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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

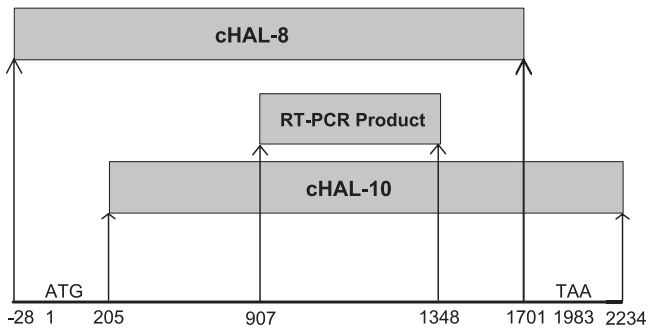

Fig. 1. Schematic relationship between the chicken hepatic histidase cDNA clones (cHAL 8 and cHAL-10) and the RT-PCR product used to screen the chicken hepatic cDNA library. Numbers correspond to nucleotide bases. The start of the protein coding sequence is indicated by nucleotide 1 (ATG, start codon),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

to obtain liver samples for RNA extraction. The Institutional Animal Care and Use Committee of the University of Georgia approved all animal procedures.

2.5. RNA extraction and Northern blot analysis

Total RNA was extracted from liver samples pooled by pen using a guanidinium isothiocyanate method [27]. Forty micrograms of total RNA was then run on an agarose/formaldehyde gel and transferred to a nylon membrane as previously described [25]. The 442-bp chicken histidase RT-PCR clone used to screen the cDNA library and a chicken glyceraldehyde-3-phosphate dehydrogenase (GAPDH) clone [25] were prepared and labeled with ^{32}P for Northern analysis as previously described [25]. The hybridization and densitometry procedures followed those described previously [25]. There were two blots for each experimental period, and the replicate samples for each dietary treatment were divided equally between the two blots. The two blots were hybridized at the same time, and exposed together on the same film. Relative mRNA expression of histidase was

