The Absolute Configuration of Sepiapterin

Sadao MATSUURA, Takashi SUGIMOTO, and Motoo TSUSUE*

Department of Chemistry, College of General Education, Nagoya University, Chikusa-ku, Nagoya 464
**Biological Laboratory, Kitasato University, Sagamihara City 228*

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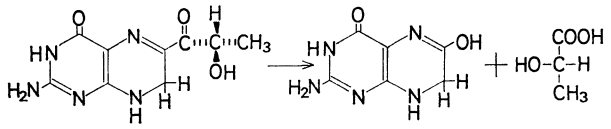
The absolute configuration of sepiapterin was determined to be 2-amino-7,8-dihydro-6-(*S*)-lactoyl-4(3*H*)-pteridinone from an analysis of the L-lactic acid produced from sepiapterin by oxidation cleavage in a sodium borate solution.

Sepiapterin, the yellow pigment contained in the eye of *Drosophila melanogaster*, was first isolated in 1954 by Forrest and Mitchel[1] and later by Viscontini and Möhlman[2] from a *sepia* mutant of the insect. Structural elucidation by Nawa[3] revealed the pigment to be 2-amino-7,8-dihydro-6-lactoyl-4(3*H*)-pteridinone (I). Sepiapterin possesses a chiral carbon at the 2-position of the side chain and has been found to be optically active,[2,4] as expected. Although no optically-active sepiapterin has been synthesized (with the exception of the recent work of Pfleiderer, see below), its racemate was produced by an unusual method starting from 2-amino-7,8-dihydro-4(3*H*)-pteridinone and 3-hydroxy-2-oxobutanoic acid.[5] It has been found that, of the



I

racemic sepiapterin isomers, only the enantiomer with the same configuration as naturally occurring sepiapterin is reduced by sepiapterin reductase.[5] However, from these results, it was not possible to reach a final determination of the configuration. Recently, Pfleiderer reported that the oxidation of 5,6,7,8-tetrahydrobiopterin yielded sepiapterin, which was found to be identical with natural sepiapterin in every respect, and thus, the configuration of the chiral carbon of sepiapterin was determined to be the (*S*)-configuration, the same as for biopterin.[7]

In this paper, an alternative approach for determining the absolute structure of sepiaterin is reported, which involves cleavage of the lactoyl group to lactic acid. Sepiapterin is known to undergo oxidative degradation to give lactic acid and 7,8-dihydroxanthopterin in a 4% sodium borate solution under aerobic conditions.[3] Since the 2-position of the side chain is not involved in the degradation reaction, its configuration is maintained throughout the reaction. Thus, it is possible to determine the absolute configuration of sepiapterin by examining the configuration of the lactic acid produced.

First, the oxidative degradation reaction was reinvestigated under a variety of conditions. It was found that sepiapterin remained practically unchanged in a phosphate buffer at pH 10.0. However, it was converted to 7,8-dihydroxanthopterin fairly slowly in a 4% sodium borate solution, as has been reported.[3] This reaction was found to be markedly enhanced by the addition

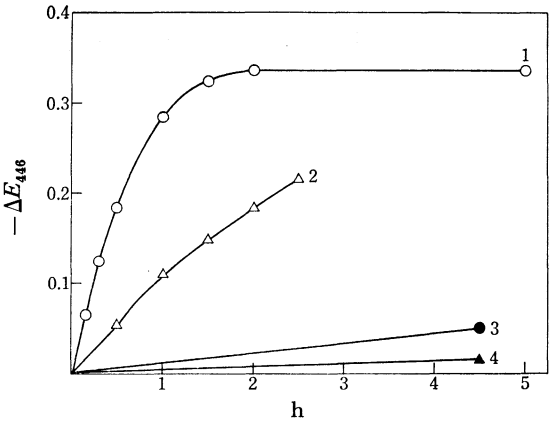


Fig. 1. Oxidative degradation of sepiapterin at 25 °C under aerobic conditions in the dark place. The initial concentration of sepiapterin was 50 μM and the reaction was monitored by reading the absorbance at 446 nm.
1: In 4% sodium borate containing cupric sulfate (25 μM).
2: In 4% sodium borate containing sodium hydroxide (0.1 M).
3: In 4% sodium borate.
4: In a 0.05M phosphate buffer at pH 10.0.

of sodium hydroxide or more effectively by a trace amount of copper(II) sulfate. This reaction was conveniently monitored spectroscopically (see Fig. 1).

Both L- and D-lactic acids can be assayed using an enzymatic method with L- and D-lactic acid dehydrogenases (LDH and D-LDH), respectively.[8] Therefore, the experimental conditions were examined using the enzymatic method in order to determine the lactic acid produced during the degradation reaction. This method employs NAD (nicotinamide-adenine dinucleotide) as a hydrogen acceptor; the amount of lactic acid can be calculated from the increment of the absorbance at 340 nm (ΔE_{340}). It was observed that the LDH activities were hindered by borate ions present in the solution (see Fig. 2), probably because the cofactor NAD is masked by borate ions due to the formation of a complex at the ribosyl moiety. However, the hindrance effects disappear upon the addition of an excess amount of D-ribose to the solution prior to the addition of NAD and the enzyme (Fig. 2).

