Properties of Caucasian Type Red Cell Acid Phosphatase Phenotypes CA and CB Found in Japanese

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Properties of Caucasian Type Red Cell Acid Phosphatase Phenotypes CA and CB Found in Japanese

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Abstract. Properties of very rare phenotypes of red cell acid phosphatase in Japanese, CA and CB, were examined and compared with those of phenotypes A, BA and B. The relative activities of enzyme assayed by using p-nitrophenyl phosphate or 4-methyl-umbelliferyl phosphate were in the order of A<BA<B<CA<CB. The thermal stabilities examined at 48°C and 52°C were also in the order of A<BA<B<CA<CB. The phenotypes CA and CB were more stable at pH 4.5-5.0 and more labile at pH 7.0-8.3 than phenotypes A, BA and B. The pH optima of phenotypes CA and CB were slightly acidic than those of phenotypes A, BA and B.

Key words : Polymorphism, Acid phosphatase, Erythrocytes, Phenotypes CA and CB, Thermal stability, pH optimum

Introduction

Polymorphism of red cell acid phosphatase (AcP) is often applied to personal identification or paternity diagnosis in forensic medicine. The polymorphism of the AcP comprising six phenotypes, A, BA, B, CA, CB and C are determined by three autosomal codomonant alleles, P^a, P^b and P^c. The phenotypes CA, CB and C are however, very rarely found in Japanese[1]. Only a few reports have so far been published on the properties of these phenotypes found in Japan. Previous reports pointed out that the CA and CB in Japanese exhibited much weaker enzyme activities than those of Caucasian, although they showed the same migration behaviors of electrophoresis as those of Caucasian[2)~4)]. That is, the enzyme activities were in the order of CA<CB<A<BA<B in Japanese, whereas in the order of A<BA<B~CA<CB<C in Caucasian[5)].

Recently samples of CA and CB were found in two Japanse living in Hamamatsu, the enzyme activities of which were similar to those of Caucasian[6)]. This report summarizes the enzyme activities, thermal and pH stabilities of these rare phenotypes.

Materials and Methods

Sample

Samples of a CA, CB and B were collected from clients of a paternity diagnosis carried out by this laboratory of legal medicine. Other samples of A, BA and B were collected from clients of other paternity diagnosis and from donored bloods of Hamamatsu Red Cross Blood Center. The number of samples examined was 5 for A, 5 for BA, 5 for B, 1 for CA and 1 for CB. After removal of plasma red cells were washed three times with 0.9% saline and kept in a freezer at −20°C until used. The hemolysates were prepared so as to contain approximately 1 g hemoglobin/100 ml of water and used throughout following experiments. The hemoglobin concentration was determined by hemoglobincyanide method[7)].

Isoelectric focusing

The type of AcP was determined by isoelectric focusing on polyacrylamide gel[8)] or on cellulose acetate membrane[9)]. Cellulose acetate membrane, Separax EF (Fuji Film), was soaked in the solution containing 1 ml Ampholine (LKB) pH 5-8 and 1 g sucrose per 20 ml distilled water. The electrode paper strips were moistened with 1 M phosphoric acid for anode and with 1 M sodium hydroxide for cathode. Samples were applied directly onto the membrane at the position 3 cm from the anode. Electrofocusing was conducted at a constant voltage of 800 V for 50 min at 10°C. Then the membrane was covered with the filter paper soaked with 0.02% 4-methyl-umbelliferyl phosphate (MUP) in 0.05 M citrate buffer (pH 6). They were

incubated for 10 min at 37°C. The isozyme bands were visualized under long wave ultraviolet light.

Enzyme assay

The activity of AcP was assayed by the method of Hopkinson et al. using 0.02 M *p*-nitrophenyl phosphate (PNPP) in 0.05 M citrate buffer, pH 6.0[6]. A mixture of 0.5 ml substrate solution and 0.4 ml of water was preheated up to 37°C and the reaction was started by adding 0.1 ml of the hemolysate. The mixture was incubated for 30 min at 37°C and the reaction was stopped by the addition of 2 ml 10% trichloroacetic acid (TCA). After centrifugation, 1 ml of the supernatant was pipetted into a clean tube and 4 ml of 0.5 N NaOH was added. The extinction at 415 nm was measured by a Shimadzu UV-200S spectophotometer. A blank was treated in a similar manner except that the hemolysate was added after the mixture of TCA. Enzyme activites of the hemolysate were expressed as μ moles *p*-nitrophenol liberated in 30 min/g hemoglobin.

