Studies on Prolidase Deficiency with a Possible Defect in Collagen Metabolism

MAMORU ISEMURA,* TADAMASA HANYU, TERUO ONO, RYOICHI IGARASHI,† YOSHIIO SATO,† FUMITAKE GEJYO,‡ RYOICHI NAKAZAWA,‡ TAKASHI MIYAKAWA,‡ TORU TAKAGI,§ YOSHINORI KUBOKI§ and SATOSHI SASAKI§

Department of Biochemistry, †Department of Dermatology and ‡Department of Internal Medicine, Niigata University School of Medicine, Niigata 951 and §Department of Biochemistry, School of Dentistry, Tokyo Medical and Dental University, Tokyo 113


Skin collagen of a female patient with prolidase deficiency was examined for the distribution of borohydride-reducible cross-links and the proportion of type III to type I collagen. Patient's skin contained after reduction more dihydroxylysinonorleucine relative to hydroxylysinonorleucine and type III collagen than expected for normally matured skin. These findings suggest that collagen of the patient's skin failed to follow a time-related normal maturation process and that collagen metabolism was disturbed. The composition of urinary collagen metabolites was also unusual. On the other hand, her asymptomatic brother with prolidase deficiency showed the normal urinary composition of collagen metabolites. It is suggested that prolidase deficiency and defect in collagen metabolism independent of it are both responsible for clinical manifestation.

In a previous paper (Isemura et al. 1979), we reported that a female patient with chronic recurring leg ulcer was suffering from deficiency of prolidase (EC 3.4. 13.9, proline dipeptidase) with imidodipeptiduria. There have been reported at least eight cases of proven or highly suspected cases of prolidase deficiency as summarized by Scriver (1978) and by Arata et al. (1979). In spite of the description of the first case of this disease as a syndrome resembling lathyrism (Goodman et al. 1968) and the suggestion of abnormal collagen metabolism in these patients (Jackson et al. 1975), few direct analyses of collagen and its metabolites of patients have been performed (Goodman et al. 1968; Isemura et al. 1979).

This paper describes the results of chemical analysis of patient's dermis and urinary hydroxylysine and its glycosides as an index of collagen metabolism.
M. Isemura et al.

(Askenasi 1975; Hanyu et al. 1979; Prockop et al. 1979). In addition, the biochemical data for the patient's uncle are included, which serve to construct a pedigree of a case of prolidase deficiency.

MATERIALS AND METHODS

Prolidase and prolinate (EC 3.4.13.8, prolyl dipeptidase) activities

These enzyme activities of erythrocytes from patient's uncle (64-year-old) were measured as described previously (Isemura et al. 1979)

Analysis of the reducible cross-links of dermal collagen

This was performed essentially according to the method as described previously (Kuboki et al. 1977). Homogenized dermis was extracted twice with 1 M NaCl in 0.05 M Tris-HCl, pH 7.4 at 4°C for 2 days and then twice with 0.5 M acetic acid. The insoluble collagen fraction (about 20 mg of dried sample) was reduced with NaB (311) (1.75 mCi). After the reaction was stopped by the addition of 0.5 M acetic acid to pH 4.0, the insoluble fraction was collected and washed with water on a millipore filter. A freeze-dried sample was hydrolyzed with 6 N HCl at 110°C for 24 hr and the radioactive cross-links were analyzed on a column (1 x 45 cm) of Hitachi ion-exchange resin No. 2630 at 60°C with 0.35 N sodium citrate buffer, pH 5.28 as an eluant. The eluate was divided into two portions by a split-stream device (Mechanic 1974). The radioactivity in one portion was determined with an Aloka RLC-601 flow-cell liquid scintillation counter, and amino acids in another portion were determined by ninhydrin reaction with a Hitachi 034 liquid chromatograph. Each radioactive cross-link was identified by comparison with standard cross-links prepared from reduced dentin collagen (Mechanic 1974; Kuboki et al. 1977).

Analysis of collagen type

Dermis was extracted twice with 1 M NaCl in 0.05 M Tris-HCl, pH 7.4 and then twice with 0.5 M acetic acid at 4°C overnight. Pepsin-soluble collagen was prepared and analyzed by interrupted sodium dodecylsulfate-polyacrylamide gel electrophoresis according to the method of Sykes et al. (1976). The ratio of (III)/[I] was determined by densitometric measurements of the gel bands stained with Coomassie brilliant blue R-250 using a Hitachi Spectrophotometer 557 equipped with a gel-scanner apparatus.

Determination of urinary hydroxylysine and its glycosides

These compounds were analyzed with an automated amino acid analyzer essentially according to the method described previously (Isemura et al. 1976; Hanyu et al. 1979). Briefly, urine samples were hydrolyzed in 2.5 N NaOH at 110°C for 20 hr. After neutralization with acetic acid, the hydrolysate was passed through a column of CG Amberlite IR-120 (H+). The column was washed with water and 8% pyridine, and eluted with 3 N NH4OH. The eluate was concentrated and analyzed on an amino acid analyzer with 0.35 N sodium citrate buffer, pH 5.20 (Hanyu et al. 1979).

