The Pest Regions Containing C-Termini of Mammalian Ornithine Decarboxylase and Histidine Decarboxylase Play Different Roles in Protein Degradation

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Proteasome 26S must recognize the PEST region-containing C-terminus of mammalian ornithine decarboxylase (ODC) monomer to proceed with degradation. We have detected PEST regions in both termini of mammalian histidine decarboxylase (HDC). In the present report, a chimaeric ODC/HDC was used to elucidate whether the PEST region-containing C-termini of ODC and HDC are exchangeable. Wild-type rat ODC had an expected antizyme and ATP-dependent degradation. This was not the case for both the chimaera and a C-terminus truncated rat ODC. Results suggest that the PEST region-containing C-terminus of rat HDC should have another role different to conferring polypeptide availability to the proteasome.© 1999 Academic Press

Polyamines are essential for macromolecular biosynthesis and cell viability along the phylogenetic scale. In most animal cells, the PLP-dependent enzyme ornithine decarboxylase (ODC, EC 4.1.1.17) is the limitant enzyme for polyamine biosynthesis. Mammalian ODC levels are strictly regulated exclusively by the modification of the protein synthesis and degradation rates. In fact, mammalian ODC exhibits one of the most rapid turnovers described for animal enzymes (10-60 min), and it is regulated by the intracellular levels of polyamines (1). Polyamines induce the expression of a protein inhibitor named antizyme, which acts as a chaperone during ATP-dependent degradation of ODC by proteasome 26S (2). After ODC-antizyme binding, the signal for ODC degradation present in the C-terminus (residues 425-461 in the rat sequence) of the monomer becomes exposed to the proteasome system (3,4). This C-terminus region is both necessary and sufficient for constitutive degradation of ODC (5). The specific signal for degradation contained in this region is a PEST sequence. PEST regions are hydrophilic sequences (between positively charged residues) enriched in P, S/T or D/E that tend to reside near the amino or carboxy termini of enzymes. PEST regions can act as constitutive or conditional signals providing mechanisms for intracellular degradation of key metabolic proteins (6).

We have previously reported (7) common protein motifs for mammalian ODC and histidine decarboxylase (HDC, EC 4.1.1.22). One of those common motifs is the presence of PEST regions. Mammalian (human, rat and mouse) HDCs have one PEST region in the N-terminus (around residues 40-70) and at least another PEST region from residues 500 on (7). On the other hand, in several proliferating models, inductions and decays of both ODC and HDC activities simultaneously occur in response to growth stimuli (8-10). These data prompted us to postulate the existence of similarities between ODC and HDC turnover as a hypothesis.

In order to elucidate whether HDC and ODC C-terminus PEST regions share a common role in protein degradation, in the present report we have constructed an ODC/HDC chimaeric protein containing the fragment 1-424 of the rat ODC sequence, lacking its own PEST region, and residues 482/656 of rat HDC. Activity and degradation patterns of wild-type and truncated (1-424) ODC, as well as of the ODC/HDC chimera transcribed and translated in vitro are compared.

MATERIALS AND METHODS

Generation of recombinant constructions. The cDNA fragment encoding for residues 1 to 424 (ODC-ΔPEST) of rat ODC and the fragment encoding for residues 482/656 of rat HDC (HDC C-terminus) were obtained by PCR. In ODC-ΔPEST, an R/H substitution was introduced at position 424, in order to introduce a new

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SacI site. The ODC-PEST fragment was cloned in PCR-Script (pODC-ΔPEST). This recombinant plasmid was digested by SacI and SacII. The HDC C-terminus encoding sequence was digested by SacI, and ligated to digested pODC-ΔPEST. Finally, the recombinant plasmid was blunt ended and ligated. Sequences were tested by sequencing.

In vitro expression and activity determinations. The original, wild rat ODC and both recombinant constructions were expressed in vitro (for 90 min) by using the TNT expression system (Promega, USA), as described previously (11). ODC activity was followed by the $^{14}$CO$_2$ released from C1-labelled ornithine, as reported elsewhere (12). For calculation of the catalytic efficiency, activity was normalized with respect to the de novo-synthesized polypeptides.

In vitro degradation experiments. Fresh in vitro expressed products (2 μl) were diluted to 100 μL with a 40 mM Tris/HCl buffer, pH 7.5, also containing 5 mM MgCl$_2$, 0.2 mM dithiothreitol (DTT), 0.1 mM cycloheximide, 25 μL of a hemin-deficient reticulocyte lysate [13], and 0.5 mM ATP, 10 mM phosphocreatine plus 5 μg of creatine kinase, as an ATP-regenerating system. For experiments in absence of ATP, the ATP-regenerating system was avoided and the addition of an ATP-consuming system was not necessary under the used conditions. The mixtures were incubated at 30°C. At different incubation times, reaction aliquots were stopped by adding SDS/PAGE sample buffer. SDS/PAGE and fluorography were carried out as described previously (14). Degradations in the presence and absence of 10 ng antizyme were always carried out in parallel. Recombinant antizyme was a kind gift from Dr. Pegg (Hershey, PA) and it was obtained and purified as described previously (15).

