SPECIES VARIABILITY OF N-TERMINAL SEQUENCE OF AVIAN ERYTHROCYTE-SPECIFIC HISTONE H5

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SUMMARY: A comparison of the N-terminal amino acid sequence of H5 isolated from chicken, quail, duck, goose and pigeon shows considerable sequence variation. With the possible exception of the very lysine-rich histone H1, H5 variation is much more extensive than that reported previously for other histones. The observed sequence diversity is in agreement with the known taxonomic grouping of these birds.

Comparative studies on the amino acid sequence of histone H2A, H2B, H3 and H4 have revealed that these proteins in evolution are extremely stable and exhibit an inordinate amount of sequence conservation (review articles 1, 2 and ref. 3, 4). On the other hand, histone H1 shows considerable sequence heterogeneity and variability (1, 2, 5).

In some specialized cells an additional basic protein has been found which is similar to histone H1 in terms of certain physico-chemical properties. Two well documented examples are: the HT histone of trout testis and the erythrocyte-specific histone (H5) of birds. The partial sequence of both histones is known and show some complementarity with histone H1 of rabbit and trout (1, 2, 6-8), particularly with respect to the distribution of basic and hydrophobic residues (2, 7). The H5 is of considerable interest from both an evolutionary and a developmental point of view. H5-like proteins have been found in fish, amphibians, reptiles and birds (1, 10-12). The H5 may be involved in the genetic restriction and chromatin condensation process of the erythrocyte during maturation. So far the partial amino acid sequence of H5 reported by Greenaway and Murray (6, 13) and Sautiere and co-workers (7, 8) is limited to the chicken histone only. In this study, we have compared the N-terminal amino acid sequence of H5 isolated from five avian species which are commonly used for biochemical studies.

MATERIALS AND METHODS

The following species were used: chicken (Gallus gallus domisticus, 3 used), duck (Cairnia moscheta, 2 used), goose (Anser anser, 2 used), pigeon (Columbia livia, 5 used) and quail (Coturinx japonica, 30 used). Chicken, duck and quail were obtained from commercial sources. Geese were obtained from Canada Agriculture and pigeons were reared at the National Research Council of Canada and a gift of Dr. A. deFreitas. Histone H5 was obtained from nuclei isolated from erythrocytes of heparinized blood. The methods for isolation of nuclei, selective extraction of H5, fractionation and purification of H5 have been described (14). For this study the CG-50 Amberlite elution profile (Fig. 1) was divided into 5 fractions prior to desaltation and lyophilization. Only the central, main fractions were used for further experimentation. The purity of each protein was assessed by electrophoresis at pH 2.7 (15) and in sodium dodecylsulphate (16).

The histone samples were hydrolyzed for 20 hr at 110 C in 6N HCl and the hydrolysates analyzed in a Beckman model 121C amino acid analyzer. The amino-terminal sequence of the five H5 fractions was determined by automatic Edman degradation (17) using a Beckman model 890C sequenator with a quadrol program No. 122974. Protein samples (1.5-6.0 mg) were dissolved in 0.5 ml of 50% formic acid containing 1 mg dithioerythritol and added to the cup and dried under vacuum. The thiazolinone derivatives or PTH derivatives were hydrolyzed separately with 6N HCl and HI (18) at 130 C for 20 hours, and the amino acid formed was analyzed with a Durrum D-500 amino acid analyzer. Identification of some PTH derivatives was also made by thin layer chromatography on silica gel plates (19).

