CHROM. 5998

ASSESSMENT OF ELECTROPHORETIC MOBILITIES OF SOME HUMAN ISOAMYLASES

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SUMMARY

Amylase isoenzymes, which are found in human serum and in the fluid from ovarian cysts, were separated by electrophoresis on agar gel. Electrophoretic mobilities in a free buffer solution were calculated for these isoamylases.

INTRODUCTION

α-Amylase (α-1,4-glucan-4-glucanohydrolase, EC 3.2.1.1), like other enzymes, occurs in several molecular forms. In the human organism amylase is produced mainly by the salivary glands and by the pancreas. (Serum amylases are hence derived[1]). Amylase is also synthesized by the intestinal mucosa[2] and also by the oviduct[3] in pathological conditions. Each tissue mentioned produces two or more isoenzymes of amylase. The properties of isoamylases are so similar that for a long time it was assumed that only one substance was involved. One of the few physicochemical properties by which isoamylases differ is their mobility in a free buffer. This paper attempts to calculate the electrophoretic mobilities of some isoamylases which are found in serum and in fluids from ovarian cysts.

EXPERIMENTAL

Materials and method

Specimens of human serum and fluid from ovarian cysts were analyzed. To assess the electro-osmotic flow a 0.1% solution of soluble starch was used.

Individual specimens were separated by electrophoresis on agar gel using the method described by KAMARÝT: 3 ml 1% agar gel in veronal buffer pH 8.4 (i = 0.05) was applied to 25 × 75 mm microscope slides. After the solution had hardened two 1 × 10 mm slits were made in it, 1.5 cm from the anodic end of the slide. The serum specimens, or specimens of the fluid from ovarian cysts, were mixed with 2% agar at a ratio of 1:1 and transferred into the slits. The separation was performed in veronal buffer pH 8.4 (I = 0.05) at a potential gradient of 4.5 V cm for 150 min

After separation the agar plate was covered with an agar-starch plate (containing 1% agar, 1% starch, hydrolyzed according to SMITHIES, and 0.05% NaCl). The plates were incubated for 1 h at 37°. The agar-starch plate was then removed and the lower agar layer was stained for 1 min in Lugol solution. The agar plate was then rinsed in distilled water and left in a wet chamber for one day, when the results were evaluated. Light strips on a dark background were found at the sites where isoamylases were present.

Serum was analyzed when it was fresh and undiluted. Fluids from ovarian cysts were either diluted to values corresponding to the amylase level of the serum or they were first lyophilized and 5 mg lyophilizate were then dissolved in 0.15 ml saline solution. This solution was then treated in the same way as the serum. The starch specimen used as an indicator of electro-osmosis (0.1 ml of 0.1% solution of soluble starch) was applied beside the specimens of serum which were separated by electrophoresis on agar gel.

After electrophoretic separation some serum specimens were stained to detect protein so that some corrections mentioned below could be made. The serum proteins were first denatured in 3% acetic acid. They were then stained in an Amide Black (0.05%) solution in methanol–water (1:1).

RESULTS AND DISCUSSION

A maximum of four isoenzymes of amylase were found in human serum (two salivary and two pancreatic), which formed five combinations. Their percentage distribution in the population and their heredity were discussed in a previous paper[5]. The combinations of isoamylases in human serum are shown in Fig. 1.

