The Microheterogeneity of Bovine Serum $\alpha_1$-Acid Glycoprotein

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ABSTRACT. The microheterogeneity in bovine serum $\alpha_1$-acid glycoprotein ($\alpha_1$AGP) was examined by crossed affino-immunoelectrophoresis with concanavalin A (Con A). In healthy cows, serum $\alpha_1$AGP revealed 2 fractions which were named fraction 1 and 2 according to the degree of binding affinity to Con A. The mean±SD of serum $\alpha_1$AGP concentration was 0.31±0.09 g/l and the relative amounts of fraction 1 and 2 were approximately 67% and 33% respectively on this method. In bovine leukemia cases, 5 out of 8 had a high serum $\alpha_1$AGP concentration of more than 0.7 g/l. On the electrophoretic pattern, the other 2 fractions (Con A weakly and non-reactive fractions, named fraction 3 and 4) were observed in 5 cases and their relative amounts of 4 fractions were 17.8–28.5%, 35.5–46.0%, 17.9–24.4% and 12.0–17.2%, respectively. In bovine leukemia virus infected cows with no clinical symptoms, however, serum $\alpha_1$AGP concentration and the electrophoretic patterns were almost the same as those in healthy cows.---KEY WORDS: cattle, Con A affinity, microheterogeneity, $\alpha_1$-acid glycoprotein.

$\alpha_1$-acid glycoprotein ($\alpha_1$AGP), well-known as an acute phase reactant in human patients, increases 2- to 4-fold in serum concentration during inflammation and cancer [5, 16, 20]. $\alpha_1$AGP have been reported to contain high amounts of carbohydrate and sialic acid [1, 7, 15]. The contents of carbohydrate in human $\alpha_1$AGP change under some conditions such as cancer or diabetes mellitus [14, 17], suggesting the microheterogeneity of $\alpha_1$AGP obtained from inflammatory and cancer cases [6].

The microheterogeneity in $\alpha_1$AGP has been investigated by concanavalin A (Con A) affinity [6, 13], since the interaction of glycoproteins with Con A was first described by Nakamura [12].

In this work we have studied on the microheterogeneity of bovine serum $\alpha_1$AGP in the bovine leukemia virus (BLV) negative or positive cows with no clinical symptoms and in the cases with bovine leukemia.

MATERIALS AND METHODS

Serum sample: Thirty three sera from BLV negative healthy cows and 22 sera from BLV positive cows with no clinical symptoms were collected. Serum samples were also obtained from 8 cases with bovine leukemia (a case of calf type, thymic type and skin type each, and 5 cases of adult type) diagnosed clinically and pathologically.

$\alpha_1$AGP assay: Standard bovine $\alpha_1$AGP and specific antiserum were prepared by technique described previously [7]. Serum $\alpha_1$AGP concentrations were determined by single radial immunodiffusion method under conditions detailed elsewhere [10]. Briefly, rabbit anti-bovine $\alpha_1$AGP in 300 µl of serum was mixed 5 ml of 1% agarose in a barbital buffer (pH 8.6, µ=0.025). The agarose-antibody solution was placed on a
methyl-α-D-mannopyranoside (Sigma Chemical Co., U.S.A) in order to dissolve the affinity precipitate. The electrophoresis in the first dimension was carried out with a voltage of 10 V/cm for 70 min, and in the second dimension with 2 V/cm for 18 hr. Then, the gel was washed with 0.9% NaCl, pressed, dried, and stained with Amido-black 10B. The area under the precipitation curves was measured by semiautomatic planimetry.

RESULTS

Serum $\alpha_1$AGP concentration: Serum $\alpha_1$AGP levels in healthy cows with or without BLV infection and in bovine cases of leukemia were shown in Fig. 1. In BLV negative healthy cows, the $\alpha_1$AGP concentration was $0.31 \pm 0.09$ g/l (mean$\pm$SD). Two of 22 BLV positive healthy cows had a slight increased level of 0.72 and 0.59 g/l, but this group had almost the same mean level of 0.25$\pm$0.14 g/l as that in healthy cows. In bovine leukemia cases, 6 of 8 had a high $\alpha_1$AGP level of more than that in healthy cows. In 4 of these cases, serum $\alpha_1$AGP concentrations increased higher than 1.20 g/l and the highest value was 3.55 g/l. However, 2 cases had 0.23 or 0.47 g/l of $\alpha_1$AGP concentration within a normal range.

