Differences in Hypoxanthine • Xanthine, Adenosine Triphosphate and Lactate Dehydrogenase Levels in Red Cells among Hemoglobin Types in Finnish Landrace Sheep

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Abstract An investigation was undertaken on the relationship between hemoglobin (Hb) types and the levels of hypoxanthine • xanthine (Hx • x), adenosine triphosphate (ATP) and lactate dehydrogenase (LDH) in the red cells of Finnish Landrace sheep. Hx • x levels in the red cells of Hb A sheep were significantly lower than those of Hb B sheep (P<0.05). Hx • x levels decreased in the order of Hb B, Hb AB and Hb A, though no significant differences were noted between types Hb A and Hb AB or between types Hb B and Hb AB. Red cell Hx • x levels had no correlation with plasma Hx • x levels, which did not show any significant difference among Hb types. ATP levels in the red cells of Hb A and Hb AB sheep were significantly lower than those of Hb B sheep (P<0.01). But the ATP levels of Hb A sheep were essentially the same as those of Hb AB sheep. The ATP values in Hb types were in the order of Hb A ≈ Hb AB < Hb B. With respect to the red cell LDH, differences in the electrophoretic pattern of LDH isozymes among Hb types could not be detected. In the enzymic activity, that of Hb A sheep was significantly higher than those of Hb B and Hb AB sheep (P<0.01). But Hb AB sheep did not differ significantly with Hb B sheep. This activity was in the order of Hb A > Hb AB ≈ Hb B. Also, the LDH activity had negative correlation with ATP values (r = -0.461, P<0.01).


Key words: hemoglobin polymorphism, sheep red cells, hypoxanthine • xanthine, adenosine triphosphate, lactate dehydrogenase

In adult sheep, there are two major components of hemoglobins (Hb) with different electrophoretic mobilities, fast-moving Hb(Hb A) and slow-moving Hb(Hb B)1,2). Both are the main components of principal Hb phenotypes(Hb A, Hb B and Hb AB) as determined genetically and differ not only in physio-chemical properties4-6) but in physiological functions7-9) as well. As one of the most typical properties, Hb A gives an oxygen dissociation curve to the left of that of Hb B, indicating to have higher affinity for oxygen than Hb B7). Among sheep with Hb A and with Hb B, great

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differences in physiological characters were found, such as with respect to hematocrit, Hb concentration and oxygen content in blood\textsuperscript{7,8,10}. Also, Hb A sheep have been shown to have higher tolerance toward hypoxia than Hb B sheep\textsuperscript{9}. The functional differences in these Hb molecular forms are thus thought to affect to relative metabolic systems, and consequently, physio-chemical capacities in metabolic systems for adaptation to changes in internal and external environments are assumed to differ among Hb types.

To obtain more detailed understanding of the functional properties of Hb molecular forms, an investigation was recently conducted on the relationship between Hb, diaphorase or glutathione types and the metabolism of methemoglobin in Finnish Landrace sheep\textsuperscript{11}. The activity of reduced glutathione in methemoglobin metabolism appeared to differ among Hb types. Attention was also directed to hypoxathine - xanthine(Hx - x), adenosine triphosphate( ATP) and lactate dehydrogenase(LDH) in the red cells of different Hb types. Hx - x levels are known to be related to oxygen content in the nucleotide metabolic pathway, in which Hx - x is oxidized to uric acid\textsuperscript{12}, and Hx in plasma has been reported to be a sensitive parameter of hypoxia in tissues. Hx - x levels in plasma and red cells may possibly be different among Hb types, since their blood shows distinctive differences in oxygen affinity, as stated above. Also, the role of ATP in red cells is known to be an allosteric modifier of Hb, as 2, 3-diphosphoglycerate(DPG) in lower animals such as fishes and amphibia\textsuperscript{13} and the ATP levels are reported to be highly correlated with LDH phenotypes in killifish\textsuperscript{14}. Though in adult sheep quite lacking in DPG, the roles of ATP to Hb, other that of an Na-K ion pump and maintaining the stability of cell membranes, and the interrelationships between ATP and LDH in red cells have not been elucidated, there may be similar phenomenon to the correlation between ATP and LDH in the killifish. In this study, an attempt was made to confirm the relationships between the Hx - x, ATP or LDH levels and the Hb types in the red cells of Finnish Landrace sheep.