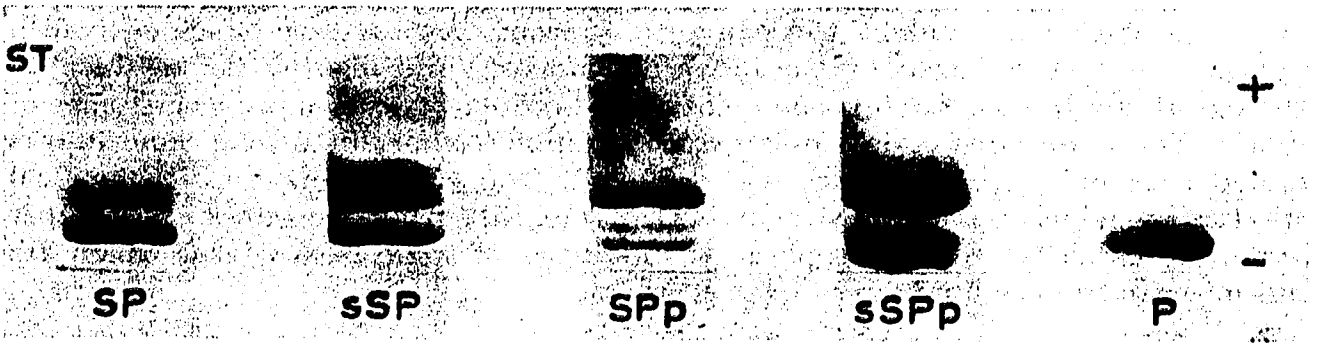

Fig. 1. Combinations of isoamylases in human serum. ST, start; SP, one salivary and one pancreatic amylase; sSP, two salivary and one pancreatic amylases; SPp, one salivary and two pancreatic amylases; sSPp, two salivary and two pancreatic amylases; P, pancreatic amylase.

In fluids from ovarian cysts two, one or no isoamylases were found. The combinations of isoamylases in fluids from ovarian cysts are given in Fig. 2.

A typical property of proteins (at a given pH) is their electrophoretic mobility in a free buffer, *i.e.* the rate at which these substances move in a solution at a given potential gradient. When using electrophoresis in a stabilizing medium, *e.g.* agar, it is important to take the influence of this medium into account in calculating the mobility. The methods for correcting for the influence of the stabilizing medium are described, *e.g.*, by MICHALEC *et al.*[6].

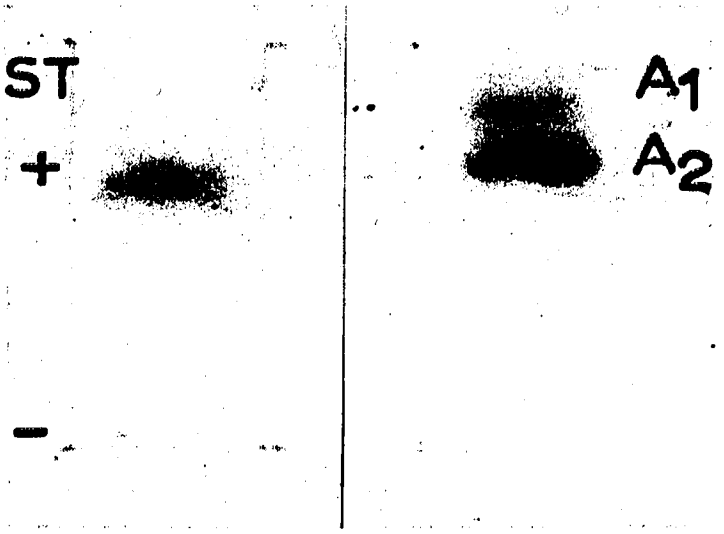

Fig. 2. Isoamylases (A_1 and A_2) in fluids from ovarian cysts. ST = start.

When calculating the mobilities of isoamylases corrections were made for the electro-osmotic flow and the influence of agar as a medium.

The electro-osmotic flow was assessed by applying, in addition to the serum, samples containing various combinations of isoamylases and starch (*i.e.* a substance carrying no electric charge). After electrophoretic separation was complete, according to the electropherograms, a reading was taken of the distance x traversed by the different isoamylases and also of the distance x_{os} covered by the starch due to electro-osmotic flow. The isoamylases would move the same distance under these identical conditions due to electro-osmotic flow. The corrected distance (the distance covered by the isoamylases due to the electric field only) was therefore found by subtracting the distance x_{os} from the assessed distance x. The corrected distance, x_{cor}, was obtained where $x_{cor} = x - x_{os}$. In the calculation x and x_{os} were measured in the direction in which the substances moved; the sign of x_{cor} therefore also gives the direction in which the isoamylases move at a given pH. However, the electro-osmotic flow is in the reverse direction as compared with that of isoamylases in an electric field under the given conditions, and the difference is considerable. This flow causes the isoamylases to move in the reverse order than the magnitude of their actual mobilities during electrophoresis on agar gel would suggest. This is apparent from Fig. 3.

