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A Cancer-Associated Lactate Dehydrogenase Is Expressed in Normal Retina

Abstract. *An unusual isozyme of lactate dehydrogenase, lactate dehydrogenase k, is found in high concentrations in cultured cells transformed by the Kirsten murine sarcoma virus and in many human cancer tissues. In experiments described here high levels of a lactate dehydrogenase k activity were detected in extracts of normal rodent retina. This activity had the same key properties as the human tumor isozyme, namely, a highly cathodic electrophoretic mobility and inhibition of enzymatic activity by oxygen and 5',5'-dipurinenucleoside tetraphosphates. Expression of this activity in the retina may be related to the high aerobic glycolysis characteristic of the retina, a metabolic feature shared with many tumors.*

Anderson and co-workers (*1, 2*) described an unusual lactate dehydrogenase (LDH), asp56/LDH_k (*3*), in cells transformed by the Kirsten sarcoma virus. This specific LDH activity is distinguished by (i) a highly cathodic electrophoretic mobility (*1, 2*); (ii) direct, reversible inhibition by physiological concentrations of oxygen (*1, 2*); (iii) direct, reversible inhibition by 5,5,-dipurinenucleoside tetraphosphates (*4*); and (iv) subunits of 56,000 daltons that are readily cleaved—without apparent loss of activity—into 35,000- and 22,000-dalton subunits (*4*). The enzyme can be induced in uninfected cultured fibroblasts by anaerobic shock (*4*) and is found in low but detectable levels in many normal tissues (*2, 4*). It is expressed at high levels in most human carcinomas and is found in serum of cancer patients (*4*).

The mammalian retina is essentially a portion of the brain specialized to transduce light into nerve impulses. The retina develops as an outgrowth of the brain walls. Early in development, before the prosencephalon is divided into telencephalon and diencephalon, the rudiments of the eye cup can be seen as a vesicle originating from the neural tube. Like other nervous tissues, the mature retina is in a nonproliferative state, that is, after differentiation is completed, no cell proliferation can be detected. The isolated retina, like many tumors, is characterized by a highly glycolytic metabolism under aerobic conditions (*5-7*). Photoreceptor cells are extraordinarily sensitive to high oxygen tension and glycolytic poisons such as iodoacetate (*6*). We report that an unusually high level of an asp56/LDH_k-like activity can be extracted from normal avian and mammalian retinas.

Retinas of mouse, rat, guinea pig, and chicken were dissected in toto and homogenized by Dounce homogenization in 0.01*M* tris buffer (*p*H 7.5) containing 0.001*M* dithiothreitol and 0.05 percent Triton X-100. Brain tissues were extracted in parallel as a control. The homogenates were centrifuged at 8000 rev/min for 45 minutes, and the supernatants were collected and assayed for asp56/LDH_k activity by using reverse-polarity electrophoresis on gels containing imidazole and borate (*1, 2*).

The amount of asp56/LDH_k-like activity extracted from rat and guinea pig retinas was far greater than the amount extracted from brain tissue from the same animals (Fig. 1A). With an integrating densitometer we determined that there was about 20 times more asp56/LDH_k-like activity in rat retina than in rat brain (200 versus 10 gel units per milligram). The concentration was approximately equal to the mean level in human carcinomas (*4*) and in fibroblasts transformed by Kirsten sarcoma virus (Fig. 1). The activity from rat retina migrated to a slightly different position than asp56/LDH_k activity from Kirsten sarcoma virus–transformed rat cells and asp56/LDH_k-like activity from guinea pig retina. In the retinas of both species at least two bands were observed that could represent either multiple isozymes of asp56/LDH_k or artifactual modifications. An unusually large amount of asp56/LDH_k-like activity was also extracted from mouse retina (Fig. 1B). Individual mouse, rat, and guinea pig retinal preparations were also assayed for

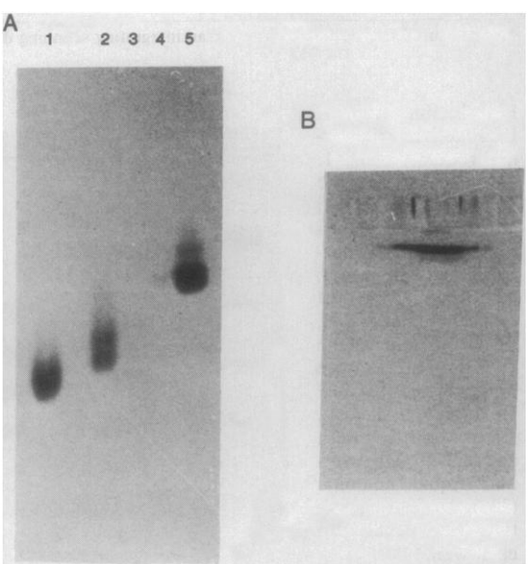

Fig. 1. Activity of asp56/LDH_k in normal rodents. (A) Lane 1, Kirsten sarcoma virus–transformed cells; lane 2, normal rat retina; lane 3, normal rat brain; lane 4, normal guinea pig brain; and lane 5, normal guinea pig retina. (B) Normal retinas pooled from C57BL/6J, BALB/c, and DBA mice. Equal amounts (30 μg) of total protein were added to each well. Gels stained in the absence of lactate showed no detectable activity.

conventional LDH activities (M4, M3H, M2H2, MH3, and H4) with normal-polarity electrophoresis on gels containing tris and glycine (Fig. 2) (*1*, *2*). The isozyme distribution was unlike that seen in the imidazole and borate gels. Avian (chicken) retina was also found to contain a high level of asp56/LDH_k-like activity, distributed approximately equally in a pattern of three bands. Chicken brain also contained a relatively high amount of asp56/LDH_k-like activity, but with a different pattern of subbands.

