Two Factors Affecting the Heat Stability of Xanthine Oxidase in Extracts of Mouse Intestine

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The heat stability at 56 C of xanthine oxidase (Xox) from mouse intestine was found to be affected by two factors: (1) Xox which had been partially digested by trypsin was less heat stable than Xox which was protected from digestion by the presence of soybean trypsin inhibitor and phenylmethylsulfonyl fluoride; (2) Xox was less heat stable in glass tubes than in polystyrene tubes and siliconized glass tubes resembled polystyrene tubes in this respect.

KEY WORDS: xanthine oxidase; mouse intestine; genetic variation; trypsin.

INTRODUCTION

A recent study of Drosophila viridis by Bernstein et al. (1973) has shown that it is possible to detect genetic variation of xanthine oxidase in this species by the technique of heat inactivation using supernatants from crude extracts of whole flies. Indeed, these workers found that more variants could be detected by heat inactivation than could be demonstrated by the well-tried technique of gel electrophoresis. In a survey of 25 laboratory inbred strains of mice (Mus musculus), one of us (Lush, 1973) had been unable to find any electrophoretic variation in xanthine oxidase (Xox) except in one strain, SF/Cam, which is a rather unusual strain recently inbred from some wild mice trapped in a coal mine. We therefore decided to see if the technique of heat inactivation could succeed in revealing genetic variation in those mice where electrophoresis

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2 These authors refer to their enzyme as "xanthine dehydrogenase"; however, there is evidence that the oxidase and the dehydrogenase activities are different aspects of the same enzyme, viz., xanthine:oxygen oxidoreductase (E.C. 1.2.3.2) (Battelli et al., 1973).
had failed. In the event, we found that our results, which at first appeared to show genetic variation between strains, were in fact due to two experimental variables that we had inadequately controlled. These variables were, first, the digestion of xanthine oxidase by endogenous trypsin in extracts of the intestinal tissue which was used as the source of the enzyme and, second, the nature of the test tubes used for the inactivations. The effects of both these variables were therefore investigated systematically and the results are presented in this report.

MATERIALS AND METHODS

Phenylmethylsulfonylfluoride (PMSF), bovine serum albumin (BSA, essentially fatty acid free), flavine adenine dinucleotide (FAD, disodium salt), trypsin (chymotrypsin free), soybean trypsin inhibitor (chromatographically prepared), and xanthine were all obtained from Sigma Ltd. Phenazine methosulfate (PMS) and nitro blue tetrazoline (NBT) were from Wessex Ltd. Tris and ferric chloride were of Analar grade. All other chemicals were of reagent grade.

Adult (2-4 months old) male mice from strain A2G/Lac were used for all experiments. They were maintained on Oxoid breeder diet and starved overnight before use. Mice were killed by stunning and decapitation and their small intestines (stomach to caecum) were placed in chilled petri dishes. The intestines were opened up, thoroughly cleansed with water, blotted on filter paper, and cut into small pieces. The pieces from one or more intestines were mixed up together before being divided into aliquots for extraction either with added trypsin or with trypsin/chymotrypsin inhibitors. The livers used for electrophoretic comparison with intestine were extracted with trypsin/chymotrypsin inhibitors.

Extraction with Added Trypsin

For extraction with added trypsin, one part tissue was homogenized in 2 parts of cold 0.05 M tris-HCl buffer, pH 8.0, containing 1 mM EDTA and 10 mg/ml BSA. Solid trypsin (1 mg/ml) was added to the homogenizing buffer just before use. After at least nine up-and-down passes in a Potter-Elvehjem homogenizer, the homogenates were centrifuged at 35,000g for 1 hr at 4 C. Supernatants were dialyzed for 3-4 hr at 4 C against 1 liter of stirred 0.05 M tris-HCl buffer, pH 8.0, containing 1 mM EDTA, 1 mM FeCl₃, 1 mM MoO₃, 4 mg/liter FAD, and 500 mg/liter PMSF. After dialysis, the supernatants were kept at -40 C and usually used for inactivation experiments the next day.