**Materials and Methods**

40 healthy ewes of Finnish Landrace sheep aged from 3 to 6 years were used and maintained at the Happy Sheep Farm in Hokkaido. Blood samples were collected into heparinized test tubes by jugular venipuncture and stored immediately in ice box (4°C). Whole blood was used within 12 hours after blood collection to determine Hb and ATP levels. The samples were separated into plasma and red cells. The red cells were washed three times with 0.9% NaCl. A portion of the packed cells was used within 24 hours to determine LDH activity by diluting the cells 30 times with the distilled water. Plasma and the remaining packed cells were stored at -20°C until use. Some of these samples for the examination of LDH were stored at 4°C. Also, the samples for electrophoresis for Hb types and LDH isozymes were prepared respectively as 2% and 50% hemolysates.

Hb concentration was measured according to the cyanmethemoglobin method\textsuperscript{12}. The concentration of Hx - x was determined according to the method described by
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CUNNINGHAM and KEAVENY\textsuperscript{13}, and ATP content, by using the ATP test kit of Boehringer Mannheim Company in West Germany. LDH activity was assayed by a modification of the method of WORBLEWSKI and LADUE\textsuperscript{14,15}.

Hb types were identified by starch–gel electrophoresis in a tris–EDTA–borate continuous buffer system (pH 8.9) as described by GHANE et al.\textsuperscript{16}. LDH isozymes were separated by starch–gel electrophoresis in a tris–borate continuous buffer system (pH 7.4)\textsuperscript{17}, and were detected by overlaying 1% agar solution (45°C) containing a reaction mixture of NAD, DL-lactic acid, MTT and PMS in 0.1 M tris buffer (pH 8.0)\textsuperscript{18} on a gel surface following electrophoresis.

Statistic analysis for the levels of Hx • x, ATP and LDH among Hb types was done by using the method of analysis of variance.

Results

Hx • x levels in plasma and red cells among different Hb types.

Plasma Hx • x levels were reported to be useful as a parameter for estimating oxygen levels in tissues\textsuperscript{19,20}. We examined on the plasma Hx • x levels in sheep of different Hb types, since oxygen levels in the tissues of Hb A sheep were considered to be lower than those of Hb B sheep. As shown in Table 1, there were no significant differences among Hb types. However, Hb A sheep showed slightly higher values than Hb B sheep.

Hx • x levels in red cells in Hb A sheep (167.76 ± 2.31 µmole/l) were significantly lower than those of Hb B sheep (178.48 ± 9.05 µmole/l, P<0.05). But that of Hb AB sheep (172.63 ± 9.23 µmole/l) was not significantly different from both those of Hb A and Hb B sheep. As a whole, the decreasing order of the red cell Hx • x values in different Hb types was Hb B, Hb AB and Hb A.

There was no correlation between Hx • x levels in plasma and red cells (r = −0.089).

Table 1. Hypoxanthine • xanthine (Hx • x), adenosine triphosphate (ATP) and lactate dehydrogenase (LDH) levels in red cells and plasma of Finnish Landrace sheep of different hemoglobin types

<table>
<thead>
<tr>
<th>Hemoglobin type</th>
<th>A</th>
<th>AB</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hx • x plasma</td>
<td>136.32±8.46 (5)</td>
<td>133.18±4.96 (20)</td>
<td>132.76±3.76 (15)</td>
</tr>
<tr>
<td>red cell</td>
<td>167.76±2.31\textsuperscript{a} (5)</td>
<td>172.63±9.23 (15)</td>
<td>178.48±9.05\textsuperscript{b} (15)</td>
</tr>
<tr>
<td>ATP red cell</td>
<td>1.72±0.39\textsuperscript{c} (5)</td>
<td>1.91±0.29\textsuperscript{c} (15)</td>
<td>2.39±0.36\textsuperscript{d} (15)</td>
</tr>
<tr>
<td>LDH red cell</td>
<td>13.90±0.49\textsuperscript{c} (5)</td>
<td>11.03±2.06\textsuperscript{d} (20)</td>
<td>10.47±1.64\textsuperscript{d} (15)</td>
</tr>
</tbody>
</table>

Mean±S.D. ( ) : Number of animals. Hx • x : µmole/l, ATP : µmole/gHb, LDH : IU/gHb. a,b,c,d : Mean within a line followed by another superscript differ significantly (a,b : P<0.05; c,d : P<0.01).
ATP and LDH levels in red cells among Hb types.

