Genetic Control of Chicken Plasma Alkaline Phosphatase Isozymes and the Contribution of Isozymes to Enzymic Activity

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A large number of studies on alkaline phosphatase (AP) of chicken plasma have been made during the past forty years. Most of those studies are on the enzymic activity and the polymorphic studies are relatively few.

In most of the polymorphic studies of chicken plasma AP, a starch gel electrophoresis has been employed. Major results by other investigators[1)2)] may be summarized as follows. AP comprises two isozymes and they are classified into two types: the one is the F type having a faster moving band in zymogram, the other is the S type having a slower moving band. The gene controlling the F type is completely dominant to the one controlling the S type.

By the neuraminidase treatment the F type can be converted to the S type, while the S type is not affected by the same treatment.[3)] This means that the enzyme of the F type has sialic acid, while that of the S type has not sialic acid.

The mean level in the enzymic activity of the F type is higher than that of the S type.[2)]

There are two conflicting arguments[4)5)] on the association of chicken AP isozyme type and egg production: first, that there is no direct association between them, and second, that S type is superior in egg production to the F type.

Above-mentioned results imply three points to be elucidated as follows.

Firstly, it is not clear yet how many isozymes chicken plasma AP is composed of. Because, in the result[6)] from our laboratory, while the zymogram of the S type had only one band by the starch gel electrophoresis, that of the F type showed distinctly two bands by the same procedure. The result suggests that if the adequate electrophoresis other than starch gel be used, the S type as well as the F type may have two bands or more. If the chicken plasma AP be composed of more than two isozymes, their genetic control should be elucidated.

Secondarily, activity difference between isozyme types has been noted only by a comparison of mean level while the details of distribution of AP activity have not been elucidated. And if possible, it is necessary to elucidate the biological meaning of the activity difference.

Thirdly, it is not clear which argument is correct on the association of isozyme type and egg production.

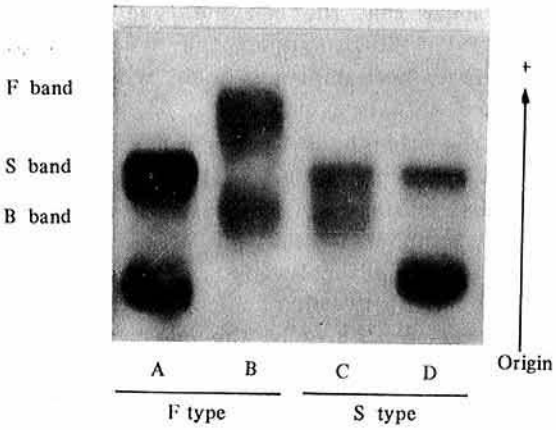
The purpose of the present study is to resolve the above three points.

Characterization and genetic control of AP isozymes[6)7)8)9)]

Horizontal polyacrylamide gel electrophoresis was used in the present study.

Chicken plasma AP comprised three isozymes. Zymogram of an individual chicken plasma had two bands, either the faster (F) or the slower (S) moving band by isozyme types and the B band having the same mobility irrespective of the isozyme types.

A migration rate of the B band, which was



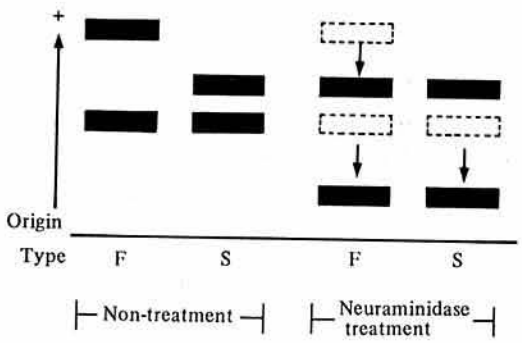
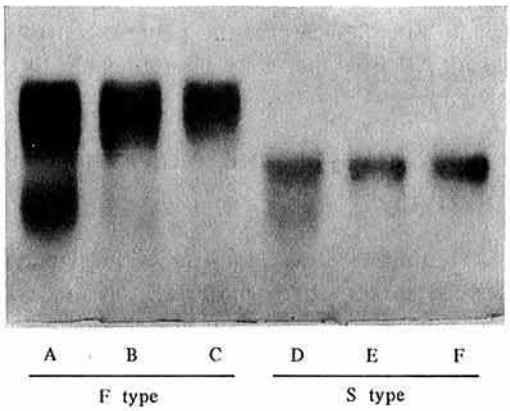


Fig. 1. Alteration of the mobility of the alkaline phosphatase isozymes in chicken plasma treated with neuraminidase. Samples B and C were untreated; samples A and D were treated with neuraminidase.

found by polyacrylamide gel, was retarded closer to the origin irrespective of the isozyme types by neuraminidase treatment, while the behaviour of the F and S bands was in accord with the result[3] observed by starch gel by the same treatment (Fig. 1). The result means that attachment of sialic acid to the enzyme of the F and S bands is due to the isozyme types, while the enzyme of B band of either type has sialic acid. Therefore, the genetic mechanism which attaches sialic acid to the F (or S) band and the B band seems to be different.