The relative activity of AcP was also assayed fluorometrically using MUP as substrate. A 3 ml assay mixture containing 1 mM MUP and 1 mM EDTA in 0.1 M citrate buffer, pH 5.5 was incubated for 5 min at 37°C. The reaction was initiated by adding 25 μl of the hemolysate to the reaction mixture. As a reference sample, 25 μl of water was added instead of hemolysate. The increase in fluorescence at 455 nm (excitation at 350 nm) was read after 15, 30, 45 and 60 min with a fluorescence spectrophotometer (Hitachi 650-10S). The enzyme activities were considered to be proportional to the net increments of fluorescence intensity in 15 min/g hemoglobin. Therefore the enzyme activities were expressed as relative ratios of the increments by assuming the activity of phenotype A as 1.0.

Thermal stability

0.1 ml of hemolysate and 0.4 ml of citrate buffer (0.1 M, pH 6.0) were pipetted into several test tubes and heated in a oil bath at 48°C and 52°C. After the incubation of various periods of time (0 to 50 min), tubes were cooled in iced water. The remaining activities for each test tube were measured using PNPP as substrate. The stability was expressed as a relative activity to the same hemolysate prior to heating.

pH stability

0.1 ml of hemolysate was pipetted into several test tubes. 40 μl of 0.2 M citrate buffers, the pH of which were ranging from 2.0 to 8.5, were added into different test tubes. The resultant pH were ranging from pH 2.5 to 8.3 Samples were held at various pH at 4°C for 20 h or at 37°C for 3 h. After pH treatment, pH of the solutions were neutralized to pH 6.0 by adding either 0.4 ml of 0.2 M citrate buffer pH 6.0 to the samples treated at pH 2.5-6.0, or 0.4 ml of 0.2 M citrate buffer pH 5.5 to the samples treated at pH 6.5-8.3. The remaining activities were measured by adding 0.5 ml of 0.02 M PNPP in 0.05 M citrate buffer pH 6.0. The stability was expressed as the relative activity to the activity of the sample treated at pH 6.0.

pH optimum

The activities of five phenotypes were measured at pH values in the range 4.0-7.0. 0.5 ml of 0.02 M PNPP in water was mixed with 0.4 ml of 0.2 M citrate buffer ranging from pH 4.0 to 7.0 and preheated up to 37°C. The reaction was strated by adding 0.1 ml of hemolysate. The activity was given as a relative activity to the activity of the same sample treated at pH 6.0.

Results and Discussion

It was noticed from the inspection of the cellulose acetate membrane that the activities CA and CB were not weaker than those of A, BA and B, as indicated by the intensity of fluorescence bands in Fig. 1. The result was confirmed by assaying the enzyme activities in whole hemolysates. That is,

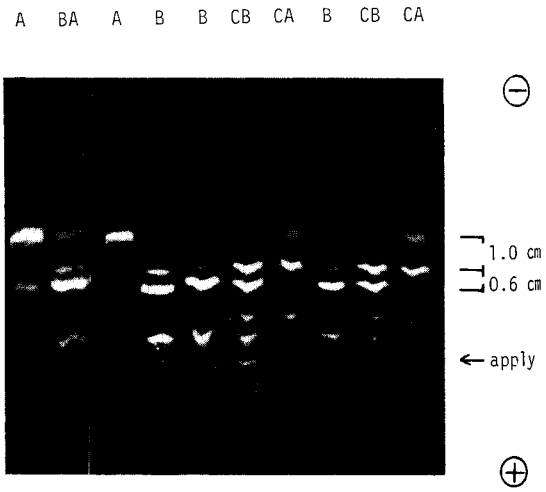

Fig. 1. Isoelectric focusing patterns on cellulose acetate membrane of red cell acid phosphatase.

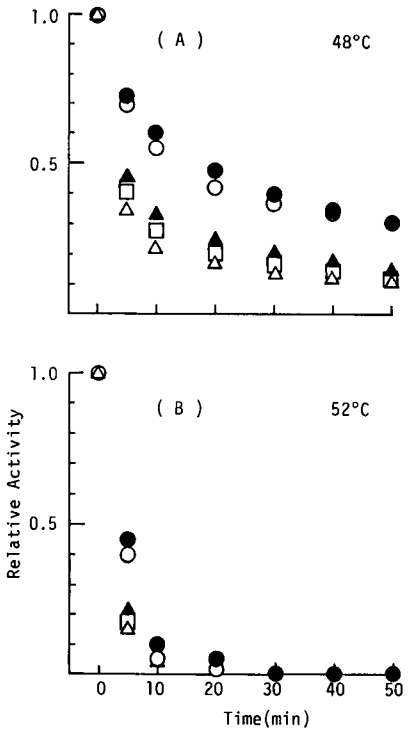

Fig. 2. Thermal stability of red cell acid phosphatase of five phenotypes.
The samples were held at (A) 48°C and (B) 52°C for various periods of time. △. A; □ BA; ▲, B; ○, CA; ●, CB.

