# ACETYLATED PEPTIDE CHAINS IN BULLFROG HEMOGLOBINS

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Globin from larval and adult bullfrogs (Rana catesbeiana) has been examined for N-terminal amino acids (Moss and Ingram, unpublished) by a modification of the phenylthiohydantoin method of Edman (Fraenkel-Conrat, et al., 1954). They recovered approximately 2 moles of PTH-valine per 65,000 mol. wt. and 2 moles of PTH-glycine per 65,000 mol. wt. for tadpole and frog globin, respectively, suggesting that the other peptide chains of these hemoglobins might be blocked. In view of reports of N-terminal acetyl groups found in other hemoglobins, initially by Schroeder, et al. (1962) in human fetal hemoglobin, tadpole and adult bullfrog hemoglobins were examined for similar groups. It is now concluded that both of these hemoglobins contain 2 moles of N-terminal acetyl per 65,000 mol. wt.

Larval and adult stages of Rana catesbeiana were purchased from the Connecticut Valley Biological Supply Co., Southampton, Mass. Blood cells were collected in cold heparinized amphibian Ringer's solution, washed five times with large volumes of

cold Ringer's solution, and lysed by freezing and thawing in four volumes of half-strength Ringer's. The lysate was shaken with 0.2 volumes of carbon tetrachloride and centrifuged in the cold for 10 minutes at 2000 g. The supernatant was then centrifuged in the cold for 30 minutes at 12,000 g, and the crude hemoglobin preparation was purified by gel filtration on Sephadex G-100 (Moss and Ingram, unpublished). Fractions which contained predominantly the major frog or tadpole bands were pooled and analyzed by analytical polyacrylamide gel electrophoresis. Hemoglobin samples used for N-terminal group analysis contained 80 to 90 per cent of the major hemoglobin component.

Acetyl groups were detected by gas chromatography. Twenty to 100 milligrams of hemoglobin were hydrolyzed with 0.25 ml of 2N HCl-methanol and distilled as described by Ludowieg and Dorfman (1959). One or two microliters of distillate were analyzed using an Aerograph Model 204-1B Gas Chromatograph, supplied with a flame ionization detector. Two different columns were used for separate experiments. Initially, samples were chromatographed on a seven-foot Poropak Q column. The column temperature was 140°C, the flow rate of nitrogen was 25 ml/min. and the hydrogen flow rate was 20 ml/min. More definitive results were later obtained using a six-foot column of 20% diglycerol absorbed on acid-washed Chromosorb W, with a column temperature of 30°C and a nitrogen and hydrogen flow rate of 20 ml/min.

Typical chromatograms for standards and hydrolyzed

hemoglobin samples analyzed on either the diglycerol column or the Poropak Q column are shown in Figure 1. The diglycerol column completely separates all components of the reaction mixture. For the operating conditions specified, methyl acetate is retained for 2.4 minutes and methanol for approxi-

