The ornithine decarboxylase domain of the bifunctional ornithine decarboxylase/*S*-adenosylmethionine decarboxylase of *Plasmodium falciparum*: recombinant expression and catalytic properties of two different constructs

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The polyamines putrescine, spermidine and spermine play an essential role in cell differentiation and proliferation. Inhibition of the rate-limiting enzymes of polyamine biosynthesis, ornithine decarboxylase (ODC) and S-adenosylmethionine decarboxylase (AdoMetDC), has been proposed as a therapeutic strategy against cancer and parasitic infections. In the case of *Plasmodium falciparum*, the causative agent of malaria tropica, this approach is especially interesting, because here both key enzymes, ODC and AdoMetDC, are combined in a bifunctional protein, ODC/AdoMetDC. This arrangement has not been found in any other organism investigated so far. We report the cloning and recombinant expression of the ODC domain of *P. falciparum* in *Escherichia coli*. First, we expressed the mere recombinant ODC domain (r*Pf*ODC). Secondly, we expressed the recombinant ODC domain in conjunction with the preceding part of the hinge region of the bifunctional ODC/AdoMetDC (r*Pf*Hinge-ODC). K_m values for L-ornithine were 47.3 μM for the r*Pf*Hinge-ODC and 161.5 μM for the r*Pf*ODC. Both recombinant enzymes were inhibited by putrescine, but the K_i value for the r*Pf*Hinge-ODC was 50.4 μM (IC$_{50}$ = 157 μM), whereas the IC$_{50}$ for the r*Pf*ODC was 500 μM. Spermidine was a weak inhibitor in both cases. α-Difluoromethylornithine inhibited the r*Pf*Hinge-ODC with a K_i value of 87.6 μM. For two novel ODC inhibitors, CGP52622A and CGP54619A, the K_i values of the r*Pf*Hinge-ODC were in the nanomolar range.

Key words: chemotherapy, malaria, polyamine.

INTRODUCTION

The diamine putrescine and the polyamines spermidine and spermine are ubiquitous organic cations, which are indispensable for cell growth and differentiation. The two rate-limiting enzymes for polyamine biosynthesis are ornithine decarboxylase (ODC; EC 4.1.1.17) and *S*-adenosylmethionine decarboxylase (AdoMetDC; EC 4.1.1.50) [1]. ODC decarboxylates L-ornithine to yield putrescine, the precursor of spermidine and spermine. The AdoMetDC produces decarboxylated *S*-adenosylmethionine. This serves as a donor of propylamine moieties, which are ligated to putrescine to form spermidine, and to spermidine to yield spermine. These reactions are carried out by spermidine synthase and spermine synthase, respectively [2]. ODC and AdoMetDC occur as two separate proteins in all organisms investigated so far. However, it was recently discovered in our laboratory that, in *Plasmodium falciparum*, both enzymes are linked to form one bifunctional ODC/AdoMetDC. The N-terminal AdoMetDC domain is joined to the C-terminal ODC domain by a hinge region [3].

P. falciparum is the causative agent of severe malaria. Resistance against the anti-malarial drugs used today is developing and spreading rapidly. For this reason, there is an urgent need to identify new compounds that attack novel targets in the parasite's metabolism [4].

Inhibition of polyamine metabolism is known to have anti-tumour and anti-parasitic effects [5,6]. The specific ODC inhibitor α-difluoromethylornithine (DFMO) is used as a trypanocidal drug against African sleeping sickness [7,8]. DFMO has also been reported to affect Plasmodia *in vitro* and *in vivo* [9–11]. Apart from DFMO, a series of analogues of 3-amino-oxy-1-propanamine have been reported to potently inhibit ODC. They were active against human T$_{24}$ bladder carcinoma both *in vitro* and *in vivo* [12,13].

We have cloned and recombinantly expressed the ODC domain of the bifunctional ODC/AdoMetDC. Two different constructs were expressed. The first comprises the *P. falciparum* ODC (*Pf*ODC) domain only. The second consists of the *Pf*ODC domain in addition to the preceding part of the hinge region of the bifunctional ODC/AdoMetDC. We analysed the catalytic properties of both recombinant proteins. Furthermore, we tested DFMO and two 3-amino-oxy-1-propanamine analogues for their potential as inhibitors of the *Pf*ODC domain.

