**The Biosynthesis of Desmosine and Isodesmosine**

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**SUMMARY**

The biosynthesis of desmosine and isodesmosine was studied with chick embryo aortas in tissue culture. Radioactivity was incorporated into desmosine and isodesmosine when uniformly labeled lysine-14C was added to the medium, but no activity could be incorporated into these amino acids from aspartic acid-14C, glutamic acid-14C, or threonine-14C. The labeled desmosine and isodesmosine obtained after the incorporation of radioactivity from lysine-14C were subjected to the action of strong sodium hydroxide solutions. This treatment liberates the pyridinium nitrogen along with its side chain in the form of lysine. The lysine thus liberated from the labeled desmosine or isodesmosine was found to contain one-fourth of the total activity of the parent compound. The distribution of radioactivity in the carboxyl carbons of these compounds was also studied. These results strongly support the hypothesis that 4 lysine molecules condense to form 1 desmosine or isodesmosine molecule.

**EXPERIMENTAL PROCEDURE**

Chick Embryo Aorta Tissue Culture—Ascending aortas (3 to 4 mm) were removed from 13- to 14-day-old chick embryos under aseptic conditions. The aortas thus removed were cultured individually in 1-ml vinyl cups (Sabri-Kal Corporation) on semi-solid medium according to the method of Wolff and Haffen (8). The desired 14C compound (1 μC) was added to the medium in each cup. The tissue culture medium contained: Tyrode’s solution, 3 parts; chick embryo extract in Tyrode’s solution, 3 parts; penicillin (1000 units) and 14C compound (1 μC), 1 part; and 1% purified agar in Tyrode’s solution, 6 parts.

The vinyl cups were placed in Petri dishes (six cups to each Petri dish) and the aortas were allowed to grow for 5 days at 39.5° ± 0.2°. In each experiment 30 to 60 aortas were used.

Preparation of Elastin—Elastin was prepared from the harvested aortas according to the method of Lansing et al. (9). The aortas were washed by dipping in glass-distilled water and were defatted by refluxing in ethanol for 1 hour and in acetone for another hour. This defatted material was then digested in 0.1 N NaOH at 98° for 60 min. The residue which remained was washed several times with glass-distilled water, followed by ethanol and finally with acetone, and was dried in a desiccator under vacuum.

Isolation of Desmosine, Isodesmosine, and Lysine—The isolated elastin was hydrolyzed with 6 N HCl in vacuum-sealed Pyrex tubes at 110° for 24 hours. The hydrolysate was dried, and desmosine, isodesmosine, and lysine were separated on the 50-cm column of a Beckman-Spinco amino acid analyzer with 0.2 N citrate buffer (pH 4.25) as described previously (6). In the experiments in which degradative studies were carried out, approximately 0.6 μmole each of nonradioactive desmosine and isodesmosine was added to the hydrolysate before applying it on to the resin. In all experiments fractions containing acidic and neutral amino acids, desmosine, isodesmosine, and lysine were collected separately and examined for radioactivity.

Alkali Degradation of Desmosine and Isodesmosine—Huntsche in 1886 (10) showed that treatment of N-methylnicotinic acid with strong alkali yields CH₃NH₂. Huff and Perlweig (11) were able to recover pyridinium nitrogen from N-methylnicotinamide as CH₃NH₂ in 97 to 98% yield by treatment with strong alkali (10 N NaOH). Since desmosine and isodesmosine are substituted pyridinium structures with one of the side chains at position 1, we studied the action of alkali on these compounds. We were able to liberate pyridinium nitrogen in the form of lysine from both desmosine and isodesmosine (Fig. 1). As described previously (3), lysine was obtained in best yield from isodesmosine (73%) by the action of 10 N NaOH at 110° in a sealed tube for 16 hours.

Partridge, Elsden, and Thomas in 1963 (1) reported the isolation of two new amino acids, named desmosine and isodesmosine, from the elastin of bovine ligamentum nuchae. Further studies by Thomas, Elsden, and Partridge (2) showed these compounds to be 1,3,4,5- and 1,2,3,5-tetra-substituted pyridinium salts, with each side chain having a carboxyl-terminal and α-amino-terminal group. The side chain at position 1 was found to be a 6-carbon straight chain (2, 3). These amino acids seem to be essential components of elastin and have been shown to be present in elastins obtained from various sources (4-6).

