# ATP and other purine nucleotides stimulate the inactivation of ornithine transcarbamylase by broken lysosomes

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Ornithine transcarbamylase (OTC) is very stable in rat liver homogenates, or mitochondria. However, pure OTC from rat or beef liver is inactivated by broken lysosomes. ATP and a number of purine nucleotides, particularly $\epsilon$-ATP, stimulate the inactivation of OTC by broken lysosomes. Inactivation of OTC by trypsin and elastase is also stimulated by $\epsilon$-ATP. Carbamoyl phosphate protects OTC against proteolytic attack by elastase or by broken lysosomes. Thus, environmental factors, e.g., ATP and carbamoyl phosphate, appear to influence OTC stability to proteases.

*Ornithine transcarbamylase* *Lysosome* *ATP* *Carbamoyl phosphate*

## 1. INTRODUCTION

Ornithine transcarbamylase (ornithine carbamoyltransferase, EC 2.1.3.3) is located in the matrix of liver mitochondria of ureotelic animals. This enzyme is very stable and, indeed, its stability to heat led to its discovery [1]. Although OTC, including intracellular transport, processing and uptake of its precursor, has been extensively studied, the degradation of the mature enzyme has not. The experiments outlined here indicate that lysosomal enzymes can inactivate OTC and that the inactivation is stimulated by purine nucleotides.

## 2. MATERIALS AND METHODS

Male Wistar rats between 200 and 300 g were used. Lysosomes, mitochondria and fractions thereof were obtained as in [2]. Mitochondria and lysosomes were disrupted before use by freezing and thawing them 10 times. Lysosomal fractions were obtained by centrifugation of broken lysosomes for 5 min at 130000 × $g$ in an air-driven centrifuge. The supernatant was used as such and the pelleted membranes were washed once and then resuspended in buffer to the original volume. Beef liver OTC and rat liver OTC were prepared as in [3,4]. Some of the experiments presented here have been done with rat liver OTC, but for practical reasons the majority of the experiments were done with OTC from beef liver. Unless otherwise specified, assays contained: 10 $\mu$g beef liver OTC, 0.2 M potassium phosphate (pH 5.5), 5 mM MSH and 1 mM nucleotides, when used, in a final volume of 0.1 ml; protein concentration was made up to 1 mg with BSA. All incubations were carried out in 1.5 ml Eppendorf centrifuge tubes at 37°C. All the results represent the average of two experiments. OTC, $\beta$-NAGASE, released amino acids and protein were determined by standard methods [4–8].

*Abbreviations:* OTC, ornithine transcarbamylase; $\beta$-NAGASE, $\beta$-*N*-acetyl-D-glucosaminidase; BSA, bovine serum albumin; $\epsilon$-ATP, 1-$N^6$-ethenoadenosine 5′-triphosphate; MSH, $\beta$-mercaptoethanol

## 3. RESULTS AND DISCUSSION

This laboratory has been interested in determining the conditions which control and/or regulate the stability, degradation and turnover of proteins, particularly mitochondrial enzymes [9–11].

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Carbamoyl-phosphate synthetase is very unstable and is inactivated by components of the inner mitochondrial membrane in combination with lysosomal enzymes [2]; it was therefore of interest to test the stability in broken cell preparations of OTC, the second enzyme of the urea cycle. We found that in homogenates and in the pH range 7.4–6.0, OTC is stable for as long as 6 h (not shown).

We tested the stability of OTC in disrupted mitochondria. These preparations show considerable proteolytic activity at neutral pH (even when the lysosomal contamination is very low [9,10]). Indeed, as shown in fig.1, there was extensive proteolysis and yet under these conditions OTC activity remained. In fact, there was a slight activation. Since it is believed that a great deal of intracellular protein degradation is carried out by lysosomes, it was of interest to test the effect of lysosomal enzymes on OTC. As illustrated in fig.2, OTC, although it is quite stable to pH 5, is inactivated fairly rapidly by broken lysosomes particularly at the lower pH values, reflecting the higher activity of lysosomal proteases in the lower pH range.