EXPERIMENTAL

Material

L-Ornithine was purchased from Sigma (Deisenhofen, Germany). Putrescine was a product of Fluka (Neu-Ulm, Germany),

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L-[1-^{14}C]ornithine (57 mCi·mmol^{-1}) of Amersham-Buchler (Braunschweig, Germany). DFMO was a gift from Marion-Merrel-Dow (Cincinnati, OH, U.S.A.). Ni^{2+}-nitrilotriacetate (Ni-NTA) agarose was obtained from Qiagen (Hilden, Germany). The StrepTactin® resin and anhydrotetracycline were purchased from the Institut für Bioanalytik (Göttingen, Germany). The CGP series of ODC inhibitors was synthesized by Novartis (Basel, Switzerland) [12,13].

Cloning of the PfODC domain

The ODC domain of the bifunctional ODC/AdoMetDC of *P. falciparum* was determined by alignment of the deduced amino acid sequence with the amino acid sequences of the ODCs of various organisms. The domain comprises the amino acids methionine 805 to tryptophan 1419 [3]. We constructed two expression plasmids. (i) The first was the ODC domain alone (bp 2413–4257 of the coding region of the bifunctional ODC/AdoMetDC) cloned into the expression plasmid pJC40 [14]. This expression construct was termed pJC40-*Pf*ODC. (ii) Second, the ODC domain and the additional 432 bp of the hinge region of the bifunctional ODC/AdoMetDC were cloned into the expression plasmid pASK-IBA7 (Institut für Bioanalytik). This expression construct comprised the bp 1981–4257 of the coding region of ODC/AdoMetDC. It was termed pASK-IBA7-*Pf*ODC.

To obtain pJC40-*Pf*ODC, the coding region of the ODC domain was amplified by PCR with *Pfu* polymerase using genomic DNA of *P. falciparum* FCBR strain as a template and the sense primer 5′-GCGCGAATTCATGGAAAAGAATTA-TAAAGAAGAAATATGG-3′ and the antisense primer 5′-GCGCGGATCCTTACCAATGTTTGTTTGGTTGCCCC-3′. The underlined nucleotides indicate restriction sites for *Eco*RI in the sense primer and *Bam*HI in the antisense primer. The PCR product was gel-purified and subcloned into the plasmid pBS+ (Stratagene) by cyclic restriction ligation. The resulting construct was transformed into *Escherichia coli* DH5α. Recombinant plasmid DNA was isolated and digested with *Eco*RI and *Bam*HI. The insert was gel-purified and subcloned into *Eco*RI/*Bam*HI-digested pJC40.

Compared with pJC40-*Pf*ODC, the insert in pASK-IBA7-*Pf*ODC was extended at its 5′ end by 432 bp of the ODC/AdoMetDC hinge region. We amplified bp 1981–4257 of the coding region of ODC/AdoMetDC by PCR using the sense primer 5′-GCGCGCGGTCTCCGCGCATGGAATATG-AAGAAAAAGATGAAGTGTATCGAAGG-3′, the antisense primer 5′-GCGCGCGGTCTCGTATCATTACCAATGTTTG-TTTGGT-TGCCCC-3′, *Pfu* DNA polymerase and genomic DNA of the *P. falciparum* FCBR strain as a template. The restriction sites for *Bsa*I (New England Biolabs) are underlined in the primer sequences. The PCR product was gel-purified and digested with *Bsa*I. It was then ligated into *Bsa*I-digested pASK-IBA7.

Both expression plasmids were sequenced to ensure that the inserts were in the correct reading frame. Sequencing methods used were the Sanger dideoxy chain-termination method on double-stranded DNA [15] and terminator cycle sequencing using Ampli*Taq* DNA polymerase (Applied Biosystems) on an ABI Prism™ automatic sequencer.

