Histochemical Journal, 9 (1977), 369–371

LETTER TO THE EDITOR

Quantitative histochemistry of esterases

Microspectrophotometry of enzyme reactions is a practical, fast and relatively easy method for studying enzyme activity *in situ*. The conditions of its applicability should be carefully studied for each of the quantitative techniques to be used. So far, they have only been established for a limited number of histo-enzymatic reactions. Using a method similar to one employed for studying the validity of acid phosphatase quantitation (Cabrini *et al.*, 1975), we have investigated a technique for demonstrating esterases in a histochemical model system.

Non-haemolized bovine serum, kept at −20°C, was used as the source of esterase. Its esterase activity (3650 IU) was determined with the method of Burlina & Galzigna (1972), which measures the activities of aryl esterase and aliesterase. A 0.25 M β-naphthyl acetate solution in a total volume of 2.5 ml, was used as substrate.

The histochemical models consisted of a solution of different serum concentrations incorporated in an acrylamide preparation in 0.1 M phosphatase buffer, pH 7.2. The films were prepared according to Van Duijn *et al.* (1967) and Van Duijn & Van der Ploeg (1972) by mixing acrylamide and bis-acrylamide with a solution of ammonium persulphate and TEMED, except that the acrylamide concentration was reduced to 7.5% in order to reduce the unspecific background staining usually produced when the films are introduced into the incubation solution. Using this concentration it is also probable that a higher substrate turnover is achieved in the models. The decrease in the polyacrylamide concentration did not, in our experience, produce an appreciable loss of incorporated enzyme (see below). The polymerization was performed at 4°C between two glass plates with a 240 ± 5 μm width spacer. Gomori's (1952) technique for demonstrating unspecific esterases was carried out on the polymerized films using α-naphthyl acetate as substrate and Fast Blue B as coupling agent. All the polyacrylamide films containing different concentrations of serum were processed simultaneously in order to avoid handling errors.

Microspectrophotometric measurements were performed in a Zeiss Cytoscan. The total magnification was x 14.3 and the wavelength 540 nm.

The microspectrophotometer can be used for measuring areas as small as 0.78 μm^2, which would amount to an assayed volume of 6.24 μm^3 if, for example, 8 μm-thick sections are used. With polyacrylamide models, the homogeneity of the reaction permitted the more comfortable measurement of larger circular areas (314 μm^2). The homogeneity of the reactions in the films was demonstrated by carrying out several measurements at different areas of each model. Using this procedure, the same optical density values were obtained.

Fig. 1 shows the optical density of the final reaction product as a function of the incubation time for each serum concentration investigated. These results indicate the validity of the microspectrophotometric quantitation of the esterase reaction. The

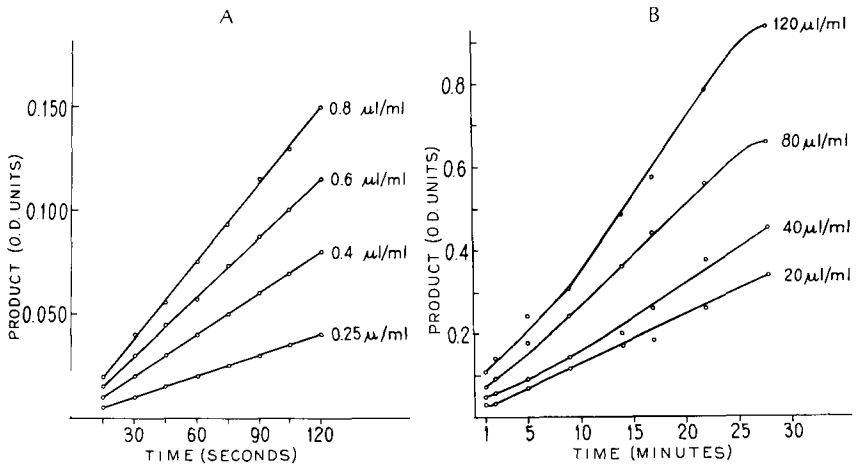

Figure 1(*A*) Variation of the product obtained (in optical density units) as a function of the incubation time. Esterase activity assayed by the method of Burlina & Galzigna (1972). Final serum concentration in a total volume of 2.5 ml. (*B*) Microspectrophotometric measurements (in optical density units) of the final reaction product as a function of the incubation time. Polyacrylamide model films containing different serum concentrations on which Gomori's histochemical technique has been carried out to demonstrate esterases.

biochemical method yielded a linear response with substrate concentration, whereas the histochemical method, under our experimental conditions, gave a linear response between 9 and 22 min. The larger slope obtained between 9 and 22 min as compared to the one registered between 1 and 9 min could be due to the difficulty the substrate may have in penetrating the tissue initially. These slope differences would, on the other hand, indicate that enzyme leakage is absent in our models. Incubation periods longer than 22 min caused saturation of the final product and gave rise to high optical densities, which cannot be measured accurately.

The reaction rates corresponding to different serum concentrations are shown in Fig. 2. In the biochemical method, the reaction rate was calculated as absorbance change during the first minute of reaction, at 313 nm. In the histochemical method, the absorbance change was calculated per minute at 540 nm, between the 9th and 22nd min corresponding to the linear part of the reaction. The extrapolated ordinate value at the origin of the linear region is larger than zero because of the unspecific background staining of the films, which also appears in the polyacrylamide controls without enzyme. The amount of serum necessary per ml of acrylamide was larger than the one used for the biochemical determination. This difference can be attributed, among other things to the path length of spectrophotometer curvette (1 cm), whereas the measured thickness of the polyacrylamide film is 40 times smaller.

The functions determined for the reaction rate of both methods have a different slope, which does not change even if differences produced by the uneven thicknesses of the measured material are corrected theoretically. This fact demonstrates the higher sensitivity of the biochemical method for detecting differences in enzyme activity. On

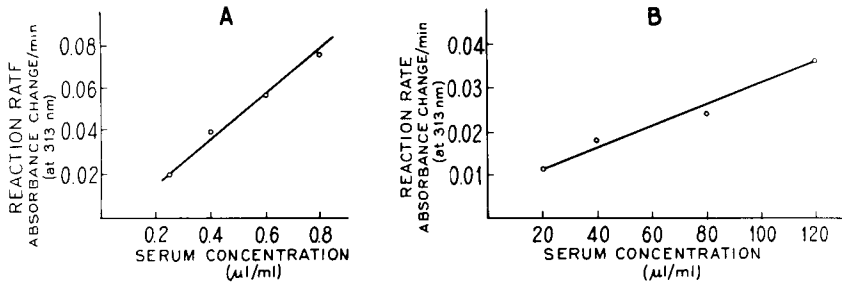

Figure 2(*A*) Biochemical reation rate (in absorption change at 313 nm/min) as a function of the final serum concentrations in the incubation mixture. (*B*) Histochemical reaction rate (in absorption change at 540 nm/min) as a function of the serum concentration incorporated into the polyacrylamide.

the other hand, microspectrophotometry of the histochemical reaction is without doubt a useful and easy technique for detecting enzymatic activities *in situ* in very small tissue areas.

The authors gratefully thank Dr Carlos Quintans for helpful discussions during the preparation of the experiments.

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Received 28 July 1976 and in revised form 14 October 1976