Red cell ATP levels in Hb A type averaged $1.72 \pm 0.39 \mu$ mole/gHb, and in Hb B type, $2.39 \pm 0.36 \mu$ mole/gHb (Table 1). The value of Hb B sheep was 1.4 times higher than that of Hb A sheep. ATP values in Hb AB type ($1.91 \pm 0.29 \mu$ mole/gHb) did not conspicuously differ with those in Hb A type, but did so with those in Hb B type ($P<0.01$).

The LDH isozymes of the red cells were separated into three bands on the anodic side of the gel. The fastest-moving band was observed to have a different staining intensity, while the other bands stained faintly. No individual variation in the number of LDH bands could be found in any of the samples tested. Thus, no differences in the electrophoretic patterns of LDH isozymes could be detected among Hb types.

The LDH activity in sheep of different Hb types is given in Table 1. Hb A sheep ($13.90 \pm 0.49$ IU/gHb) had significantly greater enzyme activity than Hb B sheep ($10.47 \pm 1.64$ IU/gHb, $P<0.01$). Also, between Hb B and Hb AB sheep ($11.03 \pm 2.06$ IU/gHb) no significant differences could be found, but Hb A and Hb AB sheep differed significantly ($P<0.01$).

A significant negative correlation was noted between LDH and ATP levels in the red cells ($r = -0.461, P<0.01$, Fig. 1.)

![Fig. 1. Correlation between lactate dehydrogenase activity and adenosine triphosphate values in sheep red cells.

●: Hb A, ○: Hb AB, □: Hb B, $r = -0.461, (P<0.05)$]
Discussion

In this study, differences in the levels of Hx·x, ATP and LDH were noted to exist between different types of Hb in the red cells of Finnish Landrace sheep. But our data could not be compared directly with those of other workers, since no reports are available on the levels of these substances in the red cells of different Hb types of sheep or other domestic animals.

As for plasma Hx·x levels in different Hb types, those of Hb A sheep were expected to exceed those of Hb B sheep, since an increase in Hx·x concentration in plasma was found to be related to decreased oxygen content in tissues19,20), and oxygen binding with Hb A is regarded as less release to tissues than binding with Hb B, due to the fact that Hb A has higher affinity for oxygen than Hb B in sheep7). But there was no significant differences in Hx·x levels in plasma between different Hb types. Hb A sheep only had slightly more Hx·x levels than Hb B and Hb AB sheep. Though this may indicate possibly that the oxygen content of Hb A sheep in tissues is lower than that of Hb B sheep, we consider it impossible to estimate exactly oxygen levels in tissues from the levels of plasma Hx·x.

The levels of red cell Hx·x in Hb A sheep were significantly lower than those of Hb B sheep. Thus oxygen consumption in Hb A red cells may possibly exceed that in Hb B red cells in the purine metabolic pathway of the resolution of Hx·x into uric acid19). Thus, Hb A sheep were considered to possess more free oxygen not combining with Hb molecules than Hb B sheep. From this, it appears possible that purine metabolism is more active in the red cells of Hb A sheep than in those of Hb B sheep.