Expression and purification of recombinant PfODC

pJC40-*Pf*ODC was transformed into *E. coli* BL21 (DE3) pLys. A single colony was picked and grown overnight at 37 °C in terrific broth medium supplemented with 50 μg/ml ampicillin

[16]. Expression was carried out in a 2 litre high-density fermenter. The overnight culture (40 ml) was added to 1.5 litres of terrific broth supplemented with 50 μg/ml ampicillin. The cultures were grown at 37 °C to a D_{600} of 1.5. Subsequently, 500 ml of terrific broth medium, 50 μg/ml ampicillin and 1 mM isopropyl β-D-thiogalactoside were added to the cultures. The incubation temperature was reduced to 30 °C. Cells were harvested 4 h after induction by centrifugation (4000 *g*; 10 min). The bacterial pellet was resuspended in binding buffer (20 mM Tris/HCl, pH 7.9, containing 5 mM imidazole, 500 mM NaCl and 0.1 mM PMSF) and sonicated. The cell lysate was centrifuged at 100000 *g* and 4 °C for 1 h. pJC40 encodes for an N-terminal His tag. The recombinant protein r*Pf*ODC was purified by chelating chromatography on Ni-NTA agarose (Qiagen) according to the manufacturer's recommendations.

pASK-IBA7-*Pf*ODC was transformed into *E. coli* BL21-CodonPlus™(DE3)-RIL (Stratagene). A single colony was picked and grown overnight in Luria–Bertani medium supplemented with 50 μg/ml ampicillin and 0.35 μg/ml chloramphenicol. The bacterial culture was diluted 1:50 and incubated at 37 °C until the D_{600} reached 0.5. Expression was induced with 200 ng/ml anhydrotetracycline. Bacteria were grown for an additional 4 h at 30 °C before being harvested. The bacterial pellet was resuspended in 100 mM Tris/HCl buffer, pH 8.0, containing 1 mM EDTA, sonicated and then centrifuged at 100000 *g* for 1 h at 4 °C. The recombinant *Pf*ODC with the partial hinge region (r*Pf*Hinge-ODC) was purified using StrepTactin® resin according to the manufacturer's recommendations.

The eluants from both constructs of recombinant *Pf*ODC were dialysed separately against 50 mM Tris/HCl buffer, pH 7.5, containing 1 mM dithiothreitol, 1 mM EDTA, 0.02% Brij-35 and 0.1 mM PMSF. Protein was determined by the method of Bradford [17], using BSA as a standard. The homogeneity of the enzyme preparation was analysed by SDS/PAGE. The proteins were revealed by Coomassie Brilliant Blue staining [16]. Recombinant expression and purification of the bifunctional ODC/AdoMetDC of *P. falciparum* was carried out as described previously [3].

N-terminal amino acid sequence of rPfODC

After purification, the eluant containing r*Pf*ODC was separated by SDS/PAGE, electrotransferred to a ProBlot™ membrane (Applied Biosystems) and stained with Coomassie Brilliant Blue. The band corresponding to the r*Pf*ODC (74 kDa) was excised and subjected to automated Edman degradation (Protein Sequencer 473A, Applied Biosystems).

Enzyme assay

Enzyme activity was assayed by measuring the production of $^{14}CO_2$ from L-[1-^{14}C]ornithine at 37 °C as described previously [18]. The standard assay contained, in a final volume of 250 μl, 50 mM potassium phosphate buffer, pH 7.5, 1 mM dithiothreitol, 1 mM EDTA, 40 μM pyridoxal 5-phosphate, 0.02% Brij-35, 100 μM ornithine (0.27 μCi of L-[1-^{14}C]ornithine) and 75 nM r*Pf*Hinge-ODC, 160 nM r*Pf*ODC or 72 nM recombinant ODC/AdoMetDC. The reaction time was 30 min. K_m values of the recombinant *Pf*ODC domains were determined by varying the concentration of the non-radioactive ornithine in the standard assay. The inhibition constants for putrescine, CGP54619A and CGP52622A were determined under standard assay conditions with varying concentrations of ornithine and the addition of various concentrations of inhibitors. The K_m value and the K_i

value for putrescine were calculated by Lineweaver–Burk plots using the software GraphPad Prism 1.0 (GraphPad Software). The K_i values of the tight-binding inhibitors CGP54619A and CGP52622A were calculated from the Morrison equation [19]. The K_i value for the irreversible inhibitor DFMO was determined by preincubation of aliquots of the r*Pf*Hinge-ODC at 37 °C with various concentrations of DFMO. At different time points, samples were withdrawn and assayed for residual enzyme activity. Kinetic analysis of the inhibition was carried out according to the method of Kitz and Wilson [20]. The inhibitory effect of spermidine on both recombinant *Pf*ODC domains and of putrescine on the r*Pf*ODC was determined under standard assay conditions, adding different concentrations of spermidine and putrescine, respectively. The IC_{50} values of r*Pf*Hinge-ODC for putrescine, CGP54619A and CGP52622A were calculated from the equation $IC_{50} = K_i(1 + [S]/K_m)$.

