# STUDIES ON THE HYDROXYLATION OF LYSINE IN VIVO

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Hydroxylysine is similar to hydroxyproline with respects to its occurrence and biosynthesis; it is found only in collagen and the hydroxylysine in collagen arises from lysine rather than from free hydroxylysine (Piez and Linkins, 1957; Sinex et al., 1959). The mechanism of the hydroxylation is not yet known.

Recently it was found by the authors (Fujimoto and Tamiya, 1962) and Prockop et al.(1962) that the atmospheric oxygen was incorporated into hydroxyproline when proline was hydroxylated in vivo. This paper describes the results of experiments with chick embryos on the source of oxygen atom of hydroxyl group of hydroxylysine. It was found that the oxygen atom was derived from water rather than from molecular oxygen in vivo.

Hen's eggs were incubated in three different ways in a vessel (9.3 l) equipped with a device to supply oxygen at a constant pressure of 1 atm. as described in the previous paper (Fujimoto and Tamiya, 1963). In experiment 1 in Table I, 25 eggs of 13-day-old were incubated with $O^{18}$-enriched air (3.8 atom % excess) for 17 hours. Fifty embryos from two incubations were combined. In experiment 2, eight eggs of 14-day-old were incubated for a week over $O^{18}$-enriched water (1.5 atom % excess, 300 ml). The $O^{18}$-content of the body fluid after the incubation was found to be 0.17 atom % excess. In experiment 3 in Table II, $T_2O$ (10 mc, 20 $\mu$l) was injected to an egg of 10-day-old through a small hole in the shell into the yolk sac and the egg incubated for four days.

After the incubation, the embryos were collected and homogenized with approximately equal amount of water. The centrifugal residue (3000 × g, 30 min.) was hydrolyzed with 6 N HCl at 110° for 24 hours. It was found that the oxygen atom in the hydroxyl group of hydroxylysine was unexchangeable with water during the hydrolysis procedure (Fujimoto and Tamiya, unpublished data). The hydrolyzate was evaporated to dryness and the residue dissolved in water (200 ml) and passed through an Amberlite

IR-120 (pyridine-form) column (Buchanan, 1957). Basic and aromatic amino acids adsorbed on the column were eluted with 2 N $NH_4OH$. The evaporation residue of the eluate was dissolved in water (50 ml) and chromatographed on an Amberlite IR-120 ($H^+$ form) column (7 $cm^2 \times 30$ cm) with 1.2 N HCl. Hydroxylysine was eluted together with phenylalanine. The aqueous solution (10 ml) of the evaporation residue was treated with an Amberlite IRC-50 ($H^+$-$Na^+$ form) column (Kunin, 1951) and the hydroxylysine on the column was eluted with 2 N $NH_4OH$. In experiment 1, hydroxyproline in the neutral amino acid fraction was separated and analyzed as described in the previous paper (Fujimoto and Tamiya, 1962).

Table I

$O^{18}$-Abundance in PTH-hydroxylysine

| Experiment | Source of $O^{18}$ | (Atom % excess) | Atom % excess in the product Found | Expected |
|---|---|---|---|---|
| 1 | $O^{18}_2$ | ( 3.8 ) | 0.030 | 0.501 † |
| 2 | $H_2O^{18}$ | (final 0.17) | 0.050 | 0.072 ‡ |

* PTH-Hydroxylysine = 5[3-Hydroxy-4-(β-phenylthioureido)butyl]-3-phenyl-2-thiohydantoin

† Calculated from the $O^{18}$-abundance in hydroxyproline isolated from the same embryos (0.334 atom % excess) assuming the $O^{18}$-content in the hydroxyl groups to be the same for both amino acids.

‡ Calculated from the $O^{18}$-abundance in water in the embryos after the incubation and assuming that the fraction of hydroxylysine synthesized during the incubation period to be 85 %, which is the observed value for hydroxyproline. It is also assumed that hydroxyl-oxygen of this fraction comes from water.

In experiments 1 and 2, hydroxylysine was converted to its phenylthiohydantoin derivative (yield 26 mg and 60 mg, respectively) according to Fraenkel-Conrat et al. (1955); infrared absorption spectra of the preparations agreed with that reported by Ramachandran et al. (1955). $O^{18}$-Abundance in the preparations was determined by a mass spectrometer, Model M-60, Process and Instruments (Brooklyn, New York) according to Rittenberg and Ponticorvo (1956).

In experiment 3 in Table II, hydroxylysine content in the hydrolyzate was estimated from its hydroxyproline content (assuming the former to be 1/12 of the latter) and the carrier (DL+allo hydroxylysine-HCl, 3 mg) was added before the isolation procedure. Hydroxylysine and lysine, which was eluted by 1.2 N HCl a little later than hydroxylysine, were analyzed for the radioactivity by a Packard Tri-Carb liquid scintillation counter.

Table II

Tritium in hydroxylysine and lysine (d.p.m./μmole)

| Experiment | Source of tritium | Hydroxylysine | Lysine |
|---|---|---|---|
| 3 | $T_2O$ | 5790* | 348 |

* Corrected for dilution by the carrier.

The results of analysis of $O^{18}$ are shown in Table I. Experiment 1 showed that $O^{18}$ of the air was not incorporated into hydroxylysine while the hydroxyproline isolated from the same embryos incorporated it. In experiment 2, an incorporation of $O^{18}$ from water into hydroxylysine was observed.

The results of tritium-analysis are shown in Table II. Experiment 3 showed that 17 times as much radioactivity of tritium was found in hydroxylysine as compared to lysine. The results with the high tritium content are in contrast to those reported for hydroxyproline (Ebert and Prockop, 1962) and hydroxylated aromatic compounds (Posner et al., 1961), which are known to be hydroxylated by the oxygenation mechanism. The result shows an incorporation of tritium into hydroxylysine from water.

Two possibilities may be considered to account for the results: (1) Hydroxylation of lysine involves a dehydrogenation-hydration mechanism rather than an oxygenation mechanism. (2) Although the lysine is hydroxylated by oxygenation, the hydroxyl group is equilibrated with water by a dehydratase (EC 4.2.1 group) reaction.

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