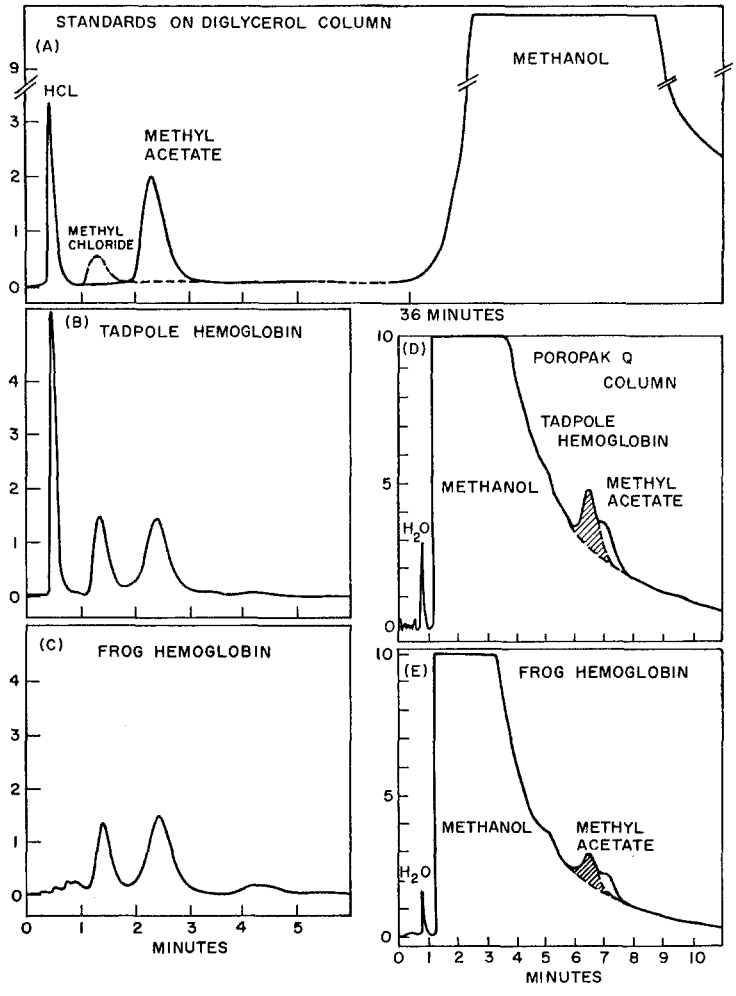


Figure 1. Typical chromatograms obtained on diglycerol and Poropak Q columns by gas chromatography. (a) Standards on diglycerol column; (b) and (c), hydrolyzed tadpole and frog hemoglobin on diglycerol column; (d) and (e), hydrolyzed tadpole and frog hemoglobin on Poropak Q column.

mately 36 minutes. Methyl chloride, a side product of the hydrolysis, is retained for 1.4 minutes. The Poropak Q column, however did not separate methyl acetate and methyl chloride completely. On this column, methanol displays a retention time of 1.2 minutes, and methyl acetate and methyl chloride have retention times of 6.5 and 7.1 minutes, respectively.

Standard amounts of methyl acetate (in methanol) were chromatographed in order to provide a calibration curve for quantitation of methyl acetate. In chromatograms obtained with the Poropak Q column, it was necessary to extrapolate the boundary of the methyl acetate peak in order to correct for

Table 1

| Column | Moles of methyl acetate recovered/65,000 mol. wt. Hb | | | |
|---|---|---|---|---|
| | Tadpole | Frog | Human (adult) | Acetylglycine |
| Poropak Q | 2.16 (2) | 1.80 (3) | ----- | 1.27 (3) |
| Poropak Q | 1.74 (2) | 1.59 (2) | <0.10 | 0.97 (3) |
| Diglycerol | 2.00 (3) | 1.70 (3) | <0.06 | 1.11 (4) |

Moles of methyl acetate recovered per 65,000 mol. wt. of hemoglobin. Each experiment represents different hemoglobin preparations hydrolyzed as described in the text. Acetylglycine is used as a control. Numbers in parentheses refer to the number of separate injections used to determine each value.

contamination by methyl chloride. As shown in Table 1, acetylglycine, used as a control, yielded an average of 1.12

moles of acetyl per mole of acetylglycine. Tadpole hemoglobin gave an average of 1.96 moles of acetyl per 65,000 mol. wt., while the yield for frog hemoglobin was an average of 1.70 moles of acetyl per 65,000 mol. wt.

Hamada, et al. (1964) have reported 2 moles of DNP-valine and 2 moles of DNP-glycine per 68,000 mol. wt. for Rana catesbeiana tadpole hemoglobin and 4 moles of DNP-glycine per 68,000 mol. wt. for the frog hemoglobin. Perhaps the differences in their results from those reported here are due to strain variations of R. catesbeiana, since it has been observed in this laboratory that R. catesbeiana tadpoles obtained from different regions of the United States display variations in their hemoglobin patterns following polyacrylamide gel electrophoresis. Elzinga (1964) found 0.93 moles of DNP-valine (corrected) per 68,000 molecular weight of unpurified tadpole hemoglobin, but no glycine end groups, and 2.1 moles of DNP-glycine (corrected) in unpurified frog hemoglobin.

Our present results, in conjunction with those of Moss and Ingram on N-terminal amino acids, strongly suggest that the major hemoglobin component of both larval and adult bullfrogs contains two N-terminal acetylated chains.

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