RESULTS AND DISCUSSION

Cloning and expression of the recombinant *Pf*ODC domain

The ODC domain of *P. falciparum* is part of the bifunctional ODC/AdoMetDC [3]. Such an arrangement of the two key enzymes in polyamine synthesis has, to date, not been detected in any other organism. However, *P. falciparum* possesses other bifunctional proteins. In its folate metabolism, the dihydrofolate reductase is joined to the thymidylate synthase (DHFR-TS), and the dihydropteroate synthase is combined with the dihydro-6-hydroxymethylpterin pyrophosphokinase [21,22]. Moreover, in two membrane proteins an ATPase domain is combined with a guanylate cyclase domain [23].

We expressed the *Pf*ODC domain in two different forms. In the pJC40-*Pf*ODC construct, we merely included the ODC domain of the bifunctional protein as it was defined by Müller et al. [3]. The recombinant *Pf*ODC domain was termed r*Pf*ODC and contained an N-terminal His tag to facilitate purification. The pASK-IBA7-*Pf*ODC construct carried the *Pf*ODC domain and in addition the preceding part of the hinge region at its 5′ end. The recombinant protein was termed r*Pf*Hinge-ODC and carried an N-terminal Strep tag (Figure 1). r*Pf*ODC and r*Pf*Hinge-ODC were purified by affinity chromatography on Ni-NTA agarose and StrepTactin® resin, respectively, and 1 litre of bacterial culture yielded about 500 μg of r*Pf*ODC or 150 μg of r*Pf*Hinge-ODC. The purity of the enzyme preparations was assessed by SDS/PAGE (Figure 2). The band representing the putative recombinant r*Pf*ODC was subjected to N-terminal amino acid sequencing, which confirmed the presence of the recombinant protein (results not shown). Western blotting with antiserum against r*Pf*ODC identified the bands running below

Figure 2   SDS/PAGE analyses of the r*Pf*ODC (I) and r*Pf*Hinge-ODC (II)

The recombinant proteins migrated to 71 and 88 kDa, respectively. Bands migrating below them were considered products of degradation. Protein was revealed by Coomassie Brilliant Blue staining.

the recombinant proteins as products of degradation (results not shown). The r*Pf*ODC has a subunit molecular mass of 74.3 kDa. (71.3 kDa *Pf*ODC domain and 3 kDa His tag). The r*Pf*Hinge-ODC, which includes 144 amino acids of the hinge region, has a molecular mass of 87.9 kDa (including 1.5 kDa Strep tag). The *Pf*ODC domain is substantially larger than most eukaryotic ODCs, which vary in their molecular mass between 43 and 55 kDa [18,24–29]. This is due to two large insertions in the *Pf*ODC domain, which interrupt the regions of homology [3]. The ODC of *Leishmania donovani* is even larger than the *Pf*ODC domain because it possesses an N-terminal extension [30]. However, this extension does not bear any homology to the hinge region of the *P. falciparum* ODC/AdoMetDC [3]. Although the overall homology between the *Pf*ODC domain and the mammalian ODC is only 21 %, the residues that are essential for dimerization and catalytic activity of the mammalian ODC are conserved in the *Pf*ODC domain [3].

Enzyme activity

Both forms of *Pf*ODC that we expressed are dependent on pyridoxal 5-phosphate, which is the essential coenzyme of ODCs [31]. Maximum specific activity of the recombinant *Pf*ODC domains is obtained at pyridoxal 5-phosphate concentrations of 10 μM and above for the r*Pf*Hinge-ODC and 50 μM and above for the r*Pf*ODC in the standard assay. Optimal activity is obtained at pH values of 7.0–8.0 for the r*Pf*ODC and pH 7.5–8.5 for the r*Pf*Hinge-ODC. The pH optimum reported for mammalian ODC is in the range of 7.0–7.8 [31]. Both *Pf*ODCs were stabilized by the addition of 0.02 % Brij-35. This feature was also reported for highly purified mammalian ODC [31]. Freezing leads to a complete loss of activity of the recombinant *Pf*ODC domains. The differential sensitivity to pyridoxal 5-phosphate and the different pH optima warrant the conclusion that there are distinctions between the catalytic features of the two recombinant *Pf*ODC domains. Analysing the enzyme activities in more detail, this becomes even more obvious: the specific activity and k_{cat} value of the r*Pf*ODC are lower than those of the r*Pf*Hinge-
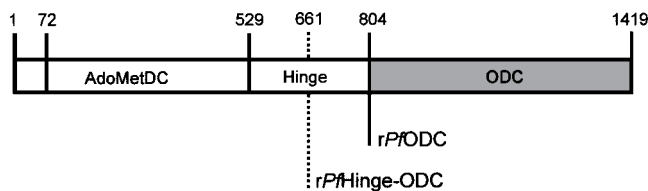

