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Molecular cloning of chicken hepatic histidase and the regulation of histidase mRNA expression by dietary protein☆

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Abstract

Chicken hepatic histidase activity varies with dietary protein consumption, but the mechanisms responsible for this alteration in activity are unclear. In the present research, the complete coding sequence and deduced amino acid sequence for chicken histidase was determined from clones isolated from a chicken liver cDNA library. The deduced amino acid sequence of chicken histidase has greater than 85% identity with the amino acid sequences of rat, mouse, and human histidase. In a series of four experiments, broiler chicks were allowed free access for 1.5, 3, 6, or 24 h to a low (13 g/100 g diet), basal (22 g/100 g diet) and high (40 g/100 g diet) protein diet. In the final experiment 5, chicks were allowed free access for 24 h to the basal, high protein diet or the basal diet supplemented with three different levels of L-histidine (0.22 g/100 g diet, 0.43 g/100 g diet or 0.86 g/100 g diet). There were no differences in the expression of the mRNA for histidase at 1.5 h, but at 3 h, histidase mRNA expression was significantly ($P<.05$) greater in chicks fed the high protein diet compared to chicks fed the low protein diet. At 6 and 24 h, histidase mRNA expression was significantly enhanced in chicks fed the high protein diet, and significantly reduced in chicks fed the low protein diet, compared with chicks fed the basal diet. Histidase mRNA expression was not altered by supplementing the basal diet with histidine. The results suggest that previously observed alterations in the activity of histidase, which were correlated to dietary protein intake, are mediated by rapid changes in the mRNA expression of this enzyme, and are not necessarily related to dietary histidine intake.

Keywords: Chicken; Histidase; Temporal change; Dietary protein

1. Introduction

Histidase (histidine ammonia-lyase, EC 4.3.1.3) deaminates L-histidine to *trans*-urocanic acid. Histidase activity has been detected in the liver and skin of a variety of vertebrate animals [1]. In the liver, urocanase converts *trans*-urocanic acid to imidazolonepropionic acid, which is subsequently converted to glutamic acid. In the skin, *trans*-urocanic acid continues to accumulate because of the absence of urocanase. *trans*-Urocanic acid does isomerize to *cis*-urocanic acid upon absorption of ultraviolet radiation. Both forms of urocanic acid have been reported to protect skin from sunburn [2–5] and to protect DNA from photomutagenesis [6]. Trans and *cis*-urocanic acid have also been reported to have important immunosuppressive activities [7–11].

In rats, hepatic histidase activity is increased as dietary protein consumption increases [12–16], but when rats are fed diets supplemented with L-histidine, hepatic histidase activity is not altered [12–14,17,18]. The increased activity of hepatic histidase in rats fed a high protein diet, or an imbalancing mixture of amino acids is associated with an increase in the mRNA concentration of hepatic histidase [14,15,19]. Interestingly, dietary protein intake does not increase the activity of skin histidase [12,13,17].

In contrast to rats, addition of 5.5% histidine to a histidine adequate control diet doubled the activity of chick hepatic histidase [20]. Subsequently, Keene and Austic [21] also reported that increasing dietary histidine levels increased chick hepatic histidase activity, but that the addition of a mixture of indispensable amino acids lacking histidine

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to a chick diet had a more pronounced effect on histidase activity than the addition of dietary histidine. The mechanisms by which dietary histidine and protein regulate the activity of chick hepatic histidase are not understood. Therefore, the goal of the present research was to obtain a cDNA clone for chicken hepatic histidase and to then determine if changes in chicken hepatic histidase activity due to dietary protein and histidine intake are preceded by changes in the amount of histidase mRNA.

2. Materials and methods

2.1. Reverse transcriptase polymerase chain reaction

Hepatic histidase cDNA sequences of rat [22], mouse [23] and human [24] were aligned, and primers for reverse transcriptase polymerase chain reaction (RT-PCR) were chosen from areas with a high degree of homology among the three species. The forward primer sequence, 5′ AAGAGGGCCTGGCACTCATC 3′, and the reverse primer sequence, 5′ CCTCCTTTGAAGGTACCACTT 3′, predicted a 442-bp product that corresponded to bases 899–1341 of the cDNA coding sequences of rat, mouse and human histidase. The primers were made by the molecular and genetics instrumentation facilities (MGIF) at the University of Georgia. Reverse transcription was performed with total RNA obtained from chick liver using the method previously described [25]. Polymerase chain reactions (PCRs) were also conducted as previously described [25], except that the annealing temperature was lowered to 45°C. The predicted sequence and orientation of the PCR product was confirmed by automated sequence analysis completed at the MGIF at the University of Georgia. To produce a sufficient quantity of the cDNA for labeling for Northern analysis, the PCR product was cloned into the pCRII vector using the TA cloning kit (Invitrogen, San Diego, CA).