Con A affino-immunoelectrophoresis: In healthy cows, serum $\alpha_1$AGP revealed 2 fractions (fraction 1 and 2) in the affinity to Con A. The fractions were numbered by the degree of binding capacity to Con A (Fig. 2a). The relative amounts of these fractions were approximately 66.9$\pm$13.9% and 33.1$\pm$13.9%, respectively (Table 1). In BLV positive healthy cows, the electrophoretic patterns were almost the same as those of negative ones, showing 2 Con A reactive fractions with the relative amounts of 58.0$\pm$11.4% and 42.1$\pm$11.4%, respectively. While these 2 fractions were
MICROHETEROGENEITY OF BOVINE $\alpha_1$-AGP

![Image](image.png)

Fig. 2. Con A affino-immunelectrophoresis patterns in serum $\alpha_1$-AGP from a BLV negative healthy cows (a) and a case with leukemia (b, Case No.3).

<table>
<thead>
<tr>
<th></th>
<th>Serum $\alpha_1$-AGP</th>
<th>$\alpha_1$-AGP (%) in fractions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>level (g/l)</td>
<td>1</td>
</tr>
<tr>
<td><strong>BLV negative</strong></td>
<td>0.309 ± 0.086</td>
<td>66.9</td>
</tr>
<tr>
<td>(n=5)</td>
<td></td>
<td>±13.9</td>
</tr>
<tr>
<td><strong>BLV positive</strong></td>
<td>0.254 ± 0.137</td>
<td>58.0</td>
</tr>
<tr>
<td>(n=22)</td>
<td></td>
<td>±11.4</td>
</tr>
<tr>
<td><strong>Leukemic case</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. 1(calf)$^{a)}$</td>
<td>3.55</td>
<td>18.2</td>
</tr>
<tr>
<td>2(adult)</td>
<td>2.20</td>
<td>25.8</td>
</tr>
<tr>
<td>3(thymic)</td>
<td>1.50</td>
<td>28.5</td>
</tr>
<tr>
<td>4(adult)</td>
<td>1.22</td>
<td>17.8</td>
</tr>
<tr>
<td>5(skin)</td>
<td>0.73</td>
<td>71.4</td>
</tr>
<tr>
<td>6(adult)</td>
<td>0.62</td>
<td>52.2</td>
</tr>
<tr>
<td>7(adult)</td>
<td>0.47</td>
<td>24.0</td>
</tr>
<tr>
<td>8(adult)</td>
<td>0.23</td>
<td>43.8</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td>1.324 ± 1.097</td>
<td>35.2</td>
</tr>
</tbody>
</table>

|                     | ±SD                  | ±19.0 | ±4.2  | ±3.0  | ±2.3  |

a) Type in bovine leukemia.

b) From Case Nos. 1, 2, 3, 4, and 7.

observed in BLV positive and negative healthy cows, the other fractions (fraction 3 and 4) indicated lower binding capacity to Con A were detected on 5 of 8 cases with leukemia (Fig. 2b). The relative amounts of fraction 1 to 4 were 17.8–28.5%, 35.5–46.0%, 17.9–24.4% and 12.0–17.2%, respectively. In remained 3 cases, however, 2 Con A reactive fractions as those in healthy cows were detected, and 2 of these cases had an slightly increased level of fraction 2 of which the relative amounts were 47.8 and 56.2%, respectively.
DISCUSSION

Serum $\alpha_1$-AGP increases in various conditions including acute or chronic inflammation, and cancer in human [5, 6, 13]. Its levels in cows ranged from 0.16 to 0.55 g/l without age-related differences in healthy state and increased from normal to 7-fold value under conditions with pneumonia and leukemia [9]. Particularly in bovine leukemia, high levels of more than 1.2 g/l were observed in 4 of 8 cases, as in the human cases with malignant diseases such as leukemia [5, 18]. Furthermore, the tumor-associated human abnormal $\alpha_1$-AGP were confirmed in the plasma of neoplastic disease [14]. In fact, the glycan structure of human $\alpha_1$-AGP in malignant diseases was estimated to be different from that in health [6].