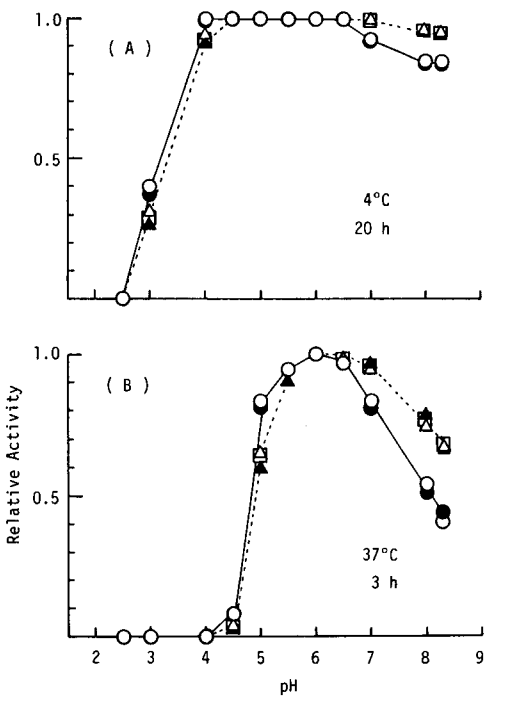

Fig. 3. pH stability of red cell acid phosphatase of five phenotypes.
The samples were held at (A) 4°C for 20 h and (B) 37°C for 3 h. The symbols distinguishing five phenotypes are the same as those in Fig. 2.

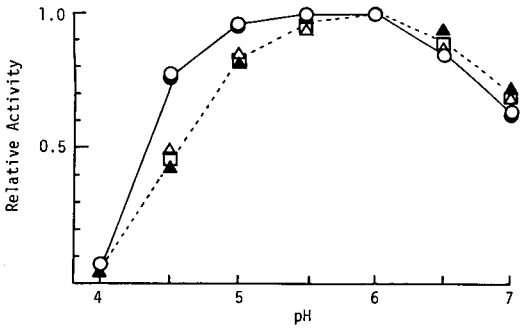

Fig. 4. pH optimum of red cell acid phosphatase of five phenotypes.
The symbols distinguishing five phenotypes are the same as those in Fig. 2.

the activities expressed as μ moles p-nitophenol liberated in 30 min at 37°C per gram hemoglobin were 104 for A, 144 for BA, 175 for B, 198 for CA and 240 for CB. The standard deviations were about 10%. Although only one sample was available for each CA and CB, their activities were almost the same as the activities of those for Caucasians[5].

The relative activities measured by fluorescence of 4-methyl-umbelliferone liberated were 1.00:1.38:1.75:2.11:2.65 for A, BA, B, CA and CB, respectively.

Thermal stabilities of five phenotypes were examined at 48°C and 52°C. The remaining activities of five phenotypes after heating for various times were shown in Fig. 2. The stabilities of five phenotypes were in the order of A<BA<B<CA<CB, although their differences were relatively small. These tendencies were the same as those reported for Caucasians[10].

The comparison of pH stabilities at 4°C and 37°C were shown in Fig. 3 (A) and (B), respectively. All phenotypes at 4°C were stable between pH 4.5 and 6.5, giving the same activities as those kept in a freezer. CA and CB were appreciably more resistant at pH 3.0 whereas they were more labile at pH 8.0 than A, BA and B. At 37°C, the activities of all samples were decreased, although the activities at pH 6.0 was taken as the standard

for each phenotype. As in the cases at 4°C, CA and CB were more resistant at pH 4.5-5.0 whereas they were more labile at pH 7.0-8.3 than A, BA and B.

The pH activity profiles shown in Fig. 4 indicated that pH optima for CA and CB were between 5.5 and 6.0 and those for A, BA and B were around 6.0.

In conclusion, the phenotype CA and CB found in Hamamatsu had quite similar enzyme activities and thermal stabilities as those found in Caucasians. The CA and CB were more stable at acidic pH and more labile at alkaline pH than A, BA and B.

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日本人に見出された白人型赤血球酸性フォスファターゼ CA型およびCB型の性質

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摘要 日本人には極めて稀な赤血球酸性フオスファターゼのCA型およびCB型の性質について，他のA，BA，B型と比較検討した．*p*-ニトロフエニルリン酸および4—メチルウンベリフエリルリン酸を基質として酵素活性を調べたところ，A<BA<B<CA<CBの順で，CA，CBの活性は強かつた．又，48℃および52℃で，熱安定性を比較したところ，同様にA<BA<B<CA<CBの順であつた．pHに対する安定性は，CA，CBはA，BA，Bに比べてpH 4.5—5.0付近で高く，pH 7.0—8.3付近で低いという結果であつた．至適pHはCA，CBがその他のものより，ほんのわずか酸性側によつていた．