Cattle Fetal Hemoglobins by Isoelectric focusing on
Polyacrylamide Gel

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INTRODUCTION

Abe et al.(1969) shown the disappearance and basis components(whose components are F₁ and F₂) of Fetal hemoglobins in cattle, by starch gel electrophoresis(SGE). Also, cattle adults hemoglobins were classified electrophoretically to the genetic variants by SGE, whose phenotypes are type Hb A, Hb B and Hb AB. In present study, Changes of cattle hemoglobins according to age were examined by isoelectric focusing on polyacrylamide gel(PAG-IEF).

MATERIALS and METHOD

Blood samples were obtained from Holstein breed, and used samples were only type Hb A(Table 1). The red cells was washed by centrifugation in 0.85% saline, and the electrophoretic samples were performed on 5% hemolysates. The hemoglobins in Holstein breed were separated by SGE, using buffer system of tris-EDTA-boric acid buffer at pH 8.7(Abe et al. 1969), and by PAG-IEF, using system at reform apart : changed from 1% ribofrabin to 50ug ammonum persulfate(Kotani et al. 1980). The pI of separated Hb bands were measured by micro pH meters, the density of those bands were measured by densitomeyters(wave length : 540nm)(Table 2).

Table 1. Number of samples

Breed	Days of age										
	1-10	11-20	21-30	31-40	41-50	51-60	61-70	71-80	81-90	91-100	101-
Hol. Breed	No. 2	3	2	1	2	3	4	3	2	3	2

Table 2. Electrophoretic conditions of isoelectric focusing on polyacrylamide gel.

a) The gel is prepared as follows:	
5.5% acrylamide-bisacrylamide solution	11.50ml
Saccharose	1.80g
Ampholine (pH6-8 range, 40%w/v, LKB)	0.85ml
Ammonum persulfate	50ug
DMAPN*	20ul
b) Electrode solution	
Anode	1% acetic acid
Cathode	1% ethylenediamine
c) Electrophoresis is performed as follows:	
The starting voltages is adjusted at 600volts, the voltage being increased to a maxmum of 1000 volts.	
Final focusing occurs after it about 6 hours.	
d) Gel size	
170 x 140 x 0.5 mm	
* 3-Dimethylaminopropionitrile	

RESULTS and DISCUSSION

In the electrophoretic changes in type Hb A according to days of age by PAG-IEF and SGE, the SGE change resembles the data reported by Abe et al.(1969). PAG-IEF revealed adults type Hb A composed of 7 bands(a, b, c, d, e, f and g) within the region from pH 7.79 to pH 7.32. However, type Hb A in age of 1 - 30 days was distinctly formed with 11 bands(a, c, e, f, j, k, l, m, n, o and p) over an extensive range from pH 7.79 to pH 6.81. Such electrophoretic changes with age suggested the bands of j, k, l, m, o and p to be the fetal hemoglobins, respectively. Furthermore, of these 6 fetal bands, the o(pI

a)

(+)

b)

(+)

Fig.1. Electrophoretic patterns in cattle hemoglobins after birth by isoelectric focusing on polyacrylamide gel (a) and starch gel electrophoresis(b).

6.87) band was presumed to be the main band in fetal hemoglobins, and the c(pI 7.61) and e(pI 7.40) bands seemed to be the main bands in adults hemoglobin. The large difference in pI of main bands between the adult and fetal hemoglobins was assumed to provide marked specificity to constitutive amino acids and their physiologic functions(Figure 1).

In the quantitative changes in the fetal and adult hemoglobins with ages, although the fetal hemoglobins has been

Fig.2. Disappeared process of isolated cattle hemoglobin bands by isoelectric focusing on polyacrylamide gel.(Densitometers : wave length is 540nm)

reported to completely disappear in approximately one to one and half months on an SGE pattern, PAG-IEF showed the presence of these isolated bands in hemoglobins of 60 - 70 days. Disappearance of these isolated bands, though differently in quantity, formed the following order : (l,m,n) $\rightarrow$ (k) $\rightarrow$ (p) $\rightarrow$ (j) $\rightarrow$ (o). The F and F components of fetal hemoglobins isolated by SGE were considered to correspond to o band(F₁) and k,j bands(F₂) of PAG-IEF. On the contrary, the amount of basic constitutive bands in an adult hemoglobin increased with age. Especially the b(pI 7.63), d(pI 7.44) and h(pI 7.32) bands, not existing in 1 - 10 days of age, appeared thereafter. The above findings suggested that the production of a, c, e and f bands which are considered to be the most basic bands in an adult hemoglobin starts from the later term embryonic stage in the order of (e) $\rightarrow$ (a,c) $\rightarrow$ (f)(Figure 2).

REFERENCE

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