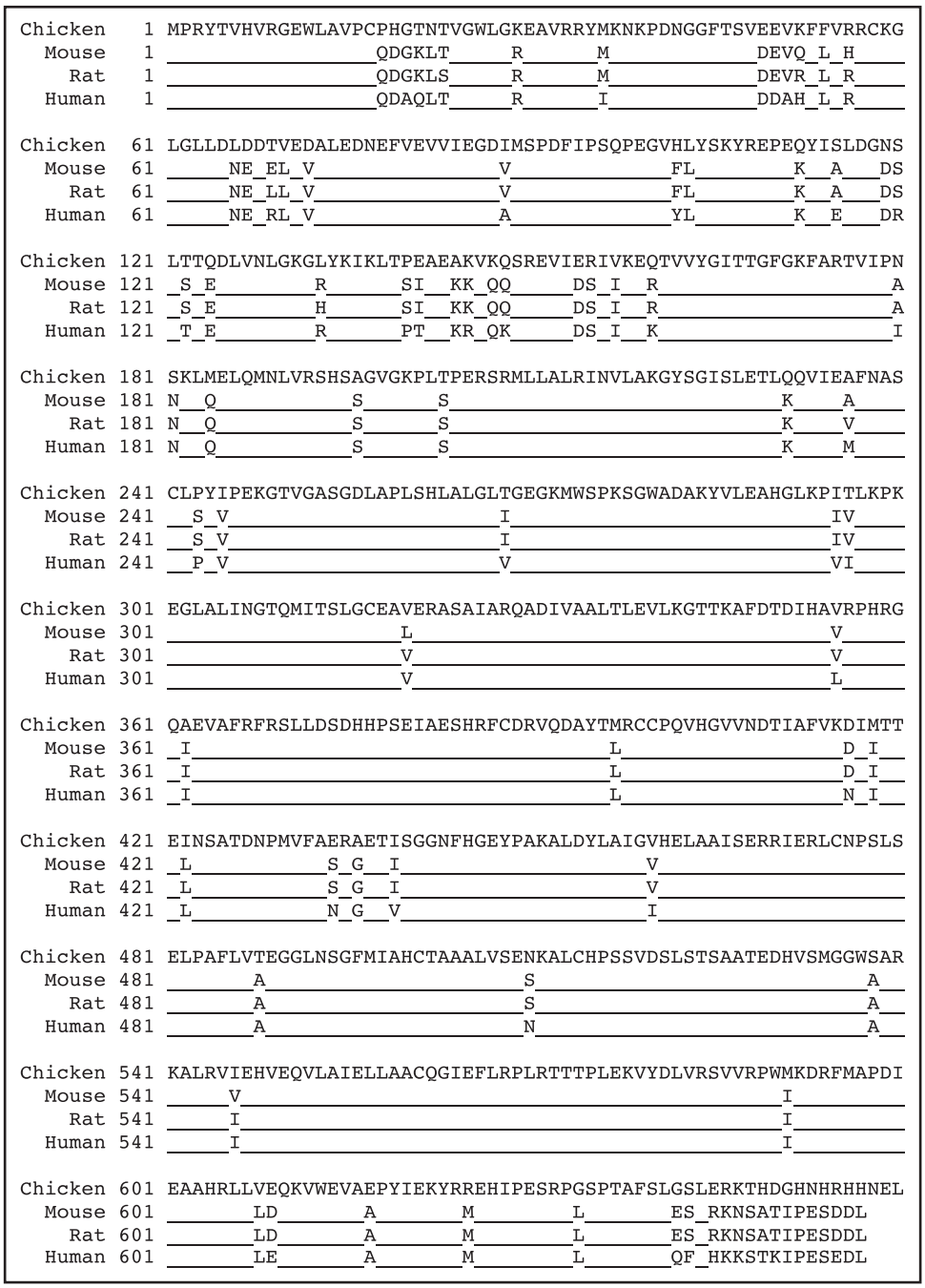

Fig. 2. Homology of the deduced amino acid sequence of chicken hepatic histidase with the histidase sequences of mouse, rat and human. For the mouse, rat and human sequences, only those amino acids that are different from the chicken sequence are shown.

determined for the samples of each blot by calculating the signal intensity of each sample relative to the strongest histidase signal, which was assigned a value of 1. Before calculation of relative histidase mRNA levels, GAPDH mRNA expression was used to correct histidase values for equality of RNA loading and transfer for each blot.

2.6. Statistical analysis

Data obtained from each experiment were subjected to ANOVA according to the General Linear Model procedure of SAS [28]. Duncan's multiple range test [29] was used to determine significant differences ($P<.05$) among the diets.

3. Results

3.1. Cloning of the chicken hepatic histidase gene

After confirming that the obtained RT-PCR product had greater than 80% nucleotide sequence homology with the corresponding nucleotide sequences for rat, mouse and human histidase, it was used to screen the chicken hepatic cDNA library. Twenty positive clones were identified after screening 3×10^5 plaque forming units. Two clones with the largest cDNA inserts, cHAL-8 and cHAL-10, were selected for sequence analysis. The reading frame for the cHAL-8 and cHAL-10 sequences was established by comparison to the published nucleotide sequences of rat [22], mouse [23] and human [24] histidase. The schematic relationship among our sequenced histidase cDNA clones and the RT-PCR product is depicted in Fig. 1. Sequence analysis revealed that the cHAL-8 clone while containing the start codon lacked 541 bp of the terminal 3′ coding region, while cHAL-10 contained the entire 3′ terminal end including the poly A tail, but lacked 196 bp from the 5′ end of the coding sequence. The two clones had an overlapping region of 1497 bp, and together contain the complete coding sequence for chicken hepatic histidase.

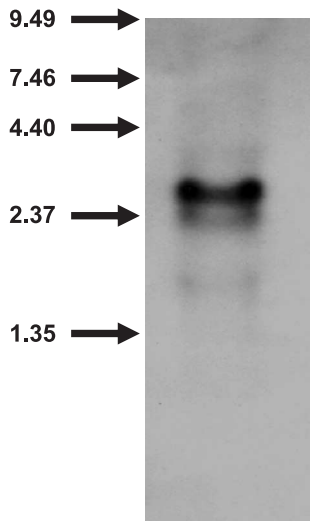

Fig. 3. Autoradiogram from a Northern analysis of histidase with total RNA from chicken liver. A 0.24- to 9.5-kb RNA ladder was run in the lane adjacent to the liver sample, and the kilobase sizes of the RNA bands are shown with arrows.

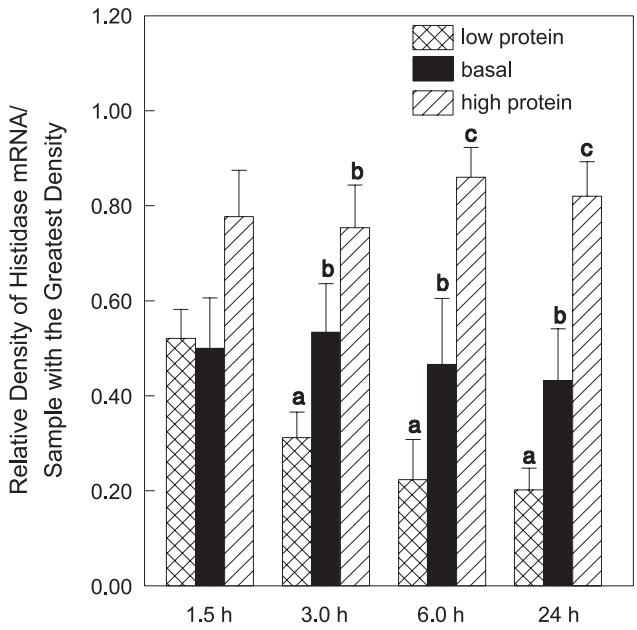

Fig. 4. The relative density of hepatic histidase mRNA of chicks fed different dietary protein concentrations [experiments 1 (6 and 24 h) and 2 (1.5 and 3 h)]. Values are means±S.E.M., $n=6$ replicate pens. Means at a time with different letters differ, $P<.05$. Note that the relative densities of histidase mRNA to one another are specific for each point and that all statistical comparisons are within a given period.

