Structural elements of ornithine decarboxylase required for intracellular degradation and polyamine-dependent regulation. Mol Cell Biol 12: 2178–2185

Ghoda L, van Daalen Wetters T, Macrae M, Ascherman D, Coffino P (1989) Prevention of rapid intracellular degradation of ODC by a carboxyl-terminal truncation. Science 243: 1493–1495

Hanson S, Adelman J, Ullman B (1992) Amplification and molecular cloning of the ornithine decarboxylase gene of *Leishmania donovani*. J Biol Chem 267: 2350–2359

Hayashi S, Murakami Y (1995) Rapid and regulated degradation of ornithine decarboxylase. Biochem J 306: 1–10

Hayashi S, Murakami Y, Matsufuji S (1996) Ornithine decarboxylase antizyme: a novel type of regulatory protein. Trends Biochem Sci 21: 27–30

Heby O, Persson L (1990) Molecular genetics of polyamine synthesis in eukaryotic cells. Trends Biochem Sci 15: 153–158

Hua SB, Li X, Coffino P, Wang CC (1995) Rat antizyme inhibits the activity but does not promote the degradation of mouse ornithine decarboxylase in Trypanosoma brucei. J Biol Chem 270: 10264–10271

Hurley JH, Lee S, Prag G (2006) Ubiquitin-binding domains. Biochem J 399: 361–372

Li X, Coffino P (1993) Degradation of ornithine decarboxylase: exposure of the C-terminal target by a polyamine-inducible inhibitory protein. Mol Cell Biol 13: 2377–2383

Mukhopadhyay R, Madhubala R (1995) *Leishmania donovani*: cellular control of ornithine decarboxylase in promastigotes. Int J Biochem Cell Biol 27: 947–952

Murakami Y, Matsufuji S, Hayashi S, Tanahashi N, Tanaka K (2000) Degradation of ornithine decarboxylase by the 26S proteasome. Biochem Biophys Res Commun 267: 1–6

Murakami Y, Matsufuji S, Kameji T, Hayashi S, Igarashi K, Tamura T, Tanaka K, Ichihara A (1992) Ornithine decarboxylase is degraded by the 26S proteasome without ubiquitination. Nature 360: 597–599

Nasizadeh S, Jeppsson A, Persson L (2003) Proteasomal degradation of a trypanosomal ornithine decarboxylase. Cell Physiol Biochem 13: 321–328

Pegg AE, Shantz LM, Coleman CS (1995) Ornithine decarboxylase as a target for chemoprevention. J Cell Biochem 58 [Suppl 22]: 132–138

Peng J, Schwartz D, Elias JE, Thoreen CC, Cheng D, Marsischky G, Roelofs J, Finley D, Gygi SP (2003) A proteomics approach to understanding protein ubiquitination. Nat Biotechnol 21: 921–926

Persson L (2006) Regulation of ornithine decarboxylase expression. In: Wang J-Y, Casero RA Jr (eds) Polyamine cell signaling. Humana Press Inc., Totowa, pp 399–414

Persson L (2007) Ornithine decarboxylase and S-adenosylmethionine decarboxylase in trypanosomatids. Biochem Soc Trans 35: 314–317

Rogers S, Wells R, Rechsteiner M (1986) Amino acid sequences common to rapidly degraded proteins: the PEST hypothesis. Science 234: 364–368

Rosenberg-Hasson Y, Bercovich Z, Ciechanover A, Kahana C (1989) Degradation of ornithine decarboxylase in mammalian cells is ATP dependent but ubiquitin independent. Eur J Biochem 185: 469–474

Rosenberg-Hasson Y, Bercovich Z, Kahana C (1991) Characterization of sequences involved in mediating degradation of ornithine decarboxylase in cells and in reticulocyte lysate. Eur J Biochem 196: 647–651

Svensson F, Ceriani C, Lövkvist Wallström E, Kockum I, Algranati ID, Heby O, Persson L (1997) Cloning of a trypanosomatid gene coding for an ornithine decarboxylase that is metabolically unstable even though it lacks the C-terminal degradation domain. Proc Natl Acad Sci USA 94: 397–402

Takeuchi J, Chen H, Coffino P (2007) Proteasome substrate degradation requires association plus extended peptide. EMBO J 26: 123–131

Voges D, Zwickl P, Baumeister W (1999) The 26S proteasome: a molecular machine designed for controlled proteolysis. Annu Rev Biochem 68: 1015–1068

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