In addition to electro-osmosis, the influence of agar on the retardation of the isoamylase movement must be taken into account. A correction factor which allows for the movement of isoamylases in this medium in relation to the mobility of the same isoamylases in free buffer (at a given pH) must be applied. This factor would include, for example, a correction for the longer pathway in agar in relation to the pathway in free buffer.

For calculating the mobility of a substance in free buffer the following relation applies:

$$u = \frac{x_{cor}}{Et} \cdot f$$

where x is the distance (corrected for electro-osmosis) covered by the substance in time t at a potential gradient of E; f is the correction factor for the medium.

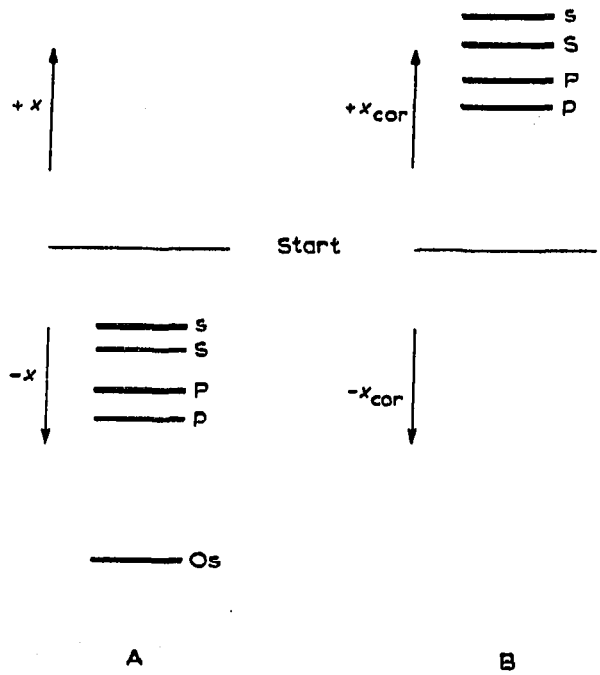

Fig. 3. Schematic presentation of the localization of isoamylases on the electropherogram. A, without corrections on electro-osmosis (x); B, after corrections on electro-osmosis (x_{cor}); s and S, salivary isoamylases; p and P, pancreatic isoamylases.

It was advantageous to apply a comparative substance of known mobility in free buffer simultaneously with the isoamylases to the agar plate. The mobility u_0 of this comparative substance is, by analogy,

$$u_0 = \frac{(x_{cor})_0}{Et} \cdot f$$

If we assume that the correction factor f is practically the same for all substances then we can derive the simple relationship from the above equation

$$u = u_0 \cdot \frac{x_{cor}}{(x_{cor})_0}$$

β-Globulin was selected as the initial comparative substance. A mobility of $u_0 = 2.8 \times 10^{-5}$ cm^2 sec^{-1} V^{-1} is reported in the literature[7] for β-globulin. Two specimens of the same serum were always separated by electrophoresis, together with the electro-osmosis indicator, *i.e.* starch. One of the serum specimens was stained to detect proteins and the second to detect amylases. Readings of the distances covered by serum proteins and isoamylases were taken from the electropherograms and were corrected for electro-osmosis. The mobilities of individual isoamylases were obtained and the results are shown in Table I.

To test the correctness of the above calculation the electrophoretic mobilities of plasma proteins were also calculated. The values obtained were compared with data reported in the literature[7]. The calculated values were found to be in agreement with these data. A schematic illustration of the mobilities of plasma proteins (our data and those reported in the literature) and of isoamylases is presented in Fig. 4.

Agar electrophoresis proved to be useful for separating isoenzymes of amylase

TABLE I

MOBILITIES OF ISOAMYLASES OF HUMAN SERUM AND FLUIDS FROM OVARIAN CYSTS, IN FREE BUFFER AT pH 8.4

Isoamylase	*Mobility* × 10^5 ($cm^2\ sec^{-1}\ V^{-1}$)
p	1.07
P	1.3
S	1.6
s	1.8
A_2	2.1
A_1	2.3

The considerable electro-osmotic flow in the reverse direction to the movement of isoamylases acts as a constant countercurrent and makes it possible to differentiate isoamylases with similar mobilities on a relatively short agar plate. By introducing corrections for electro-osmosis and the influence of agar used as the carrier medium it is possible to calculate the mobilities in free buffer, *i.e.* the mobilities of the above isoenzymes if they were separated by classical TISELIUS electrophoresis.