There has been a great deal of interest in recent years in the possibility that intracellular protein degradation is affected by or dependent on the presence of ATP [12,13]. It seemed of interest,

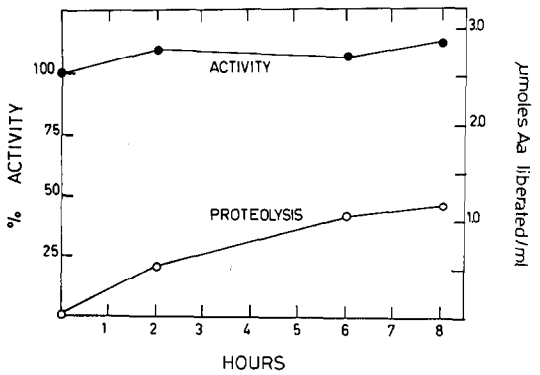


Fig.1. The effect of incubation of disrupted mitochondria on total proteolysis and on activity of ornithine transcarbamylase. In a final volume of 1 ml, each tube contained 5 mg of rat liver mitochondrial protein and 10 mM potassium phosphate buffer (pH 7.4). At the times indicated 100-μl portions were taken for assay. Lysosomal contamination was 0.008 units of β-NAGASE/ml.

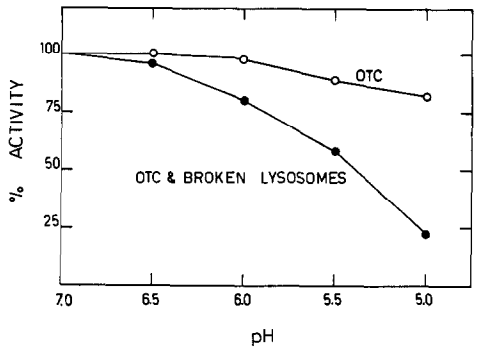


Fig.2. Action of lysosomal enzymes on rat liver OTC. Standard conditions were used except that 3 μg of rat liver OTC, broken lysosomes equal to 0.03 units of β-NAGASE and 50 mM potassium phosphate buffer were used. Incubation time was 1 h.

therefore, to see if ATP and other nucleotides affect the stability of OTC, and, whether the lysosomal effect was due to the proteases present in the soluble or in the membrane portions. As shown in table 1, broken lysosomes are more active than the supernatant. The membranes 'per se' have little activity. Moreover, as illustrated in the table, ATP in all cases stimulates the lysosomes as well as the supernatant fraction.

In view of these findings we checked a number of nucleotides with broken lysosomes or supernatants thereof. As shown in table 2, a number of purine, but not pyrimidine nucleotides, stimulate the inactivation of OTC by lysosomal enzymes.

Table 1

Stimulation by ATP of the inactivation of ornithine transcarbamylase by broken lysosomes and fractions thereof

| Additions | % activity remaining at 3 h |
|---|---|
| None | 100 |
| Broken lysosomes | 21 |
| Broken lysosomes and ATP | 7 |
| Supernatant | 44 |
| Supernatant and ATP | 23 |
| Membranes | 96 |
| Membranes and ATP | 96 |

Standard conditions and broken lysosomes (containing 0.03 units of β-NAGASE) or the equivalent fractions thereof (see section 2) were used

Table 2

The influence of purine and pyrimidine nucleotides on the stability of ornithine transcarbamylase to broken lysosomes

| Nucleotide added | % activity | Nucleotide added | % activity |
|---|---|---|---|
| None | 72 | UTP | 79 |
| ATP | 54 | UDP | 78 |
| ADP | 51 | CTP | 71 |
| AMP | 71 | CDP | 66 |
| GTP | 34 | IDP | 76 |
| GDP | 35 | $\epsilon$-ATP | 1 |

The incubations were carried out under the standard conditions (see section 2) using lysosomal supernatant equivalent to 0.08 units of $\beta$-NAGASE. The activity is that remaining after 1 h of incubation

These include GTP, GDP, ATP, ADP and particularly $\epsilon$-ATP, but not AMP or IDP. cGMP and cAMP were also found to be inactive.

That the effect of the nucleotides is concentration-dependent is exemplified in fig.3 with $\epsilon$-ATP. As already indicated, $\epsilon$-ATP seems to be most effective.