The 1983-bp coding sequence of chicken histidase predicts a protein of 660 amino acids. This predicted protein sequence has three additional amino acids at the C-terminus end, compared to sequences of rat, mouse and human (Fig. 2). The full-length coding region of chicken hepatic histidase has 83% nucleotide identity with the full-length coding regions of rat, mouse and human histidase. The deduced amino acid sequence of chicken histidase has 88%, 88% and 87% identity with the amino acid sequences of rat, mouse and human histidase, respectively. In addition, chicken histidase has the four N-glycosylation consensus sequences (Asn-X-Ser/Thr) beginning at amino acids 238, 307, 408 and 476, which are conserved across all the species. The 3′ -untranslated region of the chicken histidase cDNA sequence contains a variant of the polyadenylation hexamer (AATAAA), which starts 18 bp before the beginning of the poly A tail. The complete cDNA and the derived amino acid sequence of chicken hepatic histidase are part of the GenBank database (Accession number: AY227348).

A major histidase mRNA transcript at approximately 2.9 kb and a minor transcript slightly below it were detected by Northern analysis of total RNA derived from a liver sample of a 2-week broiler chick (Fig. 3).

3.2. Experiments 1–4

Food consumption data for these four experiments has been published previously [26]. In brief, intake in chicks fed

either the low, basal or the high protein diet did not differ from each other at 1.5, 3 and 24 h. The only significant difference in food consumption was between chicks fed the high and the low protein diet at the 6-h experimental period in experiment 1 [26].

In experiment 1, hepatic histidase mRNA expression was significantly higher in chicks fed the high protein diet, when compared to chicks fed the basal or low protein diet at both the 24- and 6-h experimental periods (Fig. 4). Chicks fed the low protein diet had significantly lower histidase mRNA expression when compared to those fed the basal protein diet.

In experiment 2, mRNA expression of histidase was significantly higher in chicks fed the basal and the high protein diet when compared to chicks fed the low protein diet at 3 h (Fig. 4). At 1.5 h, there were no significant differences in histidase mRNA expression between the three dietary treatments (Fig. 4).

The Northern analysis results for experiments 3 and 4 were the same as in experiments 1 and 2, respectively, with the expression of histidase mRNA increasing as dietary protein concentrations increased. After quantification by densitometry, correction of RNA loading and transfer with GAPDH, the mean±S.E.M. relative histidase mRNA values in experiment 3 at 6 h were 0.47±0.10, 0.71±0.06 and 0.93±0.03, and at 24 h were 0.24±0.07, 0.47±0.04 and 0.76±0.12 for the low, basal and high protein diet, respectively. In experiment 4, the mean relative histidase values after 1.5 h of feeding were 0.51±0.11, 0.58±0.11 and 0.70±0.06, and at 3 h of feeding were 0.44±0.06, 0.63±0.08 and 0.75±0.09 for the low, basal and high protein diets, respectively.

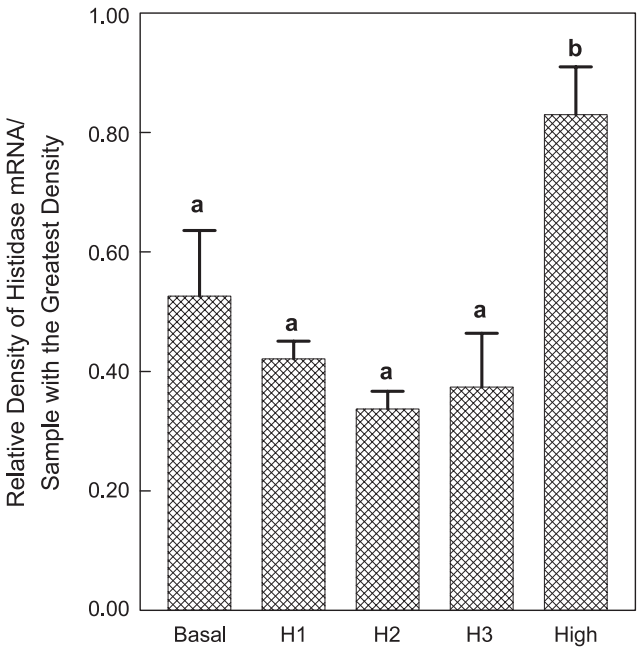

Fig. 5. The relative density of hepatic histidase mRNA of chicks fed the basal diet, high protein diet or the basal diet supplemented with either 0.22 g/100 g diet (H1), 0.43 g/100 g diet (H2) or 0.86 g/100 g diet (H3), L-histidine. Values are means±S.E.M., $n=6$ replicate pens. Means with different letters differ, $P<.05$.

