

Chicken Ornithine Transcarbamylase: Its Unexpected Expression

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Ornithine transcarbamylase (OTC), one of the enzymes of the urea cycle, is detectable in some strains of chickens, although they have no functional urea cycle. The enzyme consists of three identical subunits of 36 kd and is present in mitochondria of the kidney. Using immunoabsorbent column chromatography, we found further evidence that the enzyme is detectable as a precursor form (40 kd) in chicken brain, heart, liver, pancreas, gizzard, small intestine, and breast muscle. When an extract of small intestine containing only precursor OTC was treated with a kidney extract, the precursor was converted into OTC. This suggests that there is a tissue-specific processing protease in the kidney which splits a peptide off the precursor, causing the expression of OTC activity in this organ. However, the reason why the enzyme or its precursor is expressed in these organs is not known. The results of this study suggest that, unlike mammals, chickens are more organ specific with regard to the ability to incorporate precursor OTC into mitochondria.

KEY WORDS: chicken ornithine transcarbamylase; precursor ornithine transcarbamylase (OTC); organ-specific expression; organ-specific processing.

INTRODUCTION

Ornithine transcarbamylase (OTC) (EC 2.1.3.3), one of the urea-cycle enzymes, is a mitochondrial matrix enzyme of ureotelic animals. Recently, many attempts have been made to elucidate the mechanisms by which cellular organelles are formed, including the transportation systems and processing mechanisms of mitochondrial enzymes, such as F1-ATPase (Schatz, 1979;

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McAda and Douglas, 1982; Maccicchini *et al.*, (1979), OTC (Morita *et al.*, 1982; Argan *et al.*, 1983), and carbamyl-phosphate synthetase (Shore *et al.*, 1979; Mori *et al.*, 1979).

The chicken is an uricotelic animal having no functional urea cycle. Ornithine transcarbamylase activity varies among chicken breeds or strains. A strain established in 1972 by one of the authors shows high OTC activity in the kidney (Tsuji and Fukushima, 1983). The enzyme of this strain has nearly the same characteristics in some kinetic properties as the mammalian enzyme, and it also is located in mitochondria (Tsuji, 1983). It consists of three identical subunits of 36 kd and conserves the quarternary structure like mammalian OTC (Tsuji, 1983). Dietary induction of OTC has also been observed. However, the cause is distinct from that for mammalian OTC; in chickens a diet containing a high concentration of fat increases the amount of OTC in the kidney (Tsuji *et al.*, 1983a), whereas in mammals a diet containing a high concentration of protein increases the amount of OTC in the liver. The structural gene for OTC is encoded on the sex chromosome in mammals (Lindgren *et al.*, 1984; DeMars *et al.*, 1976). A sex difference in OTC activity has been observed in chickens, but the activity of the enzyme for the hemizygous, ZW, female is consistently higher than that for the homozygous, ZZ, male (Tsuji and Fukushima, 1983; Tsuji *et al.*, 1983b). There is no basis, therefore, for postulating that the OTC-coding gene resides on the sex chromosome, Z, in chickens.

Why is the OTC-coding gene expressed consistently in the chicken kidney despite the apparent absence of a physiological function for OTC? What is the regulatory mechanism by which OTC activity is induced? Why was OTC conserved in the chicken kidney during evolution and why does a sex difference occur? These are interesting questions because we speculate that the gene could have been lost 300 million years ago when an ancestor of the chicken lost its functional urea cycle.

This report provides evidence that the chicken OTC-coding gene is expressed in several organs and tissues and that OTC exists in a precursor form in all organs but the kidney.

MATERIALS AND METHODS

Chicks. Ten-day-old White Leghorn B-line chicks showing high OTC activity in the kidney were used in this experiment (Tsuji and Fukushima, 1983). Freshly excised brain, heart, liver, pancreas, gizzard, small intestine, breast muscle, and kidney were stored at -20°C until use.

Extraction of OTC. Each organ was homogenized in a blender with 9 vol of 0.05 M potassium phosphate buffer, pH 7.5, containing 0.1% Triton X-100. Each homogenate was centrifuged at 10,000 rpm for 10 min. Cetyltrimeth-

ylammonium bromide was added to the supernatant to a final concentration of 0.1% and stirred for 15 min. After centrifugation at 10,000 rpm for 10 min, the supernatant layer was heated at 60°C for 1 min and then cooled immediately in an ice bath. After the removal of heat-denatured proteins by centrifugation at 10,000 rpm for 10 min, the supernatant was applied to an immunoabsorbent column.

Immunoabsorbent Column Chromatography. As the antiserum against bovine liver OTC reacted strongly against chick kidney enzyme, the anti-bovine liver OTC serum prepared previously (Tsuji, 1983) was used in this experiment. The method for preparing an immunoabsorbent column has been outlined elsewhere (Tsuji *et al.*, 1985). Purified IgG from the antiserum using DE32 (Whatman Co. Ltd., Clifton, N.J.) column chromatography was coupled with BrCN-activated Sepharose 4B (Pharmacia Fine Chemicals, Uppsala). One gram of the gel, coupled with about 20 mg of IgG, could bind about 1 mg of bovine liver OTC. After the sample was applied to the column unadsorbed protein was washed out using 0.05 M sodium bicarbonate, pH 8.0, until the absorbance of the effluent at 280 nm reached 0.005. Finally, the column was washed with about 10 ml of distilled water and then bound protein was eluted with 0.5 M acetate. The effluent was lyophilized and subjected to electrophoresis on a 10% sodium lauryl sulfate-polyacrylamide gel according to the method of Laemmli (1970).

