The Mysterious World of Isozymes

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Strictly defined, isozymes are similar forms of the same enzyme that share identical functions. There are different types of isozymes that are classified according to their origin - isozymes may be encoded by several different loci (which determine subunit structure), or they may be determined by a single locus with several different alleles. If the latter, isozymes are referred to as "allozymes" (sometimes used interchangeably with "isozymes"). Many well-known enzymes and their isozymes used in electrophoretic studies function in the metabolic pathway. For example, glucophosphoisomerase (GPI or PGI) catalyzes the second step of glycolysis, by converting glucose-6-phosphate to fructose-6-phosphate. Isocitrate dehydrogenase (IDH) and malate dehydrogenase (MDH) both function in the TCA cycle.

By definition, an enzyme is a protein that is composed of an intertwining string of amino acids, giving it a specific molecular structure. It is this structure that is crucial to the enzyme's ability to function. For example, misshapen hemoglobin is unable to bind to oxygen and results in Sickle Cell Anemia. The structure of the enzyme is directly affected by hydrogen bonds and charge-relationships within the amino acid chains. Out of the twenty possible amino acids, two are negatively charged (aspartic acid and glutamic acid), two are positively charged (lysine and arginine), and the remainder are neutral. A change of just a few amino acids may alter the configuration and thus the structure and functionality of the enzyme. If the "mutated" enzyme type is still able to function normally, it can be classified as an isozyme.

Because enzymes are a type of protein and therefore are composed of amino acids, they are the direct products of DNA:

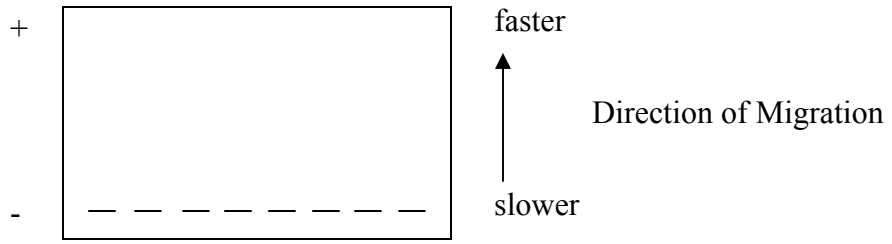
$$\text{DNA} \xrightarrow{\text{Transcription}} \text{RNA} \xrightarrow{\text{Translation}} \text{Proteins}$$

In the simplest case, one nuclear gene codes for one particular enzyme. Thus, different forms of a gene (i.e., alleles) may code for different forms of the enzyme (isozymes or allozymes, in this case). These alleles may differ in only a few nucleotide sequences or codons, and this may correspond to a different isozyme if the amino acid sequence is sufficiently changed. In diploid organisms, there will be two alleles of the gene present in an individual and thus there will be two isozymes produced. Because both parental alleles are expressed, this is known as codominance. The usefulness of isozymes as molecular markers is based on the fact that any change in the structure of an enzyme (i.e., formation of an isozyme) is directly related to a change in DNA.

Electrophoresis

On an electrophoretic gel stained for a specific enzyme, different isozymes appear as bands, which are separated from one another on the basis of charge and size. The combination of charged and uncharged amino acids results in a net charge on the protein. Because the net charge on most isozymes is negative, samples running on a gel in an electrical current typically

move towards the cathode (+) end. Band separation occurs as the smaller isozymes migrate faster through the gel than the larger molecules.



For any given individual, there should be two isozymes bands expressed, one coded for by the maternal allele and one by the paternal allele (due to codominance). If the isozymes are different (as in a heterozygotic individual), then isozymes will differ in size and their corresponding bands will be separated on the gel. If the two parental isozymes are identical (as in a homozygote), only one band will be present:

Ex. Individual A is a <u>homozygote</u>:

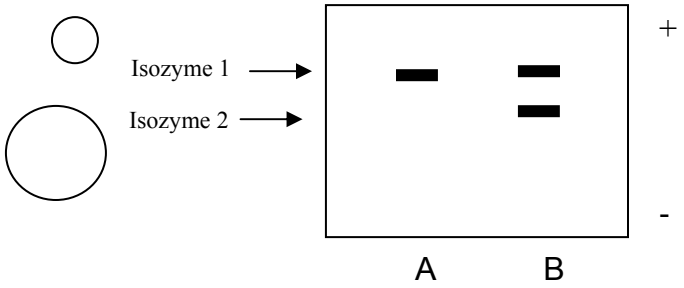
Maternal allele codes for Isozyme 1

Paternal allele codes for Isozyme 1

Individual B is a <u>heterozygote</u>:

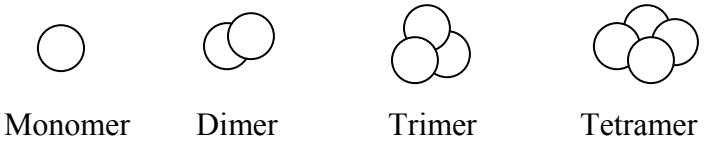
Maternal allele codes for Isozyme 1

Paternal allele codes for Isozyme 2



<u>Structural Configuration</u>

Up to this point, we have been thinking of enzymes as discrete single units, called a monomer. In fact, enzymes may be composed of more than one structural unit. An enzyme consisting of two subunits is called a dimer, and one with three subunits is a trimer, etc.