3.3. Experiment 5

Food consumption for the basal, high and the histidine supplemented diets was 38±2, 35±1, 37±2 (H1), 37±4 (H2) and 39±2 (H3) g/chick, respectively. There were no significant differences in food intake among the five dietary treatments.

Histidase mRNA expression was greater in chicks fed the high protein diet than in chicks fed the basal diet or the basal diet supplemented with histidine (Fig. 5). Addition of L-histidine to the basal diet did not increase hepatic histidase mRNA expression (Fig. 5).

4. Discussion

The nucleotide and predicted amino acid sequences of our cDNA clones were 80% and 89% identical with the respective nucleotide and amino acid sequences of rat, mouse and human histidase. Thus, we conclude that the cDNA clones we isolated truly represent chicken hepatic histidase. Interestingly, the nucleotide sequence of chicken histidase has nine additional bases at the 3′ end compared to the nucleotide sequences of rat, mouse and human histidase. The reason for the additional nucleotide bases that would code for three additional amino acids is unclear. It is worth noting that although the predicted amino acid sequence of human histidase has over 93% homology with the amino acid sequences of rat and mouse histidase, the last 16 amino acids of the C-terminal end of human histidase, however, have only 55% identity with the corresponding rat and mouse amino acid sequences. Therefore, it seems that 3′ end of the coding sequence for histidase will not be well conserved across species.

By Northern analysis of total RNA extracted from chick liver, a major histidase mRNA transcript of approximately 2.9 kb was detected. A minor transcript was detected just below the major histidase transcript. A major transcript of 2.5 kb and minor transcripts of 4.4 and 7.3 kb have been reported from Northern analysis of rat liver [22].

Previously, Keene and Austic [21] reported that when chicks were fed a protein adequate diet supplemented with a mixture of indispensable amino acids, hepatic histidase activity increased. Our results suggest that the increased histidase activity observed by Keene and Austic [21] may have been due to increased synthesis of this enzyme. Feeding a high protein diet to rats increases hepatic histidase activity [12–15], and this increase in activity has also been associated with an increase in hepatic histidase mRNA concentration [14–16].

In contrast to rats, increasing dietary histidine levels in a histidine adequate chick diet has been reported to increase hepatic histidase activity [20,21]. The present research indicates that supplementing a histidine adequate diet with

more histidine does not increase histidase mRNA expression. Even when the basal diet was supplemented with 1.5 times more histidine than was found in the high protein diet, histidase mRNA expression was not altered. Since dietary levels of histidine above the requirement do not regulate histidase activity at the pretranslational level, the previous reports of increased hepatic histidase activity may have been the result of allosteric regulation or a decrease in the rate of histidase protein degradation.

Previous studies have reported that chick [21] and rat [14] hepatic histidase activity and rat hepatic histidase mRNA levels [14–16] are increased 1–15 days after increasing dietary protein concentrations. The current research indicates that changes in dietary protein intake can alter the levels on histidase mRNA within a few hours. Such a rapid change in the mRNA levels of a metabolic enzyme due to changes in dietary protein intake have been observed in our laboratory previously. The mRNA expression of malic enzyme decreases within 3 h of feeding chicks a high protein diet [26]. Although an increase in dietary protein intake has opposite effects on the concentration of hepatic mRNA for histidase and malic enzyme, the responses may be elicited by the same mediator. The 5′ region of the chicken malic enzyme gene contains negative-acting response elements for cAMP that are responsible for the inhibition of malic enzyme transcription by glucagon [30], and the promoter region of the human hepatic histidase gene contains positive-acting response elements for cAMP [31]. In addition, Alemán et al. [32] reported that glucagon administration to rats increased hepatic histidase activity and mRNA concentrations. Thus, if the promoter region of the chicken histidase gene also contains positive-acting response elements for cAMP, then glucagon may be the mediator responsible for the effect of dietary protein on the transcription of these enzymes.

In summary, switching chicks from a basal diet to a low protein diet rapidly (3 h) decreased the level of hepatic histidase mRNA, whereas feeding a high protein diet increased its level. The mechanism by which dietary protein intake rapidly modifies the mRNA levels of histidase is not understood, but clearly, the mechanism seems to be independent of dietary histidine concentrations above its requirement.

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