Effects of red cell infusion

About 23 months after hospitalization, the patient received intravenously one unit of normal red cells three times at intervals of 3 days. One unit of red cells is an amount of those derived from 200 ml of whole blood.

Urinary excretion of dipeptides, Asp-Pro, Glu-Pro and Gly-Pro was determined as described previously (Isemura et al. 1979). Urinary hydroxylysine and its glycosides were determined as described above.
**RESULTS**

**Enzyme activities of erythrocytes from patient's uncle**

Erythrocytes of patient's uncle exhibited normal levels (Isemura et al. 1979) of prolidase and prolinase activities (5.83 and 0.140 nmole/min/mg protein, respectively). A pedigree of the present case (Fig. 1) supports the suggestion that prolidase deficiency is autosomal recessive (Scriver 1978).

---

**Reducible cross-links of dermal collagen**

Percent distributions of radioactivity in four reduced cross-links in the patient's dermal collagen were 8.6% for dihydroxylysinoonorleucine (DHLNL), 30.9% for hydroxylysinoonorleucine (HLNL), 8.6% for lysinoonorleucine and 51.9% for histidinohydroxymerodesmosine. A plot of ratios of DHLNL to HLNL as a function of the age (Fig. 2) shows that the patient's dermis contains a higher amount of DHLNL as compared with age-matched normal subjects.

---

![Pedigree of a family with prolidase deficiency. Patient's mother and brother were dead of uremia and from measles, respectively. • and ■, Female and male homozygous for prolidase deficiency; □, male heterozygous; ○, male free of prolidase deficiency; □ and □, female and male deceased, not examined.](image)

![Abundance of dihydroxylysinoonorleucine (DHLNL) relative to that of hydroxylysinoonorleucine (HLNL) in borohydride-reduced dermis at various ages. An open circle represents the ratio for the patient and closed circles for normal controls. Samples of normal femoral skin were obtained at skin graft operations.](image)
Type of collagen

Pepsin-solubilized collagen of patients dermis gave a value of 0.33 for a ratio of \(\alpha_1(III)\) to \(\alpha_1(I)\). Normal dermis derived from four 18-year-old males gave values of 0.17, 0.27, 0.27 and 0.31 for this ratio. Yields of the pepsin-soluble fraction were ranged from 40.2 to 56.1% of the total dermis collagen on the basis of a hydroxyproline content. Although the age is not strictly matched, the result was taken to suggest that the patient's dermis contained a higher proportion of type III collagen than normal dermis, in view of the fact that the ratio decreases with age (Sykes et al. 1976; Prockop et al. 1979).

Urinary hydroxylysine and its glycosides

Table 1 shows the urinary levels of hydroxylysine and its glycosides of the patient and her family. At the stage of one year after her hospitalization, no significant differences in a total amount of hydroxylysine excreted were noted between these and normal subjects. However, as shown later, a significant increase in the total hydroxylysine excretion was noted about 22 months after her hospitalization, perhaps reflecting the change of clinical condition. The ratios of glucosylgalactosylhydroxylysine (Glc-Gal-Hyl) to galactosylhydroxylysine (Gal-Hyl) of the urine samples from the patient, her elder brother and father were lower than those for normal controls, while those for the younger of her brothers and her uncle were considered to be within a normal range.

| Table 1. Urinary compositions of hydroxylysine and its glycosides |
|-------------|----------------|-----------------|------------------|
| Age (years) | Total hydroxylysine (\(\mu\)moles/g creatinine) | Glycosylation of hydroxylysine (%) | Ratio* of Glc-Gal-Hyl to Gal-Hyl |
| Patient     | 23             | 28.3            | 76.1             | 1.55 (1.53, 1.56) |
| Brother     | 26             | 24.2            | 82.3             | 2.00 (1.82, 1.92, 2.20) |
| Brother     | 30             | 20.2            | 79.9             | 1.68 (1.64, 1.65, 1.74) |
| Father      | 57             | 30.1            | 74.0             | 1.78 (1.78, 1.78) |
| Uncle       | 63             | 35.3            | 85.6             | 2.46 (2.45, 2.45, 2.49) |
| Normal controls | 27-51         | 28.4±6.45       | 75.8±6.99        | 2.16±0.18 |

* Actual values are given in parentheses.
† Taken from our previous data (Hanyu et al. 1979), mean±s.d.

Effects of red cell infusion

Since erythrocytes of the patient had essentially no activity of prolidase, normal red cell infusion may be expected to result in an altered pattern of urinary excretion of dipeptides. The results (Table 2), however, showed little effects of this potential therapy on urinary levels of imidodipeptides. No clinical improvement was observed, either. At this stage, the urinary total hydroxylysine excretion was
elevated about four times as compared with the stage of one year after hospitalization (Table 1). Red cell infusion caused no change either in this level or in the ratio of two hydroxylysine glycosides (Table 2).