This particular aspect of enzyme structure is responsible for the appearance of additional bands in the heterozygotes. As a general rule, the total number of bands in a heterozygote should consist of one more than the number of subunits in the enzyme (e.g., five bands in a tetramer).

Consider the following dimeric enzyme:

Individual A is a homozygote

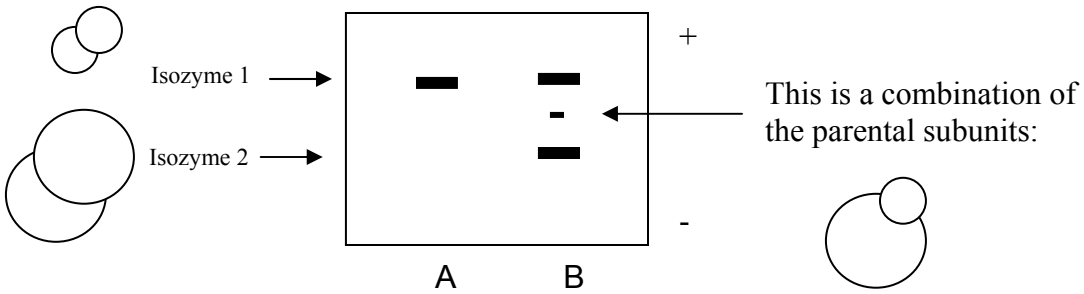
Maternal allele codes for Isozyme 1

Paternal allele codes for Isozyme 1

Individual B is a heterozygote

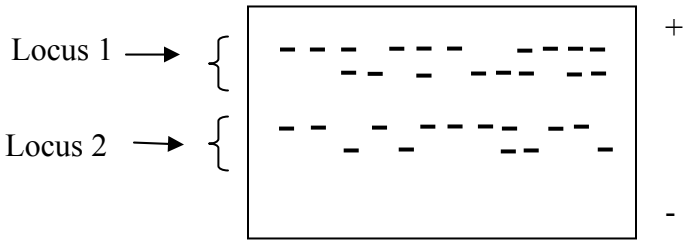
Maternal allele codes for Isozyme 1

Paternal allele codes for Isozyme 2

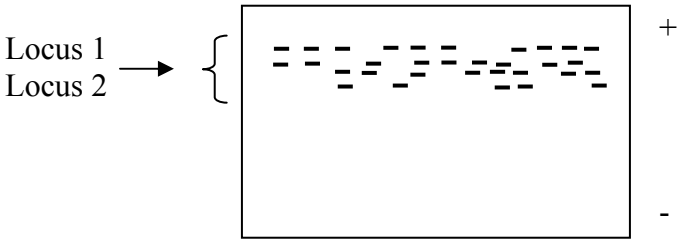


Number of Coding Loci

In addition to the structural configuration of enzymes, it is important to know the number of loci that code for the enzyme. As mentioned earlier, the simplest case is when an enzyme is encoded by only one gene. However, it is possible that more than one gene or loci may be involved. For example, hemoglobin is a tetramer with two α subunits and two β subunits (where the α and β subunits are coded by different genes). On a gel, each locus (and its associated isozymes) should be represented but it is usually possible to distinguish between different loci:

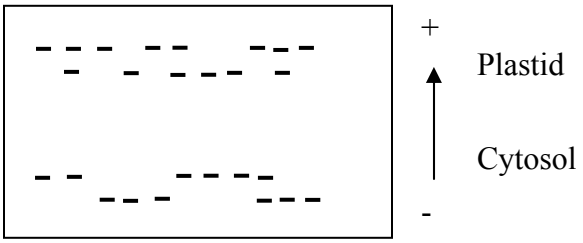


But on occasion, some loci may overlap on a gel and it is virtually impossible to differentiated among them. If a longer running time does not allow for separation of the loci, the enzyme is said to be unresolvable. For example:



Cellular Location

Another reason why different bands may be seen on a gel is that isozymes of a particular enzyme may be present in different cellular compartments. Most frequently, isozymes are found in the cytosol and plastid regions, although they may also be detected in the mitochondria and other regions of the cell. Isozymes in these different areas can be visualized on the electrophoretic gel because they occur in different locations. For example, plastid isozymes usually run faster than cytosolic isozymes:



NOTE: Sometimes isozymes from a certain cellular compartment are *not* seen on a gel because certain ingredients in the grinding buffer have degraded them. For example, the cytosolic form of MDH in violets is destroyed by the ascorbic acid in the grinding buffer.

