Isoenzymes and histochemistry of non-specific esterases in normal and cancerous skin

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Synopsis. Non-specific esterases in normal and carcinomatous skin of the mouse have been investigated electrophoretically and histochemically. Three esterase bands were obtained on electrophoresis from homogenates of normal skin; homogenates of carcinomas showed an accumulation of esterase-Ia and esterase-Ib.* However, using several ester substrates, substrate-specific patterns were demonstrated in the electrophoresis separations and histochemically in tissue sections. On the electrophoresis separations, α-naphthyl acetate, β-naphthyl acetate, 6-bromo-2-naphthyl acetate, naphthol AS acetate, naphthol AS-D acetate and naphthol AS-LC acetate gave rise to similar patterns, but with α-naphthyl propionate as substrate, more esterase-Ib was indicated and with 5-bromo-indoxyl acetate a distinctive preponderance. Peripheral or uniformly distributed staining was found histochemically in tumour epithelium using α-naphthyl acetate, α-naphthyl propionate and β-naphthyl acetate, whereas with the substrates of naphthol AS acetate, naphthol AS-D acetate and indoxyl acetate an intermediate pattern of staining related to keratinization was obtained.

Introduction

Non-specific esterases have been extensively investigated histochemically (Nachlas & Seligman, 1949; Cohen et al., 1951; Barrett, 1952; Braun-Falco, 1956; Steigleder & Löffler, 1956; Pearson & Defendi, 1957; Steigleder & Schultis, 1957; Monis & Weinberg, 1961; Oka et al., 1961; Okamoto et al., 1962; Schiffer, 1968) and electrophoretically (Markert & Hunter, 1959; Holmes & Masters, 1967a, b, 1968; Templeton, 1969; Weinstein & Mandel, 1969; Yoshimura et al., 1969, 1970), though their physiological and physicochemical significance has not been clearly established (Thompson & Whittaker, 1944; Holmes & Masters, 1967a, b; Okada & Fuji, 1968). Therefore, at present, their division into arylesterase, aliesterase and cholinesterase is based on their relative substrate specificities and the effects of inhibitors, activators and metallic ions (Augustinsson, 1961).

The electrophoretic separation of the enzymes present in tumours has not been examined in detail (Kreusser, 1966; Thyndall & Allen, 1967) and the separation of the enzymes associated with carcinogenesis has hardly been attempted. Thus it is not known whether there is any

* For explanation of nomenclature, see footnote on p. 276.
alteration in the various kinds of esterase present in these instances. The findings of enzyme histochemistry have indicated that enzymatic alterations during carcinogenesis are not specific, that is, enzyme patterns in neoplastic tissue show similar features to those in homologous normal tissue (Greenstein, 1944; Cohen et al., 1951; Oka et al., 1961; Okamoto et al., 1962). The aim of the present paper is to investigate the isoenzyme patterns and histochemical localization of non-specific esterases using several ester substrates.

Materials and methods

DDO strain adult male mice were used for the induction of tumours. After removing the hair, a 0.5% solution of 9,10-dimethyl-1,2-benzanthracene in acetone was applied to the back skin twice a week for 8 weeks. The first tumours appeared after 8 weeks, and the stages of neoplastic development were confirmed by histopathological observations. Resected tissues of normal, hyperplastic and carcinomatous skin were used for the electrophoresis and histochemical experiments.

ELECTROPHORESIS OF NON-SPECIFIC ESTERASES

Specimens were rinsed in chilled saline to remove blood and then homogenized in a 1:3 dilution (g wet wt/ml) in 0.2% cetyltrimethylammonium bromide. They were then centrifuged at 9000 g at 2-6°C for 30 min; 0.004 ml of the supernatant was pipetted into the sample slot on a polyacrylamide gel for electrophoretic analysis. The preparation of the polyacrylamide gel, buffer specifications and conditions of electrophoresis were according to the method of Ogita (1965). During electrophoresis a constant-current power supply delivered 1.2 mA per cm gel length. This current was maintained for 45 min, until the Bromophenol Blue had migrated about 4 cm towards the anode. After electrophoresis the polyacrylamide gel was incubated with substrate solutions as prepared for histochemical experiments.

Demonstration of sub-groups of non-specific esterases

The method described in a previous paper (Yoshimura et al., 1969) was used.

IDENTIFICATION OF NON-SPECIFIC ESTERASES

After electrophoresis the polyacrylamide gels were cut into strips, preincubated in a series of eserine (10^-3-10^-7 M) and diisopropylfluorophosphate (5 x 10^-4-5 x 10^-8 M) solutions of various concentrations for 15 min at room temperature, and rinsed in distilled water for 5 min before incubation in the complete substrate solution. Controls were incubated for the same time in buffer solution without substrate.

The three migration bands of non-specific esterases obtained from the electrophoresis of homogenates were symbolized as esterase-I, esterase-II and esterase-III in the order of increasing mobilities.* Esterase-I was composed of two sub-bands, which were denoted as esterase-Ia and esterase-Ib starting from the anode. Esterase-Ia, which migrated farthest to-

* In previous papers (Yoshimura et al., 1969, 1970) non-specific esterases were classified into A-, B- and C-types in the order of their increasing mobilities. This classification may be confused with a nomenclature based upon inhibitor effects and with one that has been officially not recommended (Enzyme Nomenclature, 1965, p. 126). Therefore, in the present paper, non-specific esterases are classified as esterase-I, esterase-II and esterase-III in order of their increasing mobilities. The sub-bands of A-I and A-II esterases mentioned in previous papers correspond to esterase-Ia and esterase-Ib in the present paper.