cis-Recognition and degradation of ornithine decarboxylase subunits in reticulocyte lysate

Yael ROSENBERG-HASSON, Zippi BERCOVICH and Chaim KAHANA*

Department of Molecular Genetics and Virology, Weizmann Institute of Science, Rehovot 76100, Israel

One of the most interesting characteristics of ornithine decarboxylase (ODC) is its extremely short half-life. In a recent study we have demonstrated that deletion of a C-terminal segment converts ODC into a stable protein. In the present study we have extended this observation by testing the degradation of an ODC heterodimer composed of one rapidly degraded wild-type subunit and one stable mutant subunit. Our study was motivated by the possibility of *trans*-recognition of stable subunits due to their association with labile subunits. Our results demonstrate that such an association did not confer lability upon the stable subunits, not did it stabilize the short-lived subunits.

INTRODUCTION

Ornithine decarboxylase (ODC) is the first key enzyme in the biosynthetic pathway of polyamines in mammalian cells [1]. One of the most interesting characteristics of this pyridoxal phosphate-dependent enzyme is its extremely short half-life [2]. ODC was shown to be regulated at the transcriptional, the translational and the post-translational levels [3–14]. The metabolic instability of ODC permits efficient modulation of its activity by such cellular regulatory mechanisms.

We have recently demonstrated that ODC degradation requires ATP, but is ubiquitin-independent both in intact cells and in a reticulocyte-lysate-based degradation system [15,16]. In addition, its C-terminal domain was identified as an intrinsic signal that determines the rapid intracellular degradation of the enzyme [17,18]. Deletion of this domain converts ODC into a stable protein in both intact cells and a reticulocyte-lysate-based degradation mix [17].

In the present study we have extended these observations by investigating the degradation of heterodimers composed of stable and unstable subunits. Our results demonstrate that the rapidly degraded wild-type subunit does not confer lability upon the stable subunit present in the same heterodimer. These results strongly suggest that the C-terminal of ODC acts only in *cis*, not in *trans*.

EXPERIMENTAL

Synthesis of ODC *in vitro*

ODC cDNAs representing wild-type or the stable Del-6 mutant [17] [cloned in the pGEM-1 plasmid (between the *Eco*RI (5') and *Bam*HI (3') sites] were linearized by a *Bam*HI digest, and transcribed *in vitro* with T7 RNA polymerase. Homodimers were formed by adding a single type of RNA to each reticulocyte-lysate-based translation mix. Heterodimers were formed by co-translation of both types of RNA. Translation in the reticulocyte lysate was performed in the presence of [³⁵S]methionine.

Degradation of ODC in a reticulocyte-lysate-based degradation mix *in vitro*

A 50000 c.p.m. portion of each type of *in vitro*-synthesized ODC was incubated in a reticulocyte-lysate-based degradation system [16], containing, in a final volume of 50 μ l: 40 mM-Tris/HCl, pH 7.5, 5 mM-MgCl₂, 2 mM-dithiothreitol, 0.5 mM-

ATP, 10 mM-phosphocreatine, 1600 μ g of creatine phosphokinase/ml and 20 μ l of reticulocyte lysate (prepared for degradation assays as described in [19]). After the indicated times of incubation at 37 °C, aliquots were removed and fractionated by SDS/PAGE.

Cross-linking and revelation of dimers

An aliquot (3 μ l) of the translated material was incubated for 40 min at 25 °C in 10 mM-Tris/HCl, pH 7, containing 1 mM-bis(sulphosuccinimidyl) suberate (BS³; Pierce) [20]. Cross-linking was terminated by the addition of 0.1 vol. of 0.1 M-glycine/0.1 M-Tris/HCl, pH 8.5. Cross-linked samples were fractionated by electrophoresis in an SDS/10% polyacrylamide gel.