The B band was labile to urea (4M) and heat (60°C, 10 min) treatments, while the F and S bands were stable to the same treatments (Fig. 2).



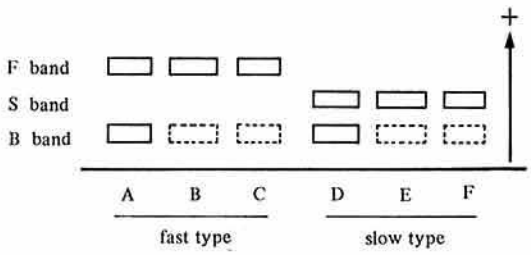


Fig. 2. Effects of urea treatment (4M urea, 37℃, 15 min) and heat treatment (60℃, 10 min) on the pattern of alkaline phospha tase isozyme from chicken plasma. Samples A and D: untreated, B and E: urea treatment, C and F: heat treatment. ——: stable; ·····: labile.

From the results of the neuraminidase, urea and heat treatments and breeding experiments, the genetic control of these bands was suggested as follows: (1) the F (or S) band and the B band are controlled by the different genes located at the different loci, (2) the gene controlling the F band is completely dominant to the one controlling the S band, (3) the B band is controlled by a single gene irrespective of isozyme types. The genetic difference in the mobility of both types obtained by starch gel is only applicable to the F or S band. Therefore, the F and S bands are corresponding to the two isozymes detected by starch gel.

Also, chicken duodenum AP comprised three isozymes and showed the same behaviour by

neuraminidase, urea and heat treatments as plasma AP. Therefore, the genetic control of plasma AP isozymes may be applicable to the duodenum AP isozymes.

Isozyme type and enzymic activity[10)11)12)]

Variation of chicken plasma AP activity was ascribed to the three major factors: isozyme type, family and sex. The first was the most important, accounting for 53% of the total variance. The residual family and sex were accounting for 9 and 5%, respectively. Eliminating the effects of family and sex, most of AP activity of the individuals with the F type showed distinctly higher value than that with the S type (Table 1). From these results, it is clear that the isozyme type exerts a powerful influence upon the AP activity. Therefore, heritability estimate will be confounded with isozyme types.

On the other hand, within the F type, the family difference in AP activity was observed. The distribution of AP activity in both types was continuous and thus it was considered to be presumably polygenic. It can be understood genetically that chicken plasma AP activity is controlled by the major gene controlling isozyme type and the quantitative gene, i.e., polygene. From our data, the heritability of AP activity within the F type was estimated to be 0.620. This estimate was higher than the values (0.04—0.36) reported by other workers. This high value may have been due to the elimination of the effect of

Table. 1. Examples of full-sib chick showing distinct differences between isozyme types in plasma alkaline phosphatase activity at 32 days of age by Kind-King method.

Genotpe		Dam No.	Off-spring No.	Sex	Iso-zyme type	Unit*	Dam No.	Off-spring No.	Sex	Iso-zyme type	Unit	Dam No.	Off-spring No.	Sex	Iso-zyme type	Unit
sire	dam															
S/S	F/S	1	1	♂	F	202.5	3	1	♂	F	139.0		4	♀	S	54.0
			2	♂	F	185.0		2	♂	F	124.0	5	1	♂	F	102.5
			3	♂	S	67.5		3	♂	S	60.0		2	♂	F	95.0
			4	♀	F	155.0		4	♂	S	70.0		3	♂	S	62.5
			5	♀	S	42.5		5	♀	F	83.5		4	♀	F	132.5
			6	♀	S	69.0		6	♀	S	45.0		5	♀	F	189.0
		2	1	♂	F	205.0	4	1	♀	F	197.5		6	♀	F	65.0
			2	♂	F	165.0		2	♀	F	97.5		7	♀	S	41.0
			3	♂	S	85.0		3	♀	F	77.5					
F/S	S/S	1	1	♂	F	125.0	3	1	♂	F	105.0	5	1	♂	F	270.0
			2	♂	S	72.5		2	♂	S	94.0		2	♂	F	220.0
			3	♂	S	69.0		3	♀	F	67.5		3	♂	F	212.5
			4	♂	S	59.0		4	♀	S	45.0		4	♂	S	65.0
			5	♀	F	82.5		5	♀	S	49.0	6	1	♂	F	167.5
			6	♀	F	82.5		6	♀	S	45.0		2	♂	F	140.0
			7	♀	S	35.0	4	1	♂	F	167.5		3	♂	S	65.0
			8	♀	S	40.0		2	♂	F	247.0		4	♂	S	65.0
		2	1	♂	F	137.5		3	♂	S	74.0		5	♂	S	45.0
			2	♂	F	89.0		4	♀	F	125.0		6	♂	S	62.5
			3	♂	S	57.5		5	♀	S	43.5		7	♀	F	81.0
			4	♂	S	55.0							8	♀	S	45.0
			5	♂	S	52.0							9	♀	S	46.5

* mg phenol per 100 ml per 15 min.

isozyme types.