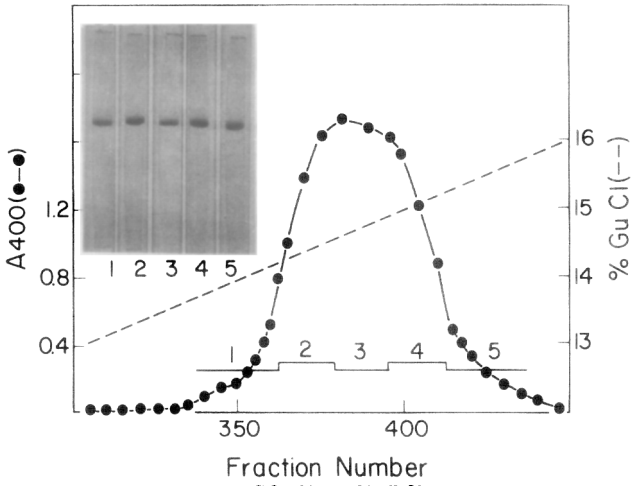

Fig. 1. Purification of erythrocyte-specific histone H5 of duck by cation-exchange chromatography. Approximately 60 mg of lyophilized basic protein extracted from isolated nuclei of mature duck erythrocytes at pH 1.8 (11) was loaded onto a CG-50 amberlite column (2.5 x 95 cm) and eluted with a guanidinium chloride (GuCl) gradient of 7 to 18% at pH 6.8. (●-●) protein concentration, turbidity at 400 nm; (---) GuCl concentration. Elution of H5 was monitored by polyacrylamide gel electrophoresis. Approximately 5-10 μg of protein from pooled fractions 1, 2, 3 etc., were subjected to electrophoresis at pH 2.7 (15).

RESULTS AND DISCUSSION

Shallow, linear-gradient chromatography has been employed in the separation of H5 from other histones, genetic variants of H5 and enzymatically modified H5 (6, 14, 20). In the present study the guanidium chloride concentration range necessary for the full elution of H5 from each of the avian types was found to be approximately the same, 12.8 to 16%. As indicated in Figure 1 for duck H5, each elution profile was routinely divided into at least 5 fractions and the homogeneity of each fraction was assessed by polyacrylamide gel electrophoresis. A comparison of the H5 proteins with chicken H5 in split gels indicated that the electrophoretic mobilities of chicken and quail were identical but not those of goose, duck, or pigeon. The H5 of pigeon showed the greatest differences; at pH 2.7 (Fig. 2) it ran a full band width slower than chicken. This difference was about the same when the proteins were electrophoresed in sodium dodecylsulphate at pH 7.0. These results suggest the

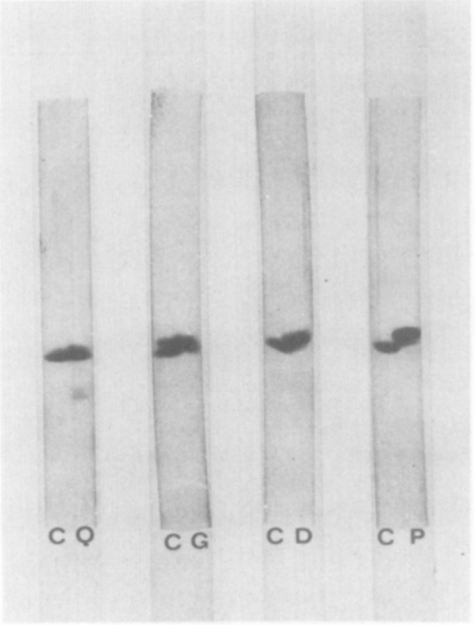

Fig. 2. Comparison of the electrophoretic mobilities of purified H5 proteins from quail (Q), goose (G), duck (D) and pigeon (P) with chicken (C) H5 by using split gels. Approximately 5-10 µg of each protein were co-electrophoresed with chicken H5 at pH 2.7 (15). A plastic divider was used to separate each of the two samples per gel.

Table 1. Amino acid composition of erythrocyte histone H5

	Moles per 100 mol of recovered amino acids				
	Chicken	Quail	Duck	Goose	Pigeon
Asx	1.8	2.4	1.7	2.1	1.6
Thr	3.1	3.6	2.0	4.0	3.5
Ser	12.4	12.2	9.8	10.1	10.8
Glx	4.0	3.4	3.6	3.3	3.2
Pro	7.8	6.5	9.3	9.1	10.9
Gly	5.0	4.5	5.8	5.4	3.7
Ala	15.9	16.0	19.0	17.3	18.2
Val	4.3	4.5	3.0	2.8	3.6
Met	0.2	0.2	0.2	0.3	0.4
Iso	3.1	2.8	3.0	3.0	4.0
Leu	4.3	4.5	3.5	3.6	3.1
Tyr	1.2	1.5	1.8	1.4	1.3
Phe	0.5	1.1	0.0	0.4	0.5
His	1.5	1.7	1.5	1.5	1.5
Lys	23.2	22.6	24.8	24.2	22.5
Arg	11.6	12.7	10.9	11.5	11.3
Total	99.9	100.2	99.9	100.0	100.1

possibility of H5 primary sequence variation, perhaps differences in net charge or molecular weight. Table 1 shows the amino acid composition of H5. The data for chicken, duck and goose are in good agreement with those already reported (7, 12, 21, 22). The amount of proline varies considerably and proline of pigeon is significantly higher than that from chicken and quail.