2.2. Cloning and sequencing

The cDNA library used was a lambda ZAP premade library (Strategene, La Jolla, CA) of approximately 2.0×10^6 primary clones made from hepatic tissue of a 7-week-old male broiler breeder. The library was screened by colony hybridization using a ^{32}P-labeled preparation of the 442-bp RT-PCR-generated clone. Positive clones containing the putative histidase cDNA were isolated and excised following the manufacturer's protocol. The largest of the positive clones were then sequenced by the dideoxy chain termination method using T_3, T_7, M_{13} and one internal oligonucleotide primers (MGIF, University of Georgia).

2.3. Experiments 1–4

Total RNA isolated from four previous experiments [26] was used to determine the effect of dietary protein on chicken hepatic histidase mRNA expression. In these previous experiments, chicks were fed either a low (13 g/100 g diet), basal (22 g/100 g diet) or a high (40 g/100 g diet) protein semipurified diet. In experiment 1, these diets were fed for 6 or 24 h, while in experiment 2, the diets were fed for 1.5 and 3 h. These two initial experiments were then duplicated in experiments 3 and 4. For each experimental time period, feed intake and body weight were determined, and liver samples were taken for RNA isolation and Northern analysis.

2.4. Experiment 5

This experiment was done to determine if the observed effects of the high protein diet on the expression of histidase mRNA were due to the increased histidine content of this diet, to the increased protein level of the diet or to a combination of both. Day old broiler chicks (Ross X Ross) were obtained from ConAgra (Athens, GA). They were housed in thermostatically controlled, electrically heated battery brooders cages with wire floors. The cages were lighted for 24 h/day. For the first week, the birds were fed a practical corn-soy starter diet. The chicks were then sorted and birds with extreme weights were discarded. The remaining birds were then distributed to 30 pens of 2 birds each, and were fed the adjusted isolated soybean protein basal diet used by Adams and Davis [26] for 5 days to allow them to acclimate to a semipurified diet.

After this adjustment period, the 30 pens were split into 5 groups, and the chicks were then fed either the adjusted basal diet, a high protein diet (40 g/100 g diet) or the adjusted basal diet supplemented with either 0.22 g/100 g diet (H1), 0.43 g/100 g diet (H2) or 0.86 g/100 g diet (H3) of L-histidine (Dyets, Bethlehem, PA). The final calculated total histidine content of each diet was 0.53 g/100 g diet, 0.75 g/100 g diet, 0.96 g/100 g diet, 1.39 g/100 g diet and 0.96 g/100 g diet for the adjusted basal, H1, H2, H3 and high protein diets, respectively. The adjusted basal and high protein diets were the same as those reported by Adams and Davis [26]. Addition of histidine to the adjusted basal diet was at the expense of cellulose. The chicks had access to these diets for 24 h, after which feed consumption and body weight were determined for each pen. The chicks were killed by cervical dislocation at the end of the experiments

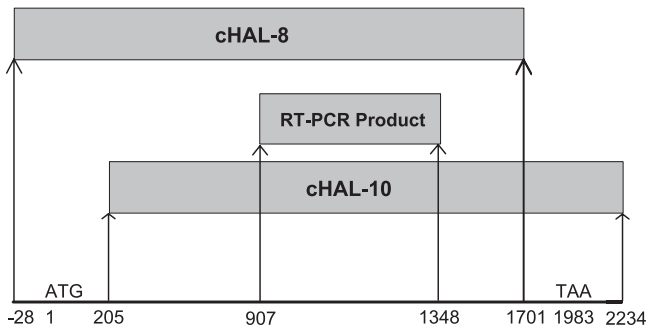

Fig. 1. Schematic relationship between the chicken hepatic histidase cDNA clones (cHAL 8 and cHAL-10) and the RT-PCR product used to screen the chicken hepatic cDNA library. Numbers correspond to nucleotide bases. The start of the protein coding sequence is indicated by nucleotide 1 (ATG, start codon), and the end of the protein coding sequence is indicated by nucleotide 1983 (TAA, stop codon).