Figure 1   Schematic organization of the *P. falciparum* ODC/AdoMetDC

Numbers correspond to amino acid residues. The AdoMetDC domain comprises amino acid residues 1–529. Residue 72 indicates the potential cleavage site for autocatalytic processing of the AdoMetDC domain. Residues 530–804 form the hinge region. The ODC domain consists of amino acids 805–1419. The dotted line at amino acid residue 661 indicates the N-terminus of the r*Pf*Hinge-ODC. The r*Pf*ODC comprises the amino acid residues 805–1419.
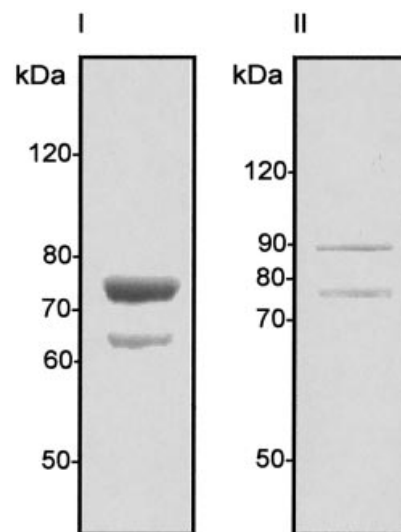
Table 1   Comparison of the properties of the r*Pf*Hinge-ODC, the r*Pf*ODC and the mammalian ODC

	r*Pf*Hinge-ODC	r*Pf*ODC	Mammalian ODC
Molecular mass (Da)	86 400	71 300	50 000–54 000*
pH optimum	7.5–8.5	7.0–8.0	7.0–7.8*
K_{cat} (min^{-1})	0.38	0.20	–
K_m for L-ornithine (μM)	47.3	161.5	30–200*
K_i for putrescine (μM)	50.4	160.5†	600‡
K_i for DFMO (μM)	87.6	–	39§

* Data from [31].
† Calculated from the equation $K_i = \text{IC}_{50}/(1 + [S]/K_m)$.
‡ Data from [24].
§ Data from [36].

ODC. The enzyme activity of the r*Pf*Hinge-ODC is linear with time and enzyme concentration for up to 40 min of incubation at 37 °C. The mean specific activity of the r*Pf*Hinge-ODC is 4.3 ± 0.54 nmol·min^{-1}·mg^{-1} ($n = 4$), resulting in $k_{cat} = 0.38$ min^{-1}. The r*Pf*ODC has a maximum specific activity of 2.9 nmol·min^{-1}·mg^{-1} and its k_{cat} is 0.2 min^{-1}. More importantly, the K_m value of the r*Pf*ODC for the substrate L-ornithine is significantly higher than the K_m value of the r*Pf*Hinge-ODC. The K_m value of the r*Pf*Hinge-ODC is 47.3 ± 10.9 μM ($n = 4$), which is comparable with that of the *Pf*ODC domain in the native bifunctional protein ($K_m = 42.4$ μM) [3]. This K_m value is similar to those of the monofunctional proteins of mammals [24,32]. Compared with the ODCs of other protozoa, however, the *Pf*ODC domain has a considerably higher affinity for its substrate [25,29,32]. The K_m value of the r*Pf*ODC for L-ornithine is 161.5 ± 61.8 μM ($n = 4$), which is about 3.5 times higher than the K_m value of the r*Pf*Hinge-ODC and the native *Pf*ODC domain.