Isozyme asp56/LDH_k can be distinguished from other LDH isozymes by its reversible inhibition by oxygen and dipurinenucleoside tetraphosphates. Accordingly, we assayed the activity bands in Fig. 1 in the presence or absence of these inhibitory factors. The retinal enzyme was indeed an asp56/LDH_k-like activity in that it was strongly inhibited by O_2, Ap4A, and Gp4G (Table 1).

It seems paradoxical that asp56/LDH_k, an enzymatic activity associated with malignant transformation, can also be found in normal retinas in the large quantities seen in tumors. The physiological role of this enzyme in normal retinas and in malignant transformation is unknown. Expression of asp56/LDH_k in the retina indicates that this isozyme, like other tumor markers, is not unique to neoplastic transformation and is apparently not growth-related. However, it may be appropriate to associate LDH_k with the metabolism of both the normal retina and many transformed cells. Many tumors and the isolated retina have a high glycolytic activity under aerobic conditions, that is, high consumption of glucose and a high production of lactic acid (*5*). Furthermore, in pig retina the total amount of oxygen extracted from choroidal and retinal blood could account for complete oxidation of only 37 percent of the extracted glucose (*7*). Specific high-level expression of an unusual LDH isozyme (LDH-C) has been described in the retina of teleost fish (*8*), although the relation of this to our finding of an asp56/LDH_k-like activity in mammalian and avian retinas remains to be determined. The fact that the asp56/LDH_k-like activity from rat retina migrates to a different position than that from Kirsten sarcoma virus–transformed rat cells suggests that these two activities are coded by different but related genes. If so, this could be related to the observation that in rats there are about 30 copies of the Kirsten homologous VL30 genes per haploid genome (*9*). Another possibility is that these activities are coded by only one gene but the product is posttranslationally modified.

Note added in proof: Since this report was submitted, Morin and Hance (*10*) proposed that LDH_k in fact represents LDH-5, with the unusual properties of LDH_k being an artifact of the imidazole-borate gel system used to resolve it. Their proposal was based on the finding of LDH_k activity in a commercial preparation of human placental LDH-5. Studies in our laboratory of this same commercial material show that it does indeed contain LDH_k activity, but that this is readily separable by conventional biochemical procedures from its LDH-5 activity. Sodium dodecyl sulfate gel analysis of the commercial enzyme show that it contains approximately 25 percent 56,000-dalton polypeptide, 65 percent 35,000-dalton polypeptide, and 10 percent other polypeptides. The 56,000-dalton polypeptide comigrates with the LDH_k activity. Independently, Evans *et al.* (*11*) found that expression of LDH_k activity is independent of LDH-5 during the cell cycle. In addition, we have found the oxygen responsiveness of retinal LDH_k to vary among species and to be due to the enzyme itself, not to an artifact of the gel assay system (*12*).

Table 1. Sensitivity of retinal asp56/LDH_k-like activity to oxygen and dinucleoside tetraphosphates. Rat retinal extract containing 100 μg of protein was electrophoresed on gels containing imidazole and borate to separate the asp56/LDH_k-like activity from other isozymes. The region of the gel containing the asp56/LDH_k-like activity was cut out and the activity was stained in the presence of the indicated compounds at room temperature.

Assay condition	asp56/LDH_k-like activity*	Percent
Plus O_2	0.0	0
Plus O_2, 5 m*M* NaCN	12.4	100
Plus O_2, 5 m*M* NaCN, 3×10^{-5} *M* Ap4A	8.3	67
Plus O_2, 5 m*M* NaCN, 8×10^{-5} *M* Ap4A	4.5	36
Plus O_2, 5 m*M* NaCN, 3×10^{-5} *M* Gp4G	5.0	40
Plus O_2, 5 m*M* NaCN, 8×10^{-5} *M* Gp4G	3.9	31
Minus O_2	4.8	39

*As measured by integrator cycles of an integrating scanning densitometer (Helena Quick Scan Jr.).

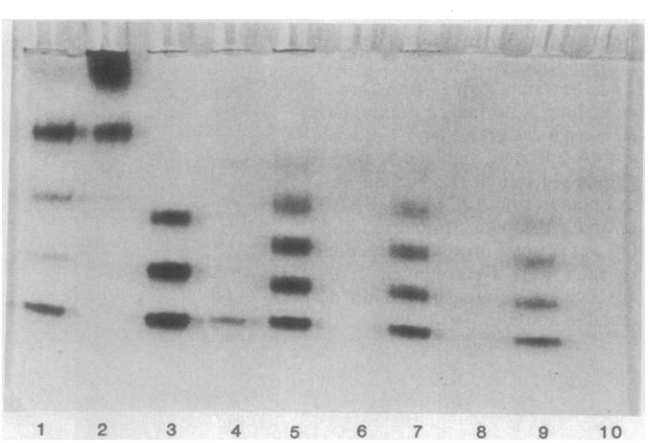

Fig. 2. Conventional LDH activities in normal rodents. Lane 1, normal rat brain; lane 2, normal rat retina; lane 3, normal guinea pig brain; lane 4, normal guinea pig retina; lane 5, C57BL/6J mouse brain; lane 6, C57BL/6J retina; lane 7, C3H/HeHa mouse brain; lane 8, C3H/HeHa retina; lane 9, DBA mouse brain; and lane 10, DBA retina. Equal amounts (30 μg) of total protein were added to each well.

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