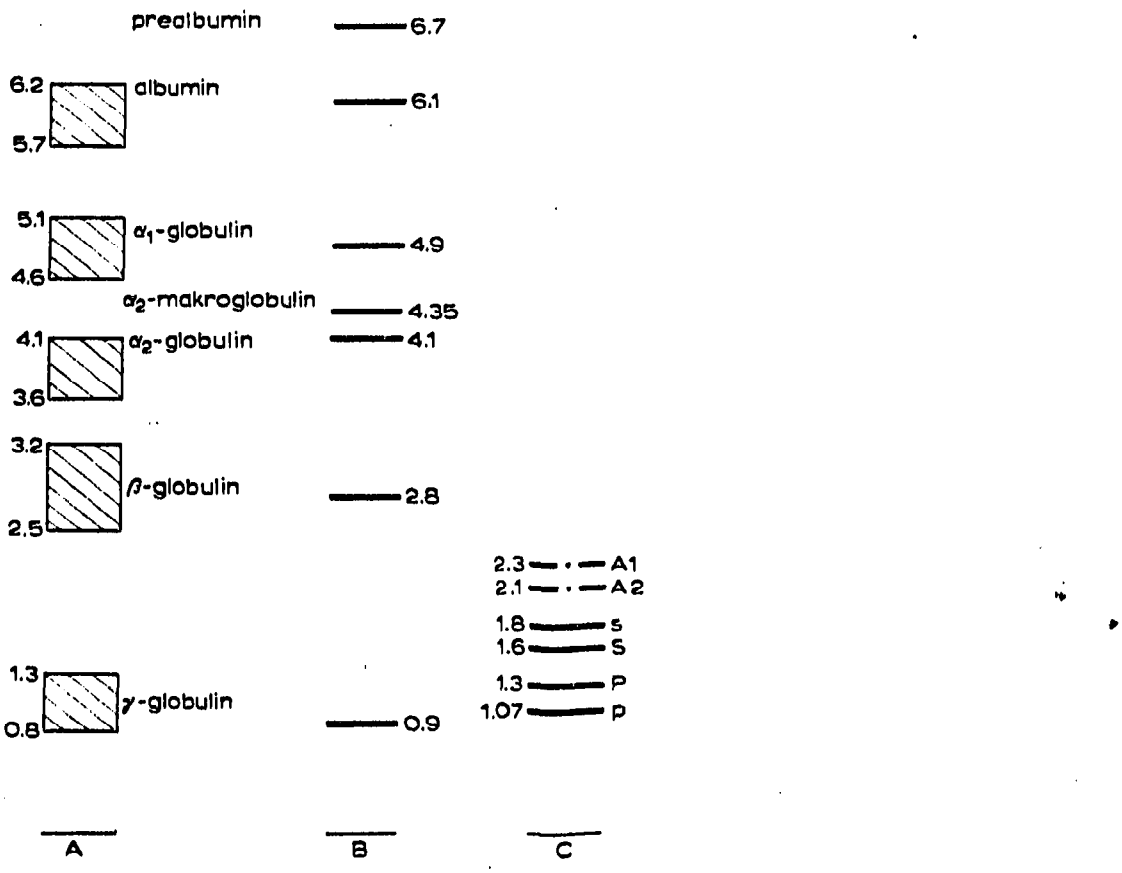

Fig. 4. Schematic presentation of mobilities (× 10^5) of plasma proteins (A, B) and isoamylases (C) in free buffer. A, data from the literature (at pH 8.6); B and C, calculated values (at pH 8.4). A_1 and A_2, isoamylases from ovarian cyst fluids; s and S, salivary amylases, p and P, pancreatic amylases from human serum.

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