Miller, Martin, and Piez (4) and Partridge et al. (5) reported the incorporation of radioactivity into desmosine and isodesmosine from lysine-14C and proposed that 4 lysine molecules condense to form 1 desmosine or an isodesmosine molecule. Miller et al. (7) have also studied the effect of copper deficiency and a lathyrogen on the desmosine, isodesmosine, and lysine content of chick aorta elastin and have shown that both factors inhibit the biosynthesis of desmosine and isodesmosine.

The experiments described in this paper provide additional evidence of direct nature in support of the hypothesis that 4 lysine molecules condense to form 1 desmosine or an isodesmosine molecule.

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and from desmosine (41%) with the action of 6 N NaOH at 110° for 70 hours. These conditions were also used for the degradation of labeled desmosine and isodesmosine. Approximately 0.4 μmole of desmosine or isodesmosine was used for this degradation. After treatment with alkali the samples were acidified with HCl, dried, and stirred with 0.5 ml of 0.2 N citrate buffer (pH 2.2) and 1.5 ml of glass-distilled water. Silica was removed by centrifugation and washed three times with 2 ml of 0.1 N citrate buffer, pH 2.2. Combined washings were placed on the hydrogen form of a column of Dowex 50-X2 (10 X 0.9 cm) to remove most of the salt present. Lysine, undegraded desmosine, or isodesmosine and some of the other degradative products were displaced with 1 M NH₃OH. The final separation of lysine was effected by paper electrophoresis on Whatman No. 3HR paper with the use of pyridine-acetate buffer, pH 3.5. The position of the liberated lysine was located by running standard lysine on both sides of the degradative products and by staining the standard lysine strips with ninhydrin. Lysine was eluted from the paper with approximately 10 ml of 0.1 N HCl, dried, and taken up in 0.5 ml of glass-distilled water. One portion (0.2 ml) was used for the estimation of lysine concentration on the 15-cm column of the Beckman-Spinco amino acid analyzer, and another portion (0.25 ml) was used for scintillation counting.

Ninhydrin Decarboxylation of Desmosine, Isodesmosine, and Lysine—Ninhydrin reaction was carried out in citrate buffer (pH 2.5) for 25 min in a boiling water bath (12). A slow stream of N₂ gas was bubbled through the reaction mixture to flush out the CO₂. The liberated CO₂ was adsorbed in a trap attached to the reaction vessel and containing 2-phenylethylamine distributed over acid-washed diatomaceous silicas (Chromosorb W 80/100, Johns Manville), as described by Stuart and Williams (13). After the completion of the reaction, the contents of the trap were transferred to a standard vial which contained the scintillation fluid, and radioactivity was counted.

Quantitative determinations of purified desmosine and isodesmosine were carried out on a 15-cm column of Beckman-Spinco amino acid analyzer. Sometimes the 50-cm column was also used for this purpose, as described previously (6).

Radioactivity Measurements—All radioactivity measurements were carried out on the Nuclear-Chicago liquid scintillation spectrometer, model 6725. Activity of CO₂ was counted as described above, according to the method of Stuart and Williams (13). All other samples were individually placed on pieces of filter paper (1 x 3 cm) and dried. These pieces of filter paper were then placed in standard vials containing 15 ml of the conventional solution for liquid scintillation counting (4 g of 2,5-diphenyloxazole (PPO) and 100 mg of 1,4-bis[2-(5-phenyloxazolyl)]benzene (POPOP) in 1.0 liter of toluene). All counts were corrected to 100% efficiency by a channels ratio technique.

Radioactive Chemicals—All amino acids were uniformly labeled. Lysine-¹⁴C and aspartic acid-¹⁴C were obtained from Merck Sharp and Dohme; glutamic acid-¹⁴C and threonine-¹⁴C were obtained from The Radiochemical Centre, Amersham, England.

![Diagram](http://www.jbc.org/)

**Fig. 1. Alkali degradation of desmosine and isodesmosine**

<table>
<thead>
<tr>
<th>Table I</th>
<th>Incorporation of radioactivity into desmosine and isodesmosine from different ¹⁴C-labeled compounds by chick embryo aortas</th>
</tr>
</thead>
<tbody>
<tr>
<td>¹⁴C-Labeled compound</td>
<td>No. of aortas</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>L-Lysine</td>
<td>56</td>
</tr>
<tr>
<td>L-Aspartic acid</td>
<td>35</td>
</tr>
<tr>
<td>L-Glutamic acid</td>
<td>30</td>
</tr>
<tr>
<td>L-Threonine</td>
<td>36</td>
</tr>
</tbody>
</table>

**RESULTS**

The results of the incorporation of radioactivity into desmosine and isodesmosine from various ¹⁴C-labeled compounds are presented in Table I. No radioactivity could be detected in desmosine, isodesmosine, or lysine from aspartic acid-¹⁴C, glutamic acid-¹⁴C, and threonine-¹⁴C. It may be pointed out that in
all these experiments a significant amount of radioactivity was present in the fraction containing acidic and neutral amino acids.

