The differential contribution of arginase and transamidinase to ornithine biosynthesis in two achromic human melanoma cell lines

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Cellular ornithine biosynthesis could be expected to play a significant role in putrescine formation and hence in growth. Two enzymes are involved in ornithine biosynthesis: arginase and transamidinase. These enzyme activities were studied in two human melanoma cell lines differing in their K_m of diamine oxidase for putrescine and in their tumorigenicity in nude mice. Arginase activity accounts for the majority of ornithine formed in the highly tumorigenic cell line, while the majority of ornithine is derived from transamidinase action in the poorly tumorigenic cell line, with concomitant formation of methyl guanidine, a potent inhibitor of diamine oxidase.

Arginase activity *Transamidinase activity* *Guanidinoacetate* *Methyl guanidine*
Ornithine biosynthesis *Human melanoma cell line*

1. INTRODUCTION

It is now well documented that increases in polyamines occur in cell growth processes [1–3]. L-Ornithine decarboxylase (L-ornithine carboxyllyase, EC 4.1.1.17) (ODC) catalyses the formation of putrescine from L-ornithine, and a large body of experimental evidence points to the key role of this enzyme ODC as the rate limiting step in the biosynthetic pathway of the polyamines. α-Difluoromethylornithine (DFMO) has been shown to represent a highly selective, enzyme-activated, inhibitor of ODC, resulting in the depletion of intracellular putrescine and spermidine content in several mammalian systems, with concomittant arrest of cell proliferation [4]. In view of this, cellular ornithine biosynthesis could be expected to play a significant role in putrescine formation and hence in growth. Two enzymes are involved in ornithine biosynthesis: arginase (L-arginine amidohydrolase, EC 3.5.3.1.) which catalyses the cleavage of L-arginine to urea and ornithine, and transamidinase (L-arginine–glycine amidinotransferase, EC 2.1.4.1) which transfers an amidine group from arginine to glycine to form guanidinoacetate and ornithine. Elevated arginase activities have been demonstrated in growing tissues and tumours [5–8], but this increased arginase activity has not always paralleled increased ODC activity [9]. It therefore seemed important to determine the contribution of transamidinase to ornithine biosynthesis under such conditions, especially as the other product of transamidinase action is guanidinoacetate which can be decarboxylated, at least theoretically, to methyl guanidine a potent inhibitor of diamine oxidase activity [10], and that alteration in diamine oxidase activity due to an altered K_m of the enzyme for putrescine takes place in tumorigenic human melanoma cells [11].

Arginase and transamidinase activities were studied in 2 human melanoma cell lines differing in their K_m of diamine oxidase and in their tumorigenicity in nude mice. This communication reports that arginase activity is the major contribution to ornithine formation in the highly tumorigenic cell line while the majority of ornithine, leading to greater formation of methyl guanidine, would appear to be derived from transamidinase action in the poorly tumorigenic cell line.

2. MATERIALS AND METHODS

L-[*guanido*-^{14}C]Arginine–HCl (56 mCi/mmol) was obtained from the Amersham International

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(Bucks) and was purified as in [12].

L-Arginine–HCl and L-glycine were purchased from Sigma Chemical Co. (St Louis MO).

Two achromic cell strains M_3Dau and M_1Dor, derived from metastatic human malignant melanoma were used. M_3Dau cells are highly tumorigenic in nude mice, while M_1Dor cells are poorly tumorigenic [13]. The homogenates for enzymic assays were obtained from cells in tissue culture as in [11].