We assessed the influence of their product, putrescine, on the activity of the recombinant *Pf*ODC domains. The K_i value of the r*Pf*Hinge-ODC for putrescine is 50.4 ± 16.8 μM ($n = 3$), and the calculated IC$_{50}$ value is 157 μM. Similar K_i values were reported for the ODC of the nematode *Panagrellus redivivus* and a membrane-associated ODC of *Caenorhabditis elegans* [18,33]. In contrast, the ODCs of mammals as well as of some lower eukaryotes are less sensitive to putrescine, the K_i value for putrescine of the ODC from rat liver being 600 μM [24,26,29,34]. Interestingly, the IC$_{50}$ value of the r*Pf*ODC for putrescine is 500 μM. This value is in line with the lower affinity for L-ornithine of the r*Pf*ODC as compared with the r*Pf*Hinge-ODC. For a summary of the properties of the r*Pf*Hinge-ODC, the r*Pf*ODC and the mammalian ODC, see Table 1. Furthermore, we tested the effect of spermidine on the recombinant *Pf*ODC domains. Spermidine is a weak inhibitor of both recombinant enzymes, and it affects them equally. In a standard assay, 1 mM spermidine reduced the activity of the r*Pf*Hinge-ODC by 61 %, the activity of the r*Pf*ODC by 63 % ($n = 2$). As far as we are aware, significant inhibition of ODC activity by spermidine has only been reported for the membrane-associated ODC of *C. elegans* [33].

These results show that there are substantial catalytic differences between the r*Pf*Hinge-ODC and the r*Pf*ODC, whereas the K_m value of the r*Pf*Hinge-ODC is comparable with that of the native ODC domain in the bifunctional protein [3]. We therefore conclude that part of the hinge region of the bifunctional ODC/AdoMetDC is important for the catalytic activity of the *Pf*ODC domain. It is conceivable that the hinge region supports the correct folding of the *Pf*ODC domain.

We have separately cloned and recombinantly expressed the ODC domain and the AdoMetDC domain (T. Krause, K. Lüersen, C. Wrenger, S. Müller and R. D. Walter, unpublished work) of the bifunctional *P. falciparum* ODC/AdoMetDC. Both domains are catalytically active, and surprisingly their catalytic features do not differ from those of the native bifunctional protein [3]. It is, therefore, unlikely that in the native bifunctional ODC/AdoMetDC the two domains exert any regulatory influence on each other. This is in contrast to the DHFR-TS of *P. falciparum*, which is a well-studied bifunctional protein. In the DHFR-TS an essential interaction between the two domains takes place, rendering it impossible to express a functional TS domain only [35].

Inhibition of enzyme activity

The inhibitor studies were carried out with the r*Pf*Hinge-ODC and, for comparison, with the recombinant bifunctional ODC/AdoMetDC. The development of DFMO by Metcalf and colleagues in 1978 [36] constitutes a milestone in polyamine research. DFMO, a derivative of the amino acid ornithine, is a specific, so-called mechanism-based inhibitor of ODC. It leads to irreversible inhibition of the enzyme by an alkylation of its active centre. DFMO is an active trypanocidal agent, which cures patients with African sleeping sickness caused by *Trypanosoma brucei gambiense* even at late stages of the disease [8]. Moreover, DFMO blocks the erythrocytic schizogony of *P. falciparum* in culture and reduces the parasitaemia in *Plasmodium berghei*-infected mice [9–11].

We determined the K_i for DFMO of the r*Pf*Hinge-ODC as 87.6 ± 14.3 μM ($n = 3$). The half-time of inactivation at saturating DFMO concentrations is 0.65 ± 0.07 min ($n = 3$; Figure 3). In comparison, mouse ODC has a K_i for DFMO of only 39 μM [36]. However, the K_i of the *T. brucei brucei* ODC for DFMO is 220 μM, and its half-time of inactivation is 2.7 min [37]. The differential effect of DFMO on trypanosomes as compared with mammalian cells can be explained by three factors. First, the parasites are rapidly dividing cells, and as such have a higher requirement for polyamines than the host cells. Secondly, trypanosomes use spermidine to synthesize trypanothione, which is essential for the maintenance of their intracellular redox state [38]. Thirdly, the ODC of *T. brucei* has a longer half life than the mammalian ODC, because it lacks a PEST sequence which leads to rapid intracellular degradation of the mammalian enzyme [2,28]. This argument can partly be applied to Plasmodia: they are also rapidly proliferating cells. The ODC of *P. falciparum* was reported to be relatively stable [9], although its C-terminus resembles a PEST region [3].