Radioactivity was incorporated into both desmosine and isodesmosine when lysine-\(^{14}\text{C}\) was added to the tissue culture medium. In this case no activity could be detected in the fraction containing acidic and neutral amino acids, indicating that no random distribution of \(^{14}\text{C}\) from lysine took place.

The results of the alkali degradation of desmosine and isodesmosine after the incorporation of radioactivity from lysine-\(^{14}\text{C}\) are presented in Table II. The specific activity of the lysine liberated by the action of alkali on desmosine or isodesmosine was found to be one-fourth of the specific activity of the parent desmosine or isodesmosine.

As shown in Table III, carbon dioxide liberated by the ninhydrin reaction from desmosine and isodesmosine was found to contain one-sixth of the total activity of the parent compound. This was also true for the lysine-\(^{14}\text{C}\) added to the medium and for the labeled lysine isolated from elastin.

**DISCUSSION**

The results reported in this paper provide direct evidence that 4 lysine molecules condense to form 1 desmosine or an isodesmosine molecule and thus support the views of Partridge et al. (5) and Miller et al. (4).

These results also provide strong evidence against the incorporation of a preformed pyridine ring (e.g. nicotinic acid) into desmosine and isodesmosine. This pathway was considered because nicotinic acid is known to be a precursor for a number of pyridine ring-containing compounds (14) and because nicotinic acid is a vitamin. According to this hypothetical scheme, one 6-carbon chain (e.g. lysine) and three 4-carbon chains (e.g. aspartic acid) could condense with nicotinic acid to form desmosine or isodesmosine; or one 6-carbon chain, two 4-carbon chains, and one 5-carbon chain (e.g. glutamic acid) could condense with the pyridine ring derived by decarboxylation of nicotinic acid. This would require that almost all the activity incorporated into desmosine and isodesmosine from lysine-\(^{14}\text{C}\) be present in the side chain attached to the pyridinium nitrogen. As shown in Table I, no activity could be detected in desmosine, isodesmosine, or lysine when L-aspartic acid-\(^{14}\text{C}\), L-glutamic acid-\(^{14}\text{C}\), or L-threonine-\(^{14}\text{C}\) was added to the medium. Radioactivity from L-lysine-\(^{14}\text{C}\) was incorporated into both desmosine and isodesmosine when lysine-\(^{14}\text{C}\) was added to the tissue culture medium, indicating that no random distribution of \(^{14}\text{C}\) from lysine took place.
lysine-\(^{14}C\) was readily incorporated into desmosine and isodesmosine. The radioactive desmosine and isodesmosine obtained after the incorporation of activity from lysine-\(^{14}C\) were subjected to the action of strong alkali. This treatment liberated the side chain attached to the pyridinium nitrogen in the form of lysine, as shown in Fig. 1. The liberated lysine (from desmosine and isodesmosine) was examined for radioactivity and was found to contain only one-fourth of the total activity (Table II). This then eliminates the possibility that a preformed pyridine ring is the precursor of desmosine and isodesmosine.

On the other hand, these results are in complete agreement with the hypothesis that 4 lysine molecules condense to form 1 desmosine or 1 isodesmosine molecule. For the formation of desmosine and isodesmosine by condensation of 4 lysine molecules, as shown in Fig. 2, one-fourth of the activity must be present in the side chain at position 1 and, lysine being an essential amino acid, no radioactivity can be incorporated into desmosine and isodesmosine from other \(^{14}C\)-labeled compounds (e.g., aspartic acid, glutamic acid).

On decarboxylation of desmosine and isodesmosine with the ninhydrin reaction, one-sixth of the total activity was found to be present in the liberated \(\text{CO}_2\) (Table III). This is also in complete agreement with the data given in Fig. 2. Lysine added to the medium was uniformly labeled and contained one-sixth of the total activity in C-1 (Table III). As shown in Fig. 2, C-1 of lysine forms the carboxyl carbons of desmosine and isodesmosine, which should contain one-sixth of the total activity.

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