Regarding the oxyhemoglobin dissociation curve of sheep, it is confirmed by HUISMAN et al.7) and other workers21,22) that the curve of Hb A is considerably to the left that of Hb B in whole blood and Hb solution. The oxygen dissociation curve of Hb is generally known to be modified by organic phosphates as DPG and ATP23). In sheep, differences in these curves of Hb A and Hb B may partially be maintained by the specific levels of organic phosphate in the red cells of each Hb type under normal physiological conditions. The ATP levels in Hb A red cells were noted to be significantly lower than those in Hb B red cells in this study, though the DPG levels in adults were too low to determine exactly24). But it is not clear from this fact whether ATP has effect on Hb function as a modifier in sheep red cells, since BUNN et al.25) reported the bulk of red cell ATP to bind to magnesium ions. Thus, ATP levels have no effect on the mediating Hb function in human beings. In sheep, the functional significance of ATP to oxygen affinity has not been demonstrated, and no considerable information is available on matters related to ATP and Hb functions to understand their interrelationships.

The red cell LDH activity in Hb A sheep was found greater than that in Hb B sheep, though the relation among electrophoretic patterns of LDH isozymes among Hb types could not be determined. This difference in LDH activity has not been found in other animals. But, from the standpoint of genetic variation in LDH isozymes,
Powers et al. have demonstrated a physiological correlation between LDH genotype and Hb function in killifish. They reported that the ATP levels expressed as ATP/Hb molar ratios under genetic control to be quite closely correlated with LDH phenotypes, and LDH homozygotes ($LDH-B^a B^a$) with low ATP levels to have higher oxygen affinity than another homozygotes ($LDH-B^b B^b$) with high ATP levels. But they did not show differences in LDH activity between the different phenotypes of this enzyme. Though red cell LDH polymorphism was not observed in any sheep during this study, and the killifish differs in taxonomy from mammalia such as sheep, this phenomenon may be help to clarify the relation between LDH and Hb functions in sheep red cells. Such differences in killifish are thought to be very similar to those in sheep, when LDH phenotypes are replaced with Hb phenotypes, since Hb A sheep with low ATP levels have higher oxygen affinity than Hb B sheep with high ATP levels. Regarding the correlation between the levels of ATP and LDH, sheep with high LDH activity and low ATP values were considered to have high affinity of Hb for oxygen, as in the case of the Hb A type. But it was not clear whether this correlation signified a direct interrelation between them in glucose metabolism or a relation between them through oxygen affinity as in the case of killifish.

By this study, it was possible to obtain some information on the relationship among Hb phenotypes in terms of different oxygen affinities and the various metabolic systems as those of glucose and purine in sheep red cells. A more detailed investigation will be conducted on Hb types to determine interrelationships with various metabolic systems in red cells.

Acknowledgement

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References

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フィニッシュランドレース羊におけるヘモグロビン型別
赤血球内のヒポキサンチン・キサンチンおよび アデノシン三磷酸値ならびに乳酸脱水素酵素 活性値の差異

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めん羊のフィニッシュランドレース種を対象に赤血球内におけるヘモグロビン型とヒポキサンチン・キサンチン (Hx•x) 値、アデノシン三磷酸 (ATP) 値および乳酸脱水素酵素 (LDH) 活性値との関係について検討した。 赤血球内の Hx•x 値に関して、Hb A 型は Hb B 型よりも統計的に有意に低かった (P<0.05)。Hb A 型と Hb AB 型および Hb B 型と Hb AB 型との間の Hx•x 値ではそれぞれ有意な差がみられなかったが、それらの値は Hb B 型、Hb AB 型および Hb A 型の順に低かった。赤血球の Hx•x 値は、Hb 型間で有意な差を認めなかった血漿中の Hx•x 値との間において相関性がなかった。赤血球内の ATP 値では、Hb A 型および AB 型は Hb B 型よりも有意に低かった (P<0.01)。

しかし Hb A 型と Hb AB 型との差は統計的に有意ではなかった。従って Hb 型別による ATP 値の順位は Hb A 型≒Hb AB 型<Hb B 型であった。赤血球内の LDH については、Hb 型間における isozyme pattern の相異は認められなかった。その活性値では、Hb A 型は Hb B 型および AB 型に比べ有意に高かった (P<0.01)。しかし Hb AB 型は Hb B 型とは有意な差ではなかった。その順位は Hb A 型>Hb AB 型≒Hb B 型であった。また LDH 活性値は ATP 値との間で有意な負の相関関係にあった (r = −0.461, P < 0.01)。

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