Con A affino-immunoelectrophoresis was a sensitive method for the investigation of microheterogenous form [3]. It has been established that the heterogeneity is resulted mainly from the differences in the carbohydrate side chains. These differences can be manifested as a change in a particular glycan structure or as differences in the properties of, for example, bi- and tri-antennary glycans [11]. Well-defined changes in the microheterogeneity of $\alpha_1$-AGP has been reported to occur in pregnancy and acute inflammation [6, 13, 19].

Normal human serum $\alpha_1$-AGP was confirmed to distribute in 4 fractions by Con A affino-immunoelectrophoresis, and 40–50% of total $\alpha_1$-AGP was occupied with the non-binding fraction [6, 13]. The serum $\alpha_1$-AGP in healthy cows was distributed in 2 reactive fractions and relative amount of the fraction 1 was about 70%. On the other hand, 5 of 8 leukemia cases (Case Nos. 1, 2, 3, 4 and 7) had 4 fractions and increased relative amounts in fraction 3 and 4. In 2 (Nos. 6 and 8) of remained 3 cases, fraction 3 and 4 were not observed, but the amount of fraction 2 was increased. However, a remained case (No. 5) had the same pattern as those of healthy cows.

The changes in the amount of each fraction detected by Con A affinity on $\alpha_1$-AGP were not relative to its serum levels and also not to BLV infection. Hansen et al. [6] demonstrated that the 3 kinds of microheterogeneity pattern in human $\alpha_1$-AGP corresponded to the 3 groups of normal health, inflammatory lung disease, and cancer of the lung, respectively. Furthermore, tumor-associated $\alpha_1$-AGP in human patients was different from that in normal health in the degree of glycosilation on $\alpha_1$-AGP [6]. Gahnberg et al. [4] showed that human $\alpha_1$-AGP was also secreted by lymphocytes and lymphoblastoid cell line. Since bovine $\alpha_1$-AGP was also secreted by lymphocytes [8] and $\alpha_1$-AGP from lymphocytes was weakly reacted with Con A, the increase of the fractions with weak or no Con A binding affinity (fraction 3 and 4) might be associated with the proliferation of lymphoblastoid cell in bovine leukemia.

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REFERENCES


要 約

牛血清α1酸性糖蛋白の多形成について：岩田祐之・井上 武・小野憲一郎*1・長谷川篤彦**1・友田 勇*1（山形大学農学部家畜衛生学教室）——牛血清α1酸性糖蛋白の多形成についてコンカバリン A (Con A) 親和性電気泳動法により検索した。健常牛の血清中α1酸性糖蛋白濃度は0.31±0.09 g/l（平均±SD）で、本法により親和性の高い2分画（分画Ⅰ及びⅡ）が認められ、その構成比は分画Ⅰは67％をまた分画Ⅱ33％であった。白血病症状例では8例中5例で血清中α1酸性糖蛋白濃度が0.7g/l以上の高値を示した。また Con A 親和性電気泳動法では、分画Ⅰ、Ⅱ以外に、Con A 親和性の低い分画Ⅲならびに親和性のない分画 IV を含めた4分画が5例で観察され、その構成比は分画Ⅰが17.8－28.5％、分画Ⅱが35.5－46.0％、分画Ⅲが17.9－24.4％、分画Ⅳが12.0－17.2％であった。なお、白血病ウィルス型で無症状の群では血清濃度ならびにCon A 親和性に健常牛と差を認めなかった。