Conversion of Precursor OTC to OTC in Vitro. Ornithine transcarbamylase was completely eliminated from the kidney extract by two passages of the extract through the immunoabsorbent column. Then the OTC-free kidney extract was added to a small intestine extract which contained only precursor OTC and the mixture was incubated at 37°C for 1 hr. The mixture was applied to an immunoabsorbent column. The protein retained on the column was eluted and subjected to electrophoresis.

RESULTS AND DISCUSSION

The OTC-coding gene is expressed in all chick organs, while OTC activity is detectable only in the kidney.

As shown in Fig. 1A, five chick organs, liver, small intestine, heart, brain, and pancreas, have a prominent polypeptide that was selectively retained on an immunoabsorbent column. Each polypeptide (shown by an arrow) is larger than the 36,000-dalton bovine OTC (shown as lane CL in each run). We presume that they are a precursor form of OTC because they are retained on the immunoabsorbent column, they are similar to some mammalian precursor OTC in size (Conboy and Rosenberg, 1981; Mori *et al.*, 1981), and the peptide in a small intestine extract can be changed into a small polypeptide

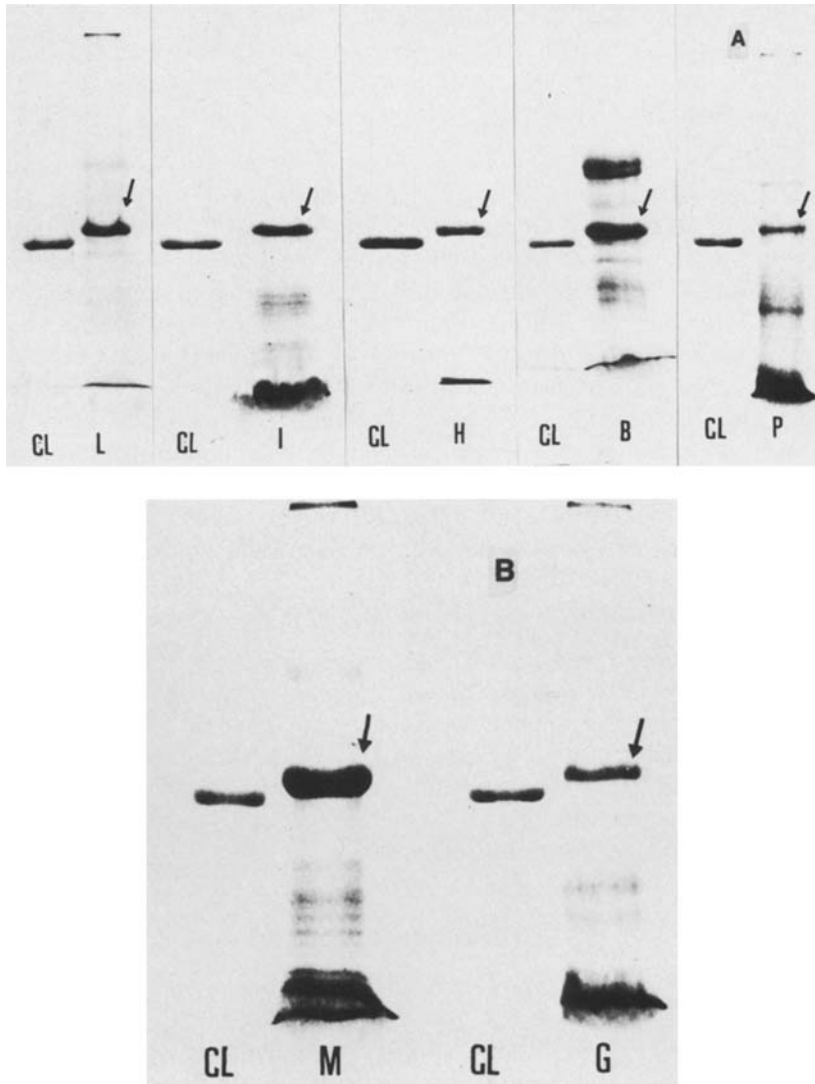


Fig. 1. Partially purified precursor OTC from different chicken organs using immunoabsorbent column chromatography. (A) L, liver; I, small intestine; H, heart; B, brain; P, pancreas. About 10 g of each tissue was used for sample preparation. (B) G, gizzard; M, breast muscle; About 40 g of each organ was used for sample preparation. CL, the bovine liver preparation, using 50 mg of tissue by the same procedure.