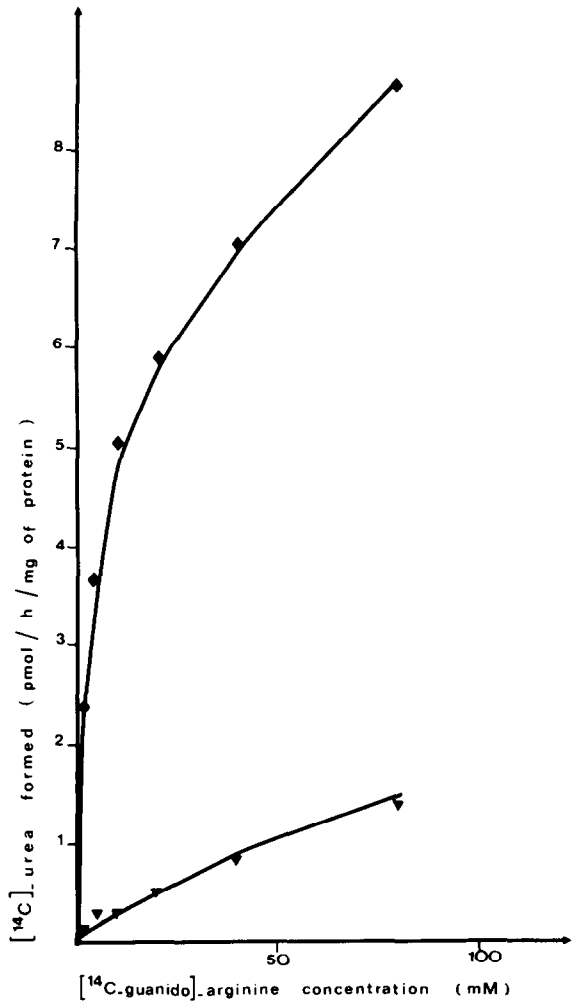

Fig.1. Arginase activity in 2 human melanoma cell lines. Cellular extracts were incubated for 120 min at 37°C at pH 9.7 in 250 μl. [*guanido*-^{14}C]Arginine was diluted with unlabeled arginine to a constant spec. act. of 5 μCi/mmol. [^{14}C]Urea released was determined as in [12]. [^{14}C]Urea formed in: (◆)M_3Dau; (▾)M_1Dor.

Arginase activity was determined at pH 9.7 by the radiometric assay developed in [12].

Transamidinase activity was determined by measuring [^{14}C]guanidinoacetate produced from L-[*guanido*-^{14}C]arginine. The 250 μl incubation volume contained 100 mM potassium phosphate buffer (pH 7.4) 25 mM L-arginine, 75 mM glycine, 1 mg cellular extract, and was incubated at 37°C for 120 min. The reaction was stopped by adding 50 μl 100% trichloroacetic acid. The mixture was then poured onto a (20 × 1 cm) column filled with Dowex 50W-X8 (100–200 mesh, H^+form). [^{14}C]-Urea, in contrast to [^{14}C]guanidinoacetate, [^{14}C]-methyl guanidine and L-[*guanido*-^{14}C]arginine is not retained by Dowex 50W-X8 (H^+) and can therefore be collected directly into scintillation vials. [^{14}C]Guanidinoacetate and [^{14}C]methyl guanidine were eluted from the resin with 40 ml 2 N HCl, L-[*guanido*-^{14}C]arginine with 20 ml 6 N HCl. The 2 N HCl fraction was taken to dryness on a rotary evaporator, the residue was dissolved in water, readjusted to pH 2.5 and applied onto a Dowex 50W-X8 (H^+) column, [^{14}C]guanidinoacetate and [^{14}C]methyl guanidine were successively eluted from the resin with 50 ml of a linear gradient of 0.5–2.0 N HCl.

Identification of each fraction was performed by comparison of the R_f of unknown with that of authentic markers by thin-layer chromatography on silica gel using as solvent *n*-butanol/acetone/glacial acetic acid/water (35:35:10:20, by vol.) [14]. The position of the authentic compounds was revealed by the Voges-Proskauer reaction [15] that of the labelled unknowns by determining the radioactive content of 1 cm fractions of the thin-layer plate.

3. RESULTS

3.1. *Arginase activity in melanoma cell lines*

To determine whether differences in arginase activity exist between M_3Dau and M_1Dor cell lines, the activity of this enzyme was measured on homogenates derived from cells at confluence. The activity of arginase in melanoma cells was higher in M_3Dau cells than in M_1Dor cells at different substrate concentrations (fig.1). These differences are not due to different localization of arginase because the assay are carried on whole cellular extract. Given that even at the optimum pH for

Table 1

Arginase and transamidinase activities in two achromic melanoma cell lines

Melanoma cell lines	pH in assay solution	[^{14}C]Urea formed (pmol . mg protein^{-1} . h^{-1})	[^{14}C]Guanidinoacetate formed (pmol . mg protein^{-1} . h^{-1})
M_3Dau	9.7	657 ± 306	72 ± 12
M_1Dor	9.7	88 ± 47	113 ± 79
M_3Dau	7.4	111 ± 42	211 ± 144
M_1Dor	7.4	38 ± 12	249 ± 143

The results are mean ± SEM of 5 replicates

arginase a small but significant amount of [^{14}C]-guanidine is used up via transamidination, the amount of [^{14}C]guanidinoacetate formed under conditions optimal for arginase was determined. 90% of the [^{14}C]guanidine group of arginine was found in urea while 10% is recovered in guanidinoacetate from M_3Dau cells while there was about an equal distribution of radioactivity in urea and guanidinoacetate from M_1Dor cells (table 1).

3.2. *Transamidinase activity in melanoma cell lines*

These results prompted us to investigate directly transamidinase activity under optimal conditions and to assess any loss of [^{14}C]guanidine via residual arginase action. Transamidinase activity in M_3Dau cells and in M_1Dor cells is not significantly different (table 1) whereas loss of [^{14}C]guanidine through urea (arginase action) is 3-fold greater ($P < 0.05$) in M_3Dau than in M_1Dor.