RESULTS AND DISCUSSION

We have recently demonstrated that deletion of the C-terminal domain encompassing amino acids 423–449 converts ODC into a stable protein, both in cells and in reticulocyte-lysate-based degradation mix ([17] and Fig. 1). Similar stabilization of ODC due to truncation of 37 C-terminal amino acids was also reported [18]. In the experiments presented in Fig. 1, wild-type ODC and the stable Del-6 mutant (lacking amino acids 423–449) were separately translated in reticulocyte lysate, and then incubated in a reticulocyte-lysate-based degradation system [16]. In contrast

Fig. 1. Degradation of ODC homodimers in a reticulocyte-lysate-based degradation mix

(a) Schematic representation of the three types of possible dimers. (b) RNA representing wild-type (WT) and Del-6 (D6) ODC were transcribed *in vitro* and translated in a reticulocyte lysate. An aliquot of each translation reaction was incubated in a reticulocyte-lysate-based degradation mix and subjected to SDS/PAGE.

Abbreviations used: ODC, ornithine decarboxylase; β -gal, β -galactosidase.

* To whom correspondence should be sent.

Fig. 2. Formation of heterodimers by co-translation of wild-type and Del-6 ODC

(a) Design of procedure for formation of ODC heterodimers. RNAs representing wild-type (WT) and Del-6 (D6) subunits were co-translated in reticulocyte lysate. The expected proportions of heterodimers and homodimers, assuming random assembly, are indicated. The three possible models for degradation of individual subunits are indicated at the bottom. (b) Separately translated and co-translated material was subjected to the cross-linking reaction and subjected to SDS/PAGE. The arrow indicates the dimers.

Fig. 3. Degradation of heterodimers in a reticulocyte-lysate-based degradation mix

RNA representing wild-type (WT) ODC or the Del-6 (D6) mutant was either separately or co-translated in reticulocyte lysate. An aliquot of each translation reaction was incubated in a reticulocyte-lysate-based degradation mix and subjected to SDS/PAGE.

with the rapid degradation of the wild-type enzyme, Del-6 was completely stable, suggesting that the deleted segment may act as a recognition signal for the proteolytic machinery that degrades ODC. *In vitro*-translated wild-type and Del-6 display almost identical activity (Y. Rosenberg-Hasson & C. Kahana, unpublished work), indicating that both are assembled into an active dimeric conformation. This implies that wild-type subunits may be assembled into a heterodimeric structure with Del-6 subunits, presenting an interesting situation in which the degradation of such an heterodimer can be investigated.

To test whether heterodimers are actually formed, wild-type and Del-6 RNAs were co-translated in reticulocyte lysate, resulting in the synthesis of equal amounts of each polypeptide (Fig. 2). A cross-linking reaction followed by fractionation in an SDS/polyacrylamide gel demonstrated that as expected about 50% of the translated material was present in heterodimers (Fig. 2).

In a wild-type homodimer, each monomer contains the destabilizing signal which is recognized in *cis*, resulting in efficient degradation (Fig. 1). In the stable Del-6 homodimer, both subunits lack the destabilizing signal and as a result are completely stable (Fig. 1). However, in the case of heterodimers,

one could also consider the possibility of *trans*-recognition of stable Del-6 subunits, owing to their association with labile wild-type subunits. We therefore set out to determine the degradation rate of the two components of the co-translated material. An aliquot of the co-translated material was incubated in a reticulocyte-lysate-based degradation mix followed by fractionation on an SDS/polyacrylamide gel. Del-6 subunits remained completely stable, whereas wild-type subunits were rapidly degraded (Fig. 3). This indicates that heterodimer formation does not confer lability upon the stable Del-6 subunits or stability upon wild-type subunits. This conclusion was further supported by demonstrating complete stability of Del-6 subunits even when wild-type subunits outnumbered those of Del-6 by a factor of 6. In addition, wild-type subunits remained unstable even when Del-6 subunits were overrepresented in the co-translated material (results not shown).

Taken together, our results indicate that ODC subunit recognition by the proteolytic machinery occurs only in *cis*, not in *trans*. However, we cannot presently distinguish between the possibility that wild-type subunits are recognized and degraded while they are in a dimeric conformation, and the possibility that recognition and degradation occurs while these subunits are in a transient monomeric state.

