Acetylated Hemoglobins in Feline Blood*

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SUMMARY

The β chain amino terminus of one or more hemoglobins in the blood of members of the Felidae family is substituted with an acetyl group. Such acetylated hemoglobins occur together with unsubstituted components in variable proportions in blood of different members of the family. In the lion, tiger, and snow leopard, the acetylated component comprises about 90% of the total hemoglobin; in the serval and caracal it comprises about 70%; in the cheetah, puma, fishing cat, and jungle cat it comprises about 50%; and, among domestic cats it comprises 10 to 50%.

The occurrence of such β chain-substituted hemoglobins as major components in animal blood is unusual. Except for the minor adult human component hemoglobin Aβ, and the minor adult human component hemoglobin Aβ, no animal hemoglobin thus far examined has been found to contain amino-blocked β (non-α) chains.

Domestic cat hemoglobins with N-acetylated β chain amino termini are insensitive to the modifying influence of organic phosphates on oxygen affinity, whereas cat hemoglobins with unsubstituted β chain amino termini are sensitive (Taketa, F., Mauk, A. G., and Lessard, J. L., J. Biol. Chem., 246, 4471 (1971)).

A number of proteins with substitutions at the amino terminus of the peptide chain are now known. An increasing number of proteins in which the substituting species is an acetyl group has been reported in recent years (1). Acetyl substitutions have been found at the α chain amino terminus of the hemoglobins of carp (2), of two frog species (3, 4), and of toad (5). The amino terminus of the γ chain of the minor human fetal hemoglobin, hemoglobin Fα, has also been shown to possess an acetyl blocking group. In addition, we have recently described the occurrence of substituted β chain amino termini in a major hemoglobin component present in cat blood (6).

Previous work in this laboratory has shown that the blood of domestic cats contains two major hemoglobins (7); one contains a substitution at the amino terminus of the β chain, and the other possesses unsubstituted β chain amino termini. The component

with blocked amino termini (HbB) comprises 10 to 50% of the total hemoglobin present in different cats (7) and differs from the other hemoglobin (HbA) in its relative insensitivity to the modulating effects of glycerate-2,3-Pd (2,3-PGA) on oxygen binding (6). The response to 2,3-PGA appears to be related at least in part to the presence or absence of unsubstituted β chain amino termini in the intact hemoglobin tetramer (6, 8–11).

The nature of the substituent at the β chain amino terminus of cat HbB has not been identified previously, but preliminary data suggested the presence of an acetyl group (12). Data are presented here that show that the substituent is indeed an acetyl group and that hemoglobins having β chains with acetylated amino termini are characteristically present as major components in the blood of all members of the Felidae which have been examined.

EXPERIMENTAL PROCEDURE

Globin was prepared from isolated cat hemoglobins A and B and from other animal hemolysates by the method of Rossi-Fanelli, Antonini, and Caputo (13).

Tryptic hydrolysis of globins was conducted for 90 min at 37°C and pH 8.0. Amino-terminal β chain tryptic peptides (βT-1) from HbA and HbB were obtained by preparative paper electrophoresis. Tryptic hydrolysis products of cat globins were applied to sheets of Whatman No. 3MM paper and electrophoretically separated in pyridine-acetic acid buffer, pH 6.4. Guide strips were cut and stained with ninhydrin to locate the separated peptide bands; the desired acidic peptides were eluted with water and then lyophilized.

Pronase hydrolysis of cat globins was conducted at pH 8.0 and 37°C for 24 hours at a globin to enzyme ratio of 100:1; negatively charged peptides produced from this digestion were obtained by passing the hydrolysis products through an AG 50W-X2 (H⁺) column as described by Marchis-Mouren and Lipmann (14).

Analyses for acetyl groups in globins and in globin peptides were performed by colorimetry as described by Ludowieg and Dorfman (15) and by gas chromatography as described by De Witt and Ingram (16). Samples containing 1 to 2 μmoles (33 to 66 mg) of globin were dissolved in 0.5-ml aliquots of an acetylated 2 N solution of HCl in methanol and heated for 4 hours at 100°C in sealed glass tubes to convert any acetyl groups present to methyl acetate. The tubes were cooled prior to opening and the contents distilled according to the method of Ludowieg and Dorfman (15). Samples of the distillate (1 μl) were injected onto a 6-foot column with 20% diglycerol adsorbed on Chromosorb W (Supelco) for anal-
The position for methyl acetate is indicated by an arrow. Preparation of samples and conditions for gas chromatographic analysis are described in the text.

Results

Fig. 1 shows the elution profile obtained from gas chromatographic analysis for methyl acetate in distillates from peptides obtained from HbA and HbB. The chromatograms are similar to those described by De Witt and Ingram (16) although occasional difficulty was encountered from an unaccountably broad elution peak for the most volatile component; in some chromatograms, the breadth of this peak tended to obscure the peak for methyl acetate.