Definite evidence for H5 variability is seen from the N-terminal amino acid sequences and the proposed structural equivalents based on the homologies shown in Fig. 3. Our sequence data for chicken H5 are in complete agreement with previously reported results (6-9). However, the heterogeneity (Gln/Arg) at residue 15, first found by Greenaway and Murray (6), was not observed in our preparations. Previous reports indicated some discrepancy in whether the N-terminal residue of H5 is blocked or not (6, 7, 23). The reason for this difference and the nature of the possible blocking group is not known. In our study the recovery of the N-threonyl residue from all five birds, as judged from the recovered α-aminobutyric acid after hydrolyzing the first thiazolinone

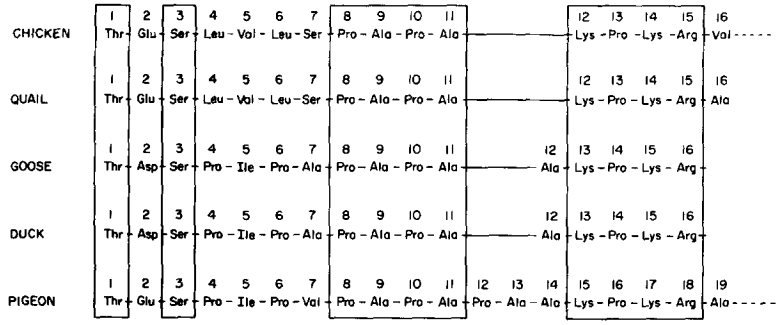

Fig. 3. The N-terminal amino acid sequence of erythrocyte-specific histone H5 from chicken, quail, goose, duck and pigeon. Sequence confined within blocks indicate homology in all H5 proteins examined.

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residue with HI (18), was lower than normally expected for an unblocked residue, and 30 to 50% of the molecules.

The N-terminal sequence of quail H5 is identical with that of chicken H5, except at residue 16 where Val is replaced by Ala in quail. This sequence similarity is rather expected since both species belong to Galliformes. The first 16 residues of H5 from goose and duck, both Anseriforms, are found to be identical. When the sequence of H5 of goose and duck are compared with those of chicken and quail, 10 residues were identical and 6 residues were different. However, in order to align the sequence Lys-Pro-Lys-Arg of positions 12-15 of chicken and quail to positions 13-16 of goose and duck one space between 11 and 12 of chicken and quail had to be made. This type of variability is further elaborated in pigeon H5; basically, its overall sequence is closer to goose and duck than to chicken and quail. However, the space between residues 11 and 12 of chicken must further be expanded to accommodate the Pro-Ala repeat observed at positions 8-11 in all of the H5 proteins. The Pro-Ala repeated sequence has also been observed in lysine-rich histones (1, 2, 5). These differences may account in part for the differences observed in the electrophoretic mobilities of these histones (Fig. 2).

The comparison of the H5 sequences made here indicates a high degree of homology (regions within blocks in Fig. 3). The invariability of these

sequences may be essential for the H5 proteins to perform their functions in chromatin. Where the sequences are not conserved the amino acid substitution is minor; the patterns of the amino acid substitutions suggests an evolutionary trend similar to that already established by taxonomic studies (24). Nevertheless, the sequence variability among the five avian species is still quite significant, particularly when chicken and pigeon H5 are compared. The species differences in the sequences of H5 may further increase or decrease when the complete sequence of these proteins is finally determined. However, it is likely that species sequence variability will decrease if the highly basic C-terminal half of this protein (1, 2, 7) is the major site for DNA interaction. At present it is apparent that H5, like H1, has also changed considerably during evolution. Whether this points to a less vital role for these histones in chromatin is presently unknown.

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