In table 3, it can be seen that both trypsin and elastase are able to inactivate OTC, and in both cases the effect is enhanced by $\epsilon$-ATP. It appears, therefore, that the stimulator effect of nucleotides

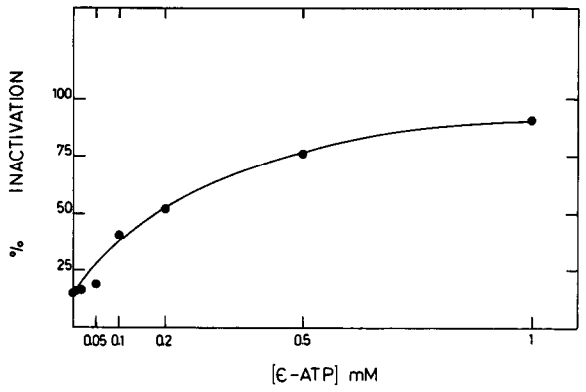


Fig.3. The effect of $\epsilon$-ATP on the inactivation of OTC by broken lysosomes. Standard conditions, except that 1.2 $\mu$g of beef liver OTC, broken lysosomes equal to 0.02 units of $\beta$-NAGASE and the indicated concentrations of $\epsilon$-ATP were used. Incubation time was 3 h.

Table 3

The effect of $\epsilon$-ATP on the inactivation of beef liver ornithine transcarbamylase by trypsin or elastase

| Nucleotide added | Protease added (% of activity left) | |
|---|---|---|
| | Trypsin | Elastase |
| None | 61 | 37 |
| $\epsilon$-ATP | 21 | 10 |

The incubations were for 1 h under the standard conditions using 80 ng of trypsin or 10 $\mu$g of elastase

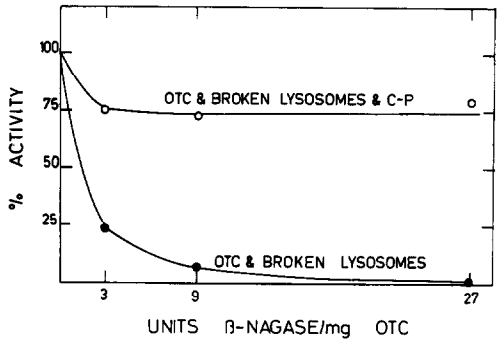


Fig.4. Inactivation of ornithine transcarbamylase by broken lysosomes and protection by carbamoyl phosphate. Each tube contained 4 $\mu$g of rat liver OTC, the indicated amounts of broken lysosomes (shown as $\beta$-NAGASE), 200 $\mu$g BSA, 50 mM potassium phosphate buffer (pH 6.0) and, when indicated, 2.8 mM carbamoyl phosphate. Other conditions were standard. Incubation time was 1 h.

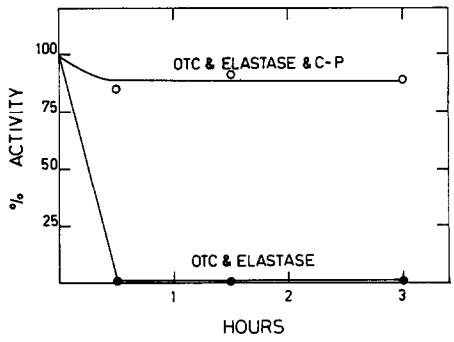


Fig.5. Effect of carbamoyl phosphate on the inactivation of rat liver OTC by elastase. The components in the incubations were as follows: rat liver OTC, 25 $\mu$g; elastase, 0.25 $\mu$g; buffer acetate–diethanolamine, 50 mM (pH 8.5) and carbamoyl phosphate, when added, 14 mM. Other conditions were standard.

is on the OTC rather than on the lysosomal enzymes.

The substrates of OTC could also induce conformational states which might alter the proteolytic response. As shown in fig.4,5, carbamoyl phosphate protects OTC very efficiently against inactivation by lysosomal enzymes or by elastase. Thus, interaction of OTC with environmental factors (substrates, cofactors, and other effectors), in addition to the proteolytic systems which are present in excess in the cell at all times [10], appears to modulate its stability and thereby affect enzyme levels by altering rates of degradation [11,15,16].

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