As Fig. 1 shows, a peak appears in chromatograms of distillates prepared from βT-1 peptides from HbB which does not occur in chromatograms of βT-1 peptide distillates from HbA; this peak occurs at the same point as that of control samples of methyl acetate and distillates from methanolysis of acetylated amino acids. Similar analysis of negatively charged peptides from a Pronase digest shows the presence of acetyl groups in samples prepared from HbB but not in those prepared from HbA. Both the tryptic and Pronase peptide analyses gave values of 0.7 to 1.10 pmoles of methyl acetate per pmole of peptide. Distillates prepared by methanolysis of undigested globin samples showed the presence of methyl acetate in HbB but not in HbA. The yield of this component from HbB amounts to approximately 0.9 to 1.0 pmole per pmole of globin (i.e., two acetyl groups per heme globin tetramer) and was in agreement with separate analysis with the hydroxamic acid assay (Table I). These results taken together with earlier data on the lack of free amino groups in HbB β chains (12) indicate that the amino-terminal β chain residues of HbB are acetylated.

Table I

<table>
<thead>
<tr>
<th>Sample</th>
<th>Amount of sample analyzed</th>
<th>Acetate</th>
<th>Methyl acetate found</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methyl acetate</td>
<td>1</td>
<td>0.099</td>
<td>0.099</td>
</tr>
<tr>
<td>Methyl acetate</td>
<td>2</td>
<td>0.186</td>
<td>0.186</td>
</tr>
<tr>
<td>Methyl acetate</td>
<td>3</td>
<td>0.288</td>
<td>0.288</td>
</tr>
<tr>
<td>N-Acetyl tyrosine ethyl ester</td>
<td>1</td>
<td>0.095</td>
<td>1.0</td>
</tr>
<tr>
<td>N-Acetyl tryptophan methyl ester</td>
<td>2</td>
<td>0.178</td>
<td>1.9</td>
</tr>
<tr>
<td>Domestic cat HbA-globin</td>
<td>2</td>
<td>0.030</td>
<td>0.3</td>
</tr>
<tr>
<td>Domestic cat HbB-globin</td>
<td>2</td>
<td>0.192</td>
<td>2.0</td>
</tr>
<tr>
<td>Lion globins</td>
<td>2</td>
<td>0.186</td>
<td>1.9</td>
</tr>
<tr>
<td>Tiger globins</td>
<td>2</td>
<td>0.178</td>
<td>1.9</td>
</tr>
<tr>
<td>Serval globins</td>
<td>2</td>
<td>0.163</td>
<td>1.7</td>
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<tr>
<td>Caracal globins</td>
<td>2</td>
<td>0.141</td>
<td>1.5</td>
</tr>
<tr>
<td>Jungle cat globins</td>
<td>2</td>
<td>0.123</td>
<td>1.3</td>
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<tr>
<td>Fishing cat globins</td>
<td>2</td>
<td>0.117</td>
<td>1.2</td>
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<tr>
<td>Puma globins</td>
<td>2</td>
<td>0.108</td>
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<tr>
<td>Cheetah globins</td>
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<td>0.103</td>
<td>1.1</td>
</tr>
<tr>
<td>Chicken globins</td>
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<td>0.030</td>
<td>0.3</td>
</tr>
<tr>
<td>Human globins</td>
<td>2</td>
<td>0.018</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Data obtained for acetyl groups in various hemoglobins with the colorimetric hydroxamic acid assay are shown in Table I. Contrary to the results of Satake et al. (18) and in agreement with those of Moss and Thompson (19), our data suggest that chicken hemoglobin is acetylated only to a minor extent, if at all. In contrast to globin from the chicken and a human adult, distillates from methanolysis of unfractionated globin samples prepared from hemolysates of all members of the Felidae gave strongly positive hydroxamic acid reactions. The relative yields of methylacetate from samples obtained from various members of the Felidae are different and are in agreement with electrophoretic data obtained from dissociation-recombination experiments that indicate the occurrence of variable proportions of hemoglobins with the HbB-type of amino-blocked β chains among multiple components that are found in various feline bloods. For example, lion and tiger blood contain two hemoglobins with the HbB type of β chains that comprise 90% of the total and a third component with the HbA type of β chain that comprises about 10%. In the caracal and serval two or more hemoglobins occur with ratios of HbB: HbA types of about 2:1. In the puma, cheetah, jungle cat, and fishing cat, the two types of components occur in about a 1:1 ratio; and among domestic cats the components are found in variable HbB: HbA ratios that range from 1:1 to 1:9. The

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results for the domestic cat HbB showed the presence of about 2.0 μmoles of acetyl per μmole of tetramer and practically none in HbA. They thus support earlier results (6) that indicated that the amino termini of the two β chains in HbB are blocked by substitution but are free in HbA.

DISCUSSION

The physiological and evolutionary significance of the occurrence of variable mixtures of acetylated and nonacetylated hemoglobins in feline blood is not known. However, feline red cells provide a system to study the mechanism of acetylation and its relationships, if any, to the process of globin chain synthesis or assembly. In addition, the physiological properties of blood containing the acetylated hemoglobin as a major component can be examined. These problems are currently being studied.

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