

Original Report

## Isozymes of hair follicles from silver fox, *Vulpes vulpes*

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### Summary

Fourteen enzymes from hair follicles of 3 foxes, *Vulpes vulpes*, have been analysed by means of isoelectric focusing. Twelve enzymes expressed activity and among these 4 revealed readable zymograms. One enzyme even showed variation. The application of using hair follicles from foxes as a source for enzymes is realistic.

### Introduction

Isozymes have been studied in blood from the silver fox *Vulpes vulpes* and a few enzymes expressed variation (Simonsen et al. 1990). However, if live animals are to be studied obtainment of blood samples requires fixation or anaesthesia. Tissue samples are even more difficult to obtain. Earlier investigations, especially in forensic medicine, used a piece of cloth with e.g. bloodstains for detection of possible persons involved in a crime. The technique used was application of a thread or a hair sac directly on the gel and the following well-established electrophoretic methods. When pulling out a hair a very small amount of muscle will be attached to the hair sac and this sample is expected to express the same enzymes as muscle tissue.

This study was undertaken to see if it was possible to obtain a sufficient amount of material from a hair follicle for a zymogram. If a zymogram was revealed it was compared to the one obtained from muscle tissue of silver fox, *Vulpes vulpes*.

### Material and methods

Muscle tissue was collected from a few foxes when removing the fur and stored at  $-80^{\circ}\text{C}$ . Just before electrophoresis the samples were thawed and homogenised in a small amount of de-ionised water. Few hairs of different colours (fully black or with a silver band) and shapes (normal or curly) were pulled out from three live animals and were immediately used for the electrophoresis.

The electrophoresis applied was iso-electric focusing as described by Simonsen et al. 1992. 1  $\mu\text{L}$  of the extract from muscle tissue was applied on the gel. The hair was cut to a size of 5-10 mm, and the part containing the hair follicle was used for the analysis. The hair was placed in the gel under a stereo microscope. After the electrophoresis, the gels were stained according to Manchenko (1994). A list of tested enzymes, their E.C. number and the pH range for the iso-electric focusing are shown in Table 1.

**Table 1.** List of enzymes tested in hair follicles from silver fox, *Vulpes vulpes*, the E. C. number and the pH range of the gels.

Enzyme	E.C. number	pH-range
Acid phosphatase	3.1.3.2	3.5 - 5
Adenosine deaminase	3.5.4.4	3.5 - 5
Aspartate aminotransaminase	2.6.1.1	4 - 6
Diaphorase	1.8.1.4	5 - 7
Esterase	3.1.1...	4 - 6
Glucosephosphate isomerase	5.3.1.9	8 - 9.5
<b><u>Glucose-6-phosphate dehydrogenase</u></b>	1.1.1.49	4 - 6 and 5 - 7
Isocitrate dehydrogenase	1.1.1.42	4 - 6
Lactate dehydrogenase	1.1.1.27	8 - 9.5
Malate dehydrogenase	1.1.1.37	3.5 - 5
Mannose phosphate isomerase	5.3.1.8	6 - 8
Peptidase C <sup>1</sup>	3.4.11 or 13...	3.5 - 5
Peptidase D <sup>2</sup>	3.4.11 or 13...	3.5 - 5
Phosphoglucomutase	5.4.2.2	6 - 8

<sup>1</sup> substrate leucyl-alanine<sup>2</sup> substrate leucyl-proline

## Results and discussion

Twelve out of 14 investigated enzymes expressed activity, see Table 2. The quality of the zymograms was acceptable for four of the enzymes, i.e. adenosine deaminase, glucosephosphate isomerase, lactate dehydrogenase and phosphoglucomutase. When comparing the zymograms obtained from the hair follicles with those obtained from muscle tissue, only the bands with heavy activity were found in the hair follicles. This may be due to the fact that the source for the enzyme activity was very small. The bands revealed by the hair follicle were congruent with the bands achieved from muscle tissue.

The glucosephosphate isomerase showed variation among the individuals investigated. This variation has not been found among the muscle samples studied so far and unfortunately, it was not possible to obtain muscle sample from the fox expressing variation in the hair follicles. However, this observation indicates that enzymes from hair follicles may be used for studies on parentage among foxes.

**Table 2.** Enzymatic activity found in hair follicles from silver fox, *Vulpes vulpes*. The number above the slash is the number of hair follicles expressing activity and the number below is the hair follicles tested.

Enzyme	Activity
Acid phosphatase	2/4
Adenosine deaminase	5/7
Aspartate aminotransaminase	0/4
Diaphorase	0/6
Esterase	2/6
Glucosephosphate isomerase	6/6
Glucose-6-phosphate dehydrogenase	6/6
Isocitrate dehydrogenase	2/6
Lactate dehydrogenase	4/4
Malate dehydrogenase	5/7
Mannose phosphate isomerase	4/6
Peptidase C <sup>1</sup>	1/4
Peptidase D <sup>2</sup>	1/4
Phosphoglucomutase	5/6

**References**

Manchenko, G.P. Handbook of detection of enzymes on electrophoretic gels. CRC Press, Boca Raton

Simonsen V., Damgaard B.M., Larsen B. & Lohi O. 1992. Genetic polymorphism of esterase in plasma of the American mink (*Mustela vison* L.). - Anim. Genet. **23**: 553-555.

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