RESULTS AND DISCUSSION

In the present decade, a great effort has been made to elucidate the structure/function relationships of ODC. A hypothesis on the structure of its PLP-binding domain has been formulated (16), essential residues for enzymic activity have been located (3,17,18), a catalytic mechanism has been proposed (19), and its unique degradation mechanism has been elucidated (1,2).

Mammalian ODC belongs to group IV of PLP-dependent L-amino acid decarboxylases (20). On the other hand, mammalian HDC belongs to group II of PLP-dependent L-amino acid decarboxylases. Thus, mammalian ODC and HDC seem to have different evolutionary origins. However, in spite of the lack of significant sequence similarities between ODCs and HDCs, we have detected common motifs among their cDNA-deduced sequences by computational analysis (7). Among these, the presence of C-terminal PEST regions could be relevant. The C-terminal PEST region of mammalian ODC behaves as a signal for ODC degradation by proteasome 26S (1,21). In fact, this region can confer a short half-life on these proteins to which it is experimentally appended, showing that

<table>
<thead>
<tr>
<th>Protein</th>
<th>$K_m$ (μM)</th>
<th>$V_{max}$</th>
<th>$V_{max}/K_m$</th>
</tr>
</thead>
<tbody>
<tr>
<td>wild type ODC</td>
<td>50</td>
<td>2.64</td>
<td>53</td>
</tr>
<tr>
<td>ODC-PEST</td>
<td>50</td>
<td>2.07</td>
<td>41</td>
</tr>
<tr>
<td>ODC/HDC</td>
<td>40</td>
<td>1.65</td>
<td>41</td>
</tr>
</tbody>
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* Given as nmoles of CO$_2$ produced per hour.

FIG. 1. Stability of wild type ODC (A) and ODC/HDC (B) chimaera in a degradation medium in the presence or absence of ATP. Bands corresponding to remaining non-degraded protein from equivalent aliquots taken after 0, 30 or 60 min of incubation are shown. Quantitative data are depicted as percentage of degradation in the presence (closed circles) or absence (open circles) of ATP.
PEST regions are transplantable proteolytic signals (6,22,23).

Primary translation product (74 kDa) of mammalian HDC mRNA has also been reported to be an unstable polypeptide, and a substrate for proteasome 26 S (24). However, the full primary sequence has two PEST region-containing termini and the exact points for processing and degradation in vivo are still unknown. Taking into account the well-known facts on ODC degradation mentioned above, a question arises: can C-terminal PEST regions of HDC substitute that of ODC? To test this hypothesis, we decided to construct an ODC/HDC chimaeric protein, containing the 1-424 region of rat ODC and residues 482-664 of rat HDC.

The chimaeric protein could be efficiently transcribed and translated in vitro and it retained ODC activity. In fact, as shown in Table 1, both affinity constants and catalytic efficiencies were very similar for wild type ODC, the ODC-ΔPEST deletion mutant and the ODC/HDC chimaeric protein. These results indicate that the C-terminus of HDC should not interfere with ODC dimerization (22). Thus, dramatic changes in the final conformation of the chimaeric enzyme cannot be suspected. It is not the case for rat HDC; in fact, its primary translation product (a 74 kDa polypeptide) is inactive (11), and it must lose 10-20 kDa to become active.

Mammalian ODC lacking its PEST region-containing C-terminus greatly reduces its access to the proteasome (1,2). If the C-terminus of HDC could be related to the proteasome 26 S as previously suggested (11,24), the degradation of the ODC/HDC chimaera, should occur by an antizyme and ATP-dependent mechanism. However, in the absence of exogenous antizyme, the decay of the band corresponding to the 74 kDa chimaera was around 25 ± 7% in one hour, but the former process was not ATP-dependent (Figure 1). The addition of 10 ng of recombinant antizyme did not significantly increase the degradation time course of the chimaera either; the contrary occurred with degradation of wild-type rat ODC (Figure 2). These results indicate that the HDC C-terminus of HDC cannot replace that of mammalian ODC to drive the protein to the proteasome, even though the rest of ODC maintains the active conformation for antizyme- and ATP-dependent degradation.

In conclusion, the data presented here suggest that the PEST region-containing C-terminus of HDC should have a role different to that described for the C-terminal PEST region of ODC. Recently, it has been proposed that the C-terminus of HDC is involved in protein sorting (25,26), but this full C-terminus fragment does not also seem to be responsible for the degradation of the polypeptide by proteasome 26 S. A further characterization of the degradation of HDC protein is in progress.

FIG. 2. Degradation patterns of wild type ODC, ODC-ΔPEST and ODC/HDC chimaera in a degradation medium containing antizyme and a regenerating system for ATP. The number below each band represents the percentage of degradation of the total protein present at zero time.

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REFERENCES