Using the present method, a 1.03 ± 0.01 molar amount of L-lactic acid and no D-lactic acid were detected from the degradation solution of sepiapterin.

From these data, it is evident that the configuration at the chiral 2-position of the side chain of sepiapterin is

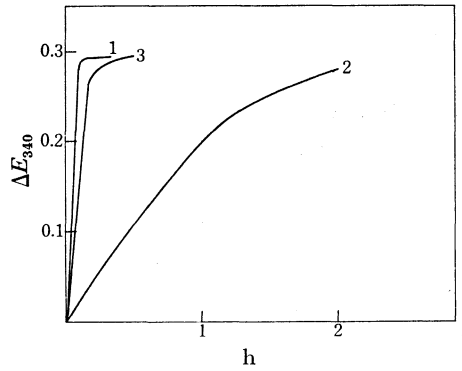


Fig. 2. Effects from borate ions and D-ribose on the dehydrogenation of L-lactic acid by a LDH–NAD system. The reaction was carried out at 25 °C in an assay solution composed of a glycine–hydrazine buffer (800 μl), 50 mM NAD (50 μl), 1 mM DL-lactic acid (100 μl), LDH (5 mg/ml; 10 μl), and distilled water (50 μl) or 4% sodium borate (50 μl).
1: In the absence of sodium borate and D-ribose.
2: In the presence of sodium borate (4%; 50 μl).
3: In the presence of sodium borate (4%; 50 μl) and D-ribose (10 mg).

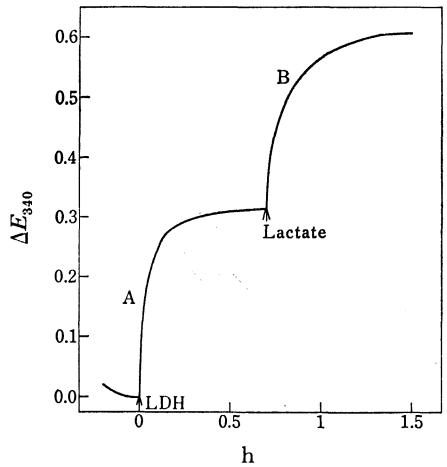


Fig. 3. Determination of L-lactic acid by a LDH–NAD system. First, the acid contained in 50 μl of the oxidative degradation solution (equivalent to 50 μmol of sepiapterin) was dehydrogenated by the system (curve A). When the reaction was complete (40 min), 50 μmole of lithium L-lactate was added (curve B) (for details, see experimental).

the (*S*)- or L-configuration as shown in the formula (I). The present conclusion is consistent with that of Pfleiderer.[7]

Experimental

Sepiapterin was obtained from *D. melanogaster sepia* by a method previously described.[4] NAD, LDH (EC.1.1.1.27; from rabbit muscle; 5 mg/ml, 550 U/mg), and D-LDH (EC. 1.1.1.28; from *Lactobacillus leichmanni*; 1 mg/ml, 300 U/mg) were purchased from Boehringer Mannheim.

Oxidation Degradation of Sepiapterin. A solution of sepiapterin (0.290 mg) in 4% sodium borate (1.22 ml; the concentration of sepiapterin was 1.00 mM) was stirred in air in the dark at 25 °C for 48 h. The sepiapterin degradated to 7,8-dihydroxanthopterin which was further oxidized to xanthopterin by air during the reaction. After completion of the reaction, the solution was adjusted to exactly 1.22 ml with water and used directly for the analysis of lactic acid.

Determination of L-*Lactic Acid.* The assay solution was composed of a glycine–hydrazine buffer (100 ml contains 7.5 g of glycine, 5.2 g of hydrazine sulfate, 0.2 g of EDTA $Na_2H_2 \cdot 2H_2O$, 51 ml of 2M-NaOH, and water to make the solution 100 ml; 840 μl), D-ribose (10%; 100 μl), NAD (50 mM; 50 μl), and 50 μl of the above reaction solution (equivalent to 50.0 μmol of sepiapterin). The assay solution was placed in both the sample and reference quartz cells which were maintained at 25 °C by a thermostatically-controlled cell holder. Then, to the sample cell was added 10 μl of LDH (5 mg/ml, 550 U/mg), and the increment of the absorbance at 340 nm (ΔE_{340}) was recorded. Within 40 min, the reaction was complete and the ΔE_{340} was found to be 0.305 (ΔE^1). Then, 50 μl of a lithium L-lactate solution (1.00 mM; equivalent to 50.0 μmol) was added to the sample cell. In about the same time as above, an additional ΔE_{340} of 0.296 (ΔE^2) was observed (Fig. 3). The amount of L-lactic acid contained in 50 μl of the oxidative degradation solution (equivalent to 50.0 μmol of sepiapterin) was determined using the following calculation:

$$\frac{0.305(\Delta E^1)}{0.296(\Delta E^2)} \times 50.0\ \mu\text{mol} = 51.5\ \mu\text{mol}.$$

By repeating the same procedures several times, it was concluded that 1.03 ± 0.01 mol of L-lactic acid was produced from sepiapterin. When D-LDH was used in place of LDH in the above procedures, no appreciable increase in absorbance at 340 nm was observed.

References

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