A recent study has demonstrated *trans*-recognition and degradation in reticulocyte lysate of stable β -galactosidase (β -gal) subunits that is due to their association with unstable subunits in the same heterotetramer [21]. The degradation of β -gal, which obeys the *N*-end rule, occurs via the ATP-and-ubiquitin-dependent proteolytic pathway [21]. By contrast, we have demonstrated that, both in intact cells and in reticulocyte-lysate ODC, degradation is ATP-dependent but ubiquitin-independent [15,16]. Our present results support the above distinction by demonstrating a different mechanism of action of the two proteolytic machineries. Alternatively, lack of *trans*-recognition in the degradation of ODC may represent an intrinsic property of this protein. The degradation of the β -gal was shown to be governed by two determinants, one being the *N*-terminal amino acid and the other being the ubiquitination site [21]. It is possible that, like β -gal, ODC also contains distinct recognition and degradation determinants. However, in the case of ODC both signals may reside within the segment deleted in the stable Del-6 variant. A more detailed dissection of this region may permit distinction between these two possibilities.

This work was supported in part by grant 88-00241 from the United States-Israel Binational Foundation, Jerusalem, Israel, by a fund for basic research administered by the Israeli Academy of Science and Humanities, by a grant from the Leo and Julia Forchheimer Centre for Molecular Genetics at the Weizmann Institute of Science, by the Jakubskind-Cymerman research grant, and by a grant from the Revson Foundation. C.K. is a Research Career Development awardee of the Israel Cancer Research Fund (U.S.A.).

REFERENCES

1. Pegg, A. E. & Williams-Ashman, H. G. (1981) in *Polyamines in Biology and Medicine* (Morris, D. R. & Marton, L. J., eds.), pp. 3-42, Marcel Dekker, New York.
2. Russell, D. H. & Snyder, S. H. (1969) *Mol. Pharmacol.* **5**, 253-262.
3. Berger, F. G., Szymanski, P., Read, E. & Watson, G. (1984) *J. Biol. Chem.* **259**, 7941-7946.
4. Kahana, C. & Nathans, D. (1984) *Proc. Natl. Acad. Sci. U.S.A.* **81**, 3645-3649.
5. Kontula, K. K., Torkkali, T. K., Bardin, C. W. & Jänne, O. A. (1984) *Proc. Natl. Acad. Sci. U.S.A.* **81**, 731-735.
6. McConlogne, L., Gupta, M., Wu, L. & Coffino, P. (1984) *Proc. Natl. Acad. Sci. U.S.A.* **81**, 540-544.
7. Katz, A. & Kahana, C. (1987) *Mol. Cell. Biol.* **7**, 2641-2643.
8. Kahana, C. & Nathans, D. (1985) *J. Biol. Chem.* **260**, 15390-15393.
9. Hölttä, E. & Pohjanpelto, P. (1986) *J. Biol. Chem.* **261**, 9502-9608.

10. Persson, L., Holm, I. & Heby, O. (1988) *J. Biol. Chem.* **263**, 3528–3533
11. Isomaa, V. V., Pajunen, A. E. I., Bardin, C. W. & Janne, O. A. (1983) *J. Biol. Chem.* **258**, 6735–6740
12. Heller, J. S., Fong, W. F. & Canellakis, E. S. (1976) *Proc. Natl. Acad. Sci. U.S.A.* **73**, 1858–1862
13. Kitani, T. & Fujisawa, H. (1981) *J. Biol. Chem.* **259**, 10036–10040
14. Kahana, C. & Nathans, D. (1985) *Proc. Natl. Acad. Sci. U.S.A.* **82**, 1673–1677
15. Rosenberg-Hasson, Y., Bercovich, Z., Ciechanover, A. & Kahana, C. (1989) *Eur. J. Biochem.* **185**, 469–474
16. Bercovich, Z., Rosenberg-Hasson, Y., Ciechanover, A. & Kahana, C. (1989) *J. Biol. Chem.* **264**, 15949–15952
17. Rosenberg-Hasson, Y., Bercovich, Z. & Kahana, C. (1991) *Eur. J. Biochem.*, in the press
18. Ghoda, L., Van Daalen Wetters, T., Macrae, M., Ascherman, D. & Coffino, P. (1989) *Science* **243**, 1493–1495
19. Ciechanover, A., Hod, Y. & Hershko, A. (1978) *Biochem. Biophys. Res. Commun.* **81**, 1100–1105
20. Staros, J. V. (1982) *Biochemistry* **21**, 3950–3955
21. Johnson, E. S., Gonda, D. K. & Varshavsky, A. (1990) *Science* **346**, 287–291

Received 16 November 1990/24 January 1991; accepted 12 February 1991