**Table 2. Effects of red cell infusion on patient's urinary composition**

<table>
<thead>
<tr>
<th>Urine sample</th>
<th>Total hydroxylysine (μmoles/g creatinine)</th>
<th>Glycoxylation of hydroxylysine (%)</th>
<th>Ratio of Glc-Gal-Hyl to Gal-Hyl</th>
<th>Asp-Pro Glu-Pro Gly-Pro (mmoles/g creatinine)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>116.6</td>
<td>85.4</td>
<td>1.36</td>
<td>3.75</td>
</tr>
<tr>
<td>2</td>
<td>115.9</td>
<td>83.6</td>
<td>1.47</td>
<td>3.41</td>
</tr>
<tr>
<td>3</td>
<td>100.6</td>
<td>82.7</td>
<td>1.41</td>
<td>3.38</td>
</tr>
</tbody>
</table>

About 22 months after hospitalization, the patient received one unit of red cells three times at each 3-days interval. Urine samples collected just before infusion (1), three days (2) and 24 days (3) after the last infusion were analyzed for hydroxylysine, its glycosides and three dipeptides. Values are from duplicate determinations.

**DISCUSSION**

Although clinically heterogeneous, skin manifestations are characteristic and skin fragility appears to be a prominent aspect in the prolidase deficiency (Scrivener 1978; Arata et al. 1979; Isemura et al. 1979). Fig. 3 shows chronic

![Fig. 3. The skin lesions on the patient’s legs at hospitalization.](image-url)
recurring ulcers on legs of the 23-year-old patient (Gejyo et al. 1980). The role of prolidase deficiency in the skin pathology has not been clearly defined. Since the disease accompanies hyperimidodipeptiduria, the defect in collagen metabolism caused by continuous loss of proline has been suggested to account for the pathogenesis (Jackson et al. 1975; Arata et al. 1979). In spite of this suggestion, amino acid analyses of patient's skin collagens have failed to reveal any significant difference from normal samples (Goodman et al. 1968; Isemura et al. 1979). The present analyses, however, indicated the unusual compositions in collagen cross-links and in collagen type of the patient's skin, although age-related changes of these parameters make it difficult to deduce any definitive conclusion. As shown in Fig. 2, the ratio of two hydroxylysine-derived borohydride-reduced cross-links, DHLNL/HLNL decreases with age in normal dermis. Similar observation has been reported in the case of the scar tissue (Bailey et al. 1975). It was also shown that DHLNL is the predominant cross-link derivative in young dermal scar (Forrest et al. 1972).

It is well documented that the ratio of type III to type I collagen decreases after birth with age and that a fibrotic tissue contains more type III collagen than is present originally in the tissue (Sykes et al. 1976; Prockop et al. 1979). Thus, the patient's skin collagen appears to fail to follow the time-related changes of collagen cross-links and collagen type distribution, resulting in a disturbed maturation of the dermal tissue. At present, it is not known whether these disturbed changes in the patient's skin collagen is primary in itself or is produced secondarily by the ulceration accompanied with recurrent infection. There is also a possibility that any drug used for therapy such as steroid hormone may have induced these changes.

Analysis of hydroxylysine glycosides of the patient's urine showed that a ratio of Glc-Gal-Hyl to Gal-Hyl decreased as compared with normal urine samples (Table 1). Normal urine samples from three 21-year-old males have been reported to give values of 1.9, 2.0 and 2.1 for this ratio (Isemura 1976). Although it is difficult to correlate this finding with the pathogenesis of the disease because of the lack of information of catabolism of hydroxylysine glycosides, this finding again seems to indicate that the collagen metabolism is not following the normal process in the patient. In view of the fact that acid-soluble collagen has a lower ratio of Glc-Gal-Hyl to Gal-Hyl than insoluble collagen (Spiro 1967; Isemura et al. 1976), the result may suggest that the pre-matured collagen is degraded predominantly.

The finding that the patient's father and 30-year-old brother also showed lower value for this ratio (Table 1) may be considered to indicate that this disturbance of collagen metabolism is heritable. It has been puzzling that there are cases of prolidase deficient persons with hyperimidodipeptiduria yet without clinical symptoms (Arata et al. 1979; Isemura et al. 1979). In our case, onset of the disease was at 8 years of age and her prolidase deficient brother is asymptomatic even at 26 years of age. There seems to be little possibility that he will develop any clinical manifestation in the future. Thus, prolidase deficiency and
resulting impaired recycling of proline appear not to be solely responsible for development of clinical manifestations. Therefore, additional defect(s) should be considered to be involved in this disease. Since urinary composition of hydroxylysine glycosides of this brother appears to be normal in contrast with the patient, a possible candidate for this is the heritable disorder of collagen metabolism independent of prolidase deficiency, which results in the abnormal urinary composition of hydroxylysine glycosides. Such defect would lead to develop various clinical manifestations (Scriver 1978; Isemura et al. 1979) by affecting connective tissues systemically including blood vessels.

Acknowledgment

This work was supported in part by a Grant-in-Aid from the Niigata University Science Foundation to MI.

References