In view of residual enzyme activity of transamidinase when arginase is determined in optimal conditions and vice versa, the total contribution of [*guanido*-^{14}C]arginine to [^{14}C]guanidinoacetate formation and to [^{14}C]urea formation was assessed at both pH-values. Table 2 shows that for M_3Dau cells 73.1% of L-[*guanido*-^{14}C]arginine is metabolized via arginase and 26.9% via transamidinase whereas for M_1Dor cells these values are 25.8% and 74.2%, respectively. Thus in M_3Dau cells highly tumorigenic in nude mice, ornithine is apparently synthetized preferentially by the arginase pathway while in M_1Dor the poorly tumorigenic line, the transamidinase pathway is predominant.

Table 2

Contribution of arginase and transamidinase to ornithine biosynthesis in two melanoma cell lines

Melanoma cell lines	Total act.[a]	Arginase act. (%)	Transamidinase act. (%)
M_3Dau	1051	73.1	26.9
M_1Dor	488	25.8	74.2

[a](pmol [^{14}C]guanidinoacetate + [^{14}C]Urea . mg protein^{-1} . h^{-1})

3.3. *Separation and identification of labelled reaction products of transamidinase activity*

Guanidinoacetate can be theoretically decarboxylated to methyl guanidine, a potent inhibitor of diamine oxidase [10]. It was therefore of interest to determine whether methyl guanidine was formed under the incubation conditions described. Accordingly, the reaction products were separated by column chromatography on Dowex 50W-X8 (H^+ form) in 2 steps and identified as in section 2. Table 3 shows that the amount of [^{14}C]methyl guanidine formed is greater in M_1Dor cells than in M_3Dau cells. The identity in R_f was verified in another solvent system: isopropanol/formic acid/water (80:4:20, by vol.).

4. DISCUSSION

It appears from these results that ornithine can be derived in these two human melanoma cell lines through the action of both arginase and transamidinase. However, the contribution of these enzymes to ornithine biosynthesis may differ in these cell lines. Thus, arginase activity is 7.5-fold greater in M_3Dau than in M_1Dor, but, their transamidinase activity is roughly equivalent.

A net preference for the arginase pathway in M_3Dau cells is seen at pH 9.7 and is further evidenced at pH 7.4, the optimal pH for transamidi-

Table 3

Methyl guanidine formation from guanidinoacetate in melanoma cell lines

Melanoma cell lines	Methyl guanidine (% formation)
M_3Dau	44.10 ± 4.6 (3)
M_1Dor	93.72 ± 2.5 (3)

Mean ± SE; data represent the average of 3 determinations

nase, where the residual arginase activity contributes to the formation of 111 pmol urea, i.e., due to the stoichiometry of the reaction, 34% of the ornithine formed.

However, the residual transamidinase activity which is still manifest at pH 9.7 contributes to form 113 pmol guanidinoacetate in M_1Dor cells. These 113 pmol guanidinoacetate are roughly equivalent to the amount of urea and hence to the amount of ornithine formed at pH 9.7. This indicates that in this cell line, even under conditions optimal for arginase activity, about half of the ornithine is formed through the transamidinase pathway. Thus, regardless of whether the assays are performed under optimal pH conditions, it seems reasonable to conclude that arginase activity is the major contribution to ornithine formation in M_3Dau cells, while the majority of ornithine in M_1Dor cells would appear to be derived from transamidinase action. This difference in arginine metabolism between the two cell lines is illustrated on table 2.

Another way of expressing this different utilization of arginine between these 2 cell lines is the guanidinoacetate/urea action under optimal conditions. In M_3Dau this ratio is 0.3 while in M_1Dor it is ~3, a 10-fold difference: this ratio is of the same order of magnitude as that obtained for these 2 cell lines in culture in arginine-deprived medium to which L-[*guanido*-^{14}C]arginine was added (not shown).

Guanidinoacetate, the other product of transamidinase action, is not metabolically inert. In fact the possibility of its subsequent decarboxylation to methyl guanidine was verified in these 2 cell lines. In M_1Dor methyl guanidine formation accounted for 93.72% of the metabolites formed from guanidinoacetate, while in M_3Dau methyl guanidine accounted for only 44.10% of the metabolites formed from guanidinoacetate. Since methyl guanidine has been shown to be an inhibitor of diamine oxidase [10], the decreased activity of this enzyme in M_1Dor reported in [11] may be due to the increased production of methyl guanidine through this pathway.

The verification of this relationship should provide an approach for directly testing the effect of methyl guanidine on the tumorigenicity of human melanoma cells.

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