A few years ago, a series of highly potent ODC inhibitors was synthesized. The compounds are analogues of 3-amino-oxy-1-propanamine [12,13]. Members of this group of ODC inhibitors were reported to inhibit rat liver ODC with an IC$_{50}$ in the nanomolar range. They are active against the growth of human T$_{24}$ bladder carcinoma cells *in vitro*, and some compounds are tumoristatic in mice bearing solid T$_{24}$ tumours. The cellular growth arrest appears to be due to polyamine depletion, because it is reversed by spermidine. Furthermore, cells resistant to 3-amino-oxy-2-fluoro-1-propanamine have amplified *ODC* genes [12,13]. CGP52622A, which belongs to this series, is a reversible inhibitor of the *Pf*ODC. It inhibits the r*Pf*Hinge-ODC with a K_i value of 20.4 ± 8.1 nM ($n = 3$). The calculated IC$_{50}$ value is 63.5 nM, which is comparable with that of the recombinant bifunctional ODC/AdoMetDC (31.7 ± 2.8 nM, $n = 3$). The inhibitory effect of the second compound which we tested, CGP54619A, is even more pronounced. It reversibly inhibits the
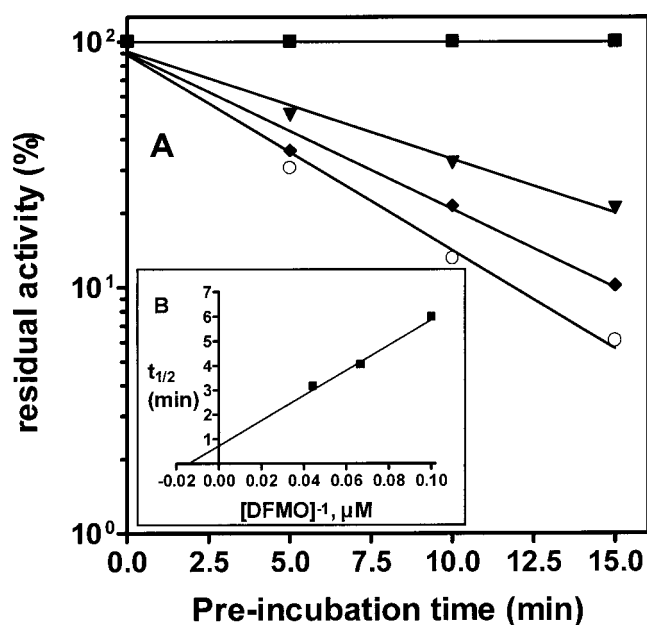
Figure 3 Representative experiment for the time- and concentration-dependent inhibition of the r*Pf*Hinge-ODC with DFMO

(**A**) The r*Pf*Hinge-ODC was preincubated without (■) or with 10 (▼), 15 (◆) or 22.5 μM (○) DFMO for the times indicated. Residual ODC activity was then assayed as described in the Experimental section. (**B**) The half lives ($t_{1/2}$) of ODC activity in the presence of DFMO were determined and plotted against the reciprocals of the inhibitor concentrations. The K_i value was determined to be 69.2 μM, $t_{1/2}$ was 0.75 min.

r*Pf*Hinge-ODC with a K_i value of 7.9 ± 2.1 nM ($n = 3$). Again, the calculated IC_{50} value of 25 nM fits well with the IC_{50} value determined for the recombinant ODC/AdoMetDC (18.6 ± 1.3 nM, $n = 3$). In view of the extremely low K_i values, we propose that CGP52622A and CGP54619A should be further evaluated as possible anti-malarial agents.

In summary, we have cloned, recombinantly expressed and kinetically characterized the ODC domain of the bifunctional ODC/AdoMetDC in two forms. The catalytic differences between the recombinant *Pf*ODC domain that includes part of the hinge region, and the recombinant *Pf*ODC domain without the hinge region, imply that the hinge region is important for correct folding and activity of the *Pf*ODC domain. Interestingly, the recombinant r*Pf*Hinge-ODC has catalytic properties comparable with the native *Pf*ODC domain. We have tested inhibitors of ODC against this recombinant domain, and identified CGP52622A and CGP54619A as highly effective compounds. They should be assessed for their potential as anti-malarial drugs.

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