A similar phenomenon was recognized also in chicken plasma leucine aminopeptidase (LAP) activity. Both AP and LAP activity differences between isozyme types might be related to the attachment of sialic acid to the enzyme molecules, because their mobility differences between isozyme types are due to the presence or the absence of the attachment of sialic acid.

The total AP activity of the F or S type was little affected by 4M urea treatment in spite of the unstableness of the B band. It is considered that the B band inactivated by urea restores the activity when the urea concentration was reduced. The AP activity was reduced by the heat (60°C, 10 min) treatment. The reduction may be primarily due to the loss of the activity of the B band.

Biological meaning of activity difference[7)9)11)]

The objective of this study was to determine the cause of the observed higher activity for the F type in the young chicken.

In the present study, the difference in AP activity of the young chicken between isozyme types was found to be due to the different activities of the F and S bands, but not due to the activity of the B band of either type. These enzymes of the F and S bands differed in the attachment of sialic acid to the enzyme protein. However, by the neuraminidase treatment, no change of AP activity was found in spite of the release of sialic acid. On the other hand, Chang & Moog[13)14)] found that the purified F_1 and F_2 enzymes in pooled chicken duodenum AP were indistinguishable in Michaelis constant (Km) and optimal pH. These F_1 and F_2 bands of chicken duodenum can be considered to be identical with the F and S bands, respectively, of the chicken plasma, because both agree in the apparent migration rate and in the effect of the neuraminidase treatment. From these result, the activity difference between F and S bands may be considered to be due to the different amount of the enzyme present, but not due to the differences in enzyme characterization.

The different amount of the enzyme present might be due to the differences in the release rate of AP into the plasma, or in the survival time of the enzymes in circulation, and/or in their combinations. The first hypothesis is on the basis of the assertion of Eylar[15)] that the carbohydrate might serve as a passport for transporting glycoprotein from the cell. Therefore, the enzyme protein of the F band might be easily released by the aid of sialic acid into the plasma. A second hypothesis is proposed from the facts that the proteins having sialic acid *in vitro* were effectively bound to the liver cell membrane by the neuraminidase treatment[16)] and that the biological half-life of the protein having sialic acid was shortened by the same treatment.[17)] According to this hypothesis, the survival time of the enzymes of the S band might be shorter in circulation than that of the F band because of the absence of sialic acid.

Further studies are necessary to establish which of these two hypotheses may explain this difference in activity. It appears difficult to prove the first of the two hypotheses, because the albumin, which has no carbohydrate, is released abundantly into the plasma.

Isozyme type and egg production[18)]

There have been two viewpoints on the association of chicken plasma AP isozyme type and egg production. Firstly, on the basis of considerable variation in the gene frequencies of F type found among 14 commercial egg production strains, Engh and Wilcox[4)] suggested that there was no direct association between isozyme type and egg production. Secondarily, Csuka and Petrovsky[5)] reported that the phenotypic frequencies of S type were higher than those of F type in two groups of chickens selected for higher egg production. They also noted that individual with S type had significantly higher egg yield than

Table 2. Paired full-sib comparisons of egg numbers between isozyme types in a White Plymouth Rock strain

Year hatched	Test age (day)	Genotype		Nunmber of dams	Egg number		t	p
		Sire	Dam		FS type	SS type		
1974	338	S/S	F/S	13	131.4±3.3(36)*	130.3±4.4(14)	0.761	ns
1975	406	S/S	F/S	21	165.4±3.0(47)	159.6±4.4(35)	1.974	ns

* Mean ± standard error; figures in brackets indicate the number of birds used.

those with F type.

A limitation in the experiment of Engh and Wilcox is that the gene frequencies of F type in the initial populations are not specified. The gene frequencies of the selected lines might have been affected by those in the initial populations. In the experiment of Csuka and Petrovsky, the difference in egg production was determined by a simple comparison of the mean levels for the isozyme types, and therefore, might have been disturbed by family effect.

The present study was conducted to clarify which of these two viewpoints is correct by using a paired comparison between isozyme types within dam families to eliminate the family effect.

No significant difference in egg numbers was found between isozyme types even in the paired comparison eliminating the effect of family as shown in Table 2. No characteristic trend between genotypes was observed in monthly percent egg production. Therefore, the present result supports the conclusion of Engh and Wilcox that AP isozyme type is not directly related to egg production. Also, this is supported by the results that the Michaelis constant (Km) and pH of chicken AP isozymes have been found to be indistinguishable.[13][14] However, further studies are necessary to establish the relationship between AP activity and egg production, because AP activity is considered to be controlled by isozyme type and polygenes, and furthermore, recent investigations[10] in humans, quail and so on suggest that AP may act *in vitro* as inorganic pyrophosphatase.

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