corresponding to bovine OTC (shown in Fig. 3). Chicken kidney OTC consists of three identical subunits of 36 kd and its size is the same as that of bovine liver OTC (Tsuji, 1983). The difference in size between the polypeptide and bovine liver OTC is about 4 kd, in accordance with the size of the leader peptide of mammalian precursor OTC (Conboy and Rosenberg 1981; Mori *et al.*, 1982; Rosenberg *et al.*, 1983). The chromatogram of each organ shows several bands besides the main bands of the precursor peptide and OTC. These bands could not be seen consistently in every trial, so that they seem to be nonspecific polypeptides retained on the column. Figure 1B shows chromatograms of breast muscle (lane M) and gizzard (lane G) extracts. Both extracts clearly show a polypeptide corresponding to the precursor OTC of 40 kd, but the amount of this protein was about one-fourth of that in other organs. Approximately 40 g of breast muscle and gizzard, in contrast to about 10 g of other tissues, was used for each preparation. It seems likely, therefore, that the precursor OTC is much more abundant in mitochondrion-rich organs, such as the heart, the liver, and the brain, than in other tissues.

Figure 2 illustrates the results of an experiment using a kidney extract in which a polypeptide of 36 kd, the same size as bovine OTC (Marshall and Cohen, 1972), was detected instead of the apparent precursor 40-kd polypeptide. The result suggests that the chicken kidney contains a specific protease that splits off the leader peptide of precursor OTC. It is possible that the precursor OTC is immediately transported after translation and incorporated



Fig. 2. Partially purified OTC from the kidney using immunoabsorbent column chromatography. CL, bovine liver OTC; K, chicken kidney OTC. About 1 g of kidney tissue was used for the preparation.

into mitochondria and processed to OTC as observed in the liver of mammals (Mori *et al.*, 1982; Rosenberg *et al.*, 1983). As OTC activity is detected only in the kidney in chickens, the expression of enzyme activity may relate to the ability of each organ to process precursor OTC.

When a small intestine extract was incubated with a kidney extract (Fig. 3), two protein bands were clearly observed: one corresponding to precursor OTC from the small intestine extract and the other corresponding to OTC. This result further suggests that the kidney extract contains a specific processing protease which converts a precursor enzyme to OTC.

From these results it is concluded that the OTC-coding gene is expressed in almost all organs of the chick and is translated to a polypeptide. However, only in the kidney is the polypeptide processed to OTC. When the relative amount of bovine liver OTC is expressed as 800, that of chick kidney is 40, that of precursor OTC in other chicken organs, the liver, the heart, the brain, and the pancreas, is 4, and that of the muscle is only 1. The amount of enzyme



Fig. 3. Conversion of precursor OTC in a small intestine extract to OTC by an extract of the kidney. Lane 1, kidney OTC; Lane 2, small intestine precursor OTC; Lane 3, precursor and OTC from a small intestine extract incubated with a kidney extract. A small intestine extract was incubated with a kidney extract to determine whether the latter could convert precursor OTC to OTC. Kidney extract OTC was completely eliminated prior to incubation by two passages through an immunoabsorbent column. About 10 g of small intestine and kidney was used in this experiment.

or precursor therefore is variable among organs. This suggests that the OTC-coding gene does not have an "all-or-none" expression but is expressed with quantitative differences in different organs. This suggests that the suppression of gene expression is incomplete as has been observed in the case of the δ -crystalline gene (Thomson *et al.*, 1981; Agata *et al.*, 1983). If this is true for other genes as well as the OTC-coding gene, we should consider changes of gene regulation during evolution and differentiation not only from a qualitative viewpoint, but also from a quantitative one.

It has been shown that precursor OTC prepared from rat liver OTC mRNA *in vitro* is integrated into mitochondria in the presence of the reticulocyte lysate system whether the mitochondria are prepared from different organs of rat or from pigeon liver (Mori *et al.*, 1981). Neither inter- nor intraspecies specificity is, therefore, postulated, and each organ would seem to have a similar processing protease(s) to incorporate the precursor into mitochondria (Mori *et al.*, 1981; Takiguchi *et al.*, 1983; Morita *et al.*, 1982). In chickens, however, the precursor OTC seems to be processed in the kidney and not in other organs. This clearly shows that chickens, as opposed to mammals, have an organ-specific processing system for the precursor OTC. It is difficult to explain why the chick liver precursor OTC is not processed in the liver, while a rat precursor OTC is processed and incorporated into mitochondria of the pigeon liver (Takiguchi *et al.*, 1983).

The chicken kidney appears to have a specific protease which splits off the leader peptide of the precursor OTC. This leads us to speculate that during evolution an ancestor of the chicken lost a functional urea cycle in the liver due to a disturbance in the incorporation of precursor enzymes into mitochondria and that the transcriptional efficiency subsequently was reduced by mutations of each structural gene or regulatory regions of each gene. Mutation of the regulatory gene is one possibility in view of the fact that the OTC-coding gene has been conserved in chickens over the past 300 million years. Alternatively, each organ may express different OTC genes consisting of a multigene family, and each precursor OTC may possess different leader sequences which cannot be processed, except in the kidney. However, since the kidney extract processed the small intestine precursor to OTC, the latter case seems unlikely.

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