To make matters even more difficult, plastid and cytosol isozymes for a given enzyme may differ in their subunit composition. For example, SOD is in the dimer form in the cytosol and plastid regions, and is a tetramer in the mitochondria. Thus, it is important to know both the cellular locations of the enzyme of interest, and its structural configuration.

Ploidy Level

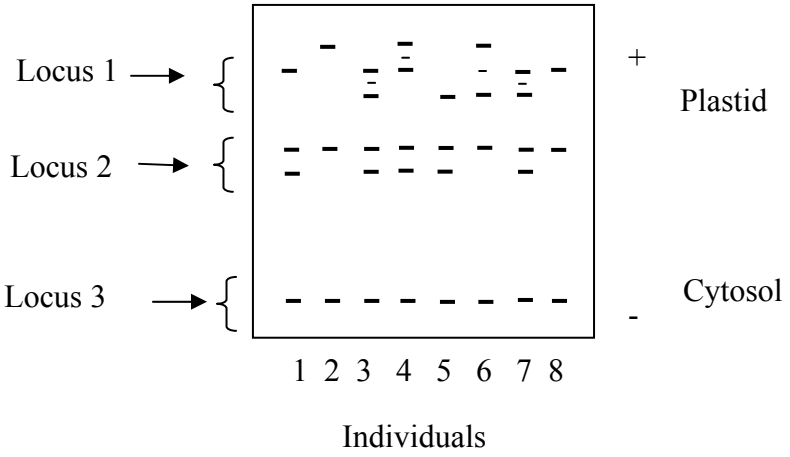
In addition to the enzyme structure, the cellular location, and the number of loci involved in coding for the enzyme, the last important aspect of understanding isozymes is the ploidy level of the animal or plant being analyzed. The above examples have been presented for diploid individuals, but it is also possible to use isozyme electrophoresis to characterize haploid or polyploid individuals. In the case of the latter, more bands will be visible on the gel (representing the different allelic combinations). For more information on polyploidy, please see Weeden and Wendel (1989).

Summary

To summarize, there are four important aspects that should be known about any particular isozyme system before the gels can be scored correctly:

1. Structural configuration - monomer, dimer, etc.
2. Number of loci involved
3. Cellular location - cytosol, plastid, mitochondria, etc.
4. Ploidy level of the species

As a final example to incorporate all of the above information, consider eight individuals of a diploid plant species in which samples have been stained for a hypothetical enzyme (XYZ):



In this case, enzyme XYZ is found in both the plastid and cytosol compartments of the cell. In the plastid, the enzyme is encoded by two different loci - locus 1 is a dimer and locus 2 is a monomer. In the cytosol area, enzyme XYZ is only encoded by one locus, which shows no variation and is referred to as monomorphic (which results in an unknown structure - the enzyme could be a monomer or a dimer). Technically, each different band level within a locus refers to a different isozymes. For example, locus 1 in the plastid has three isozymes (fast, slow, and intermediate isozymes), while locus 2 of the plastid only has two isozymes (fast and slow isozymes - seen most easily in the heterozygote). With experience and practice, the scoring of difficult gels such as this becomes easier.

Ending Remarks

Although isozyme electrophoresis is a powerful technique, it is not without potential problems. First, isozymes are assumed to be selectively neutral, but this is debatable because of the prominent role that isozymes play in metabolism. Isozymes may therefore be under high selective pressures. In fact, several studies have found that particular isozymes are correlated with specific traits. For example, Stockwell and Mulvey (1998) discovered that a specific PGDH isozyme was correlated with high salinity tolerance in desert pupfish. Polymorphism of PGDH has also been found to be correlated with latitude in *Drosophila spp.*, dark respiration rates in perennial ryegrass, and salinity tolerance in other fish species (see references in Stockwell and Mulvey, 1998). In *Colias* butterflies, individuals with different GPI genotypes tend to fly at different temperatures (see Mitton, 1989 and references therein).

Another concern in using isozymes is that the number of isozyme systems represent only a very small fraction of the total number of enzymes within an organism. It must be remembered that any study of genetic variation based on isozyme data is limited in this regard. And last of all, enzymes and their isozymes may be genetically linked in some cases (i.e. in linkage disequilibrium), such that they cannot be used as independent molecular markers. However, there are now statistical methods that allow for the testing of linkage disequilibrium.

Researchers who are embarking into the mysterious world of isozymes must weigh the relative costs and benefits of this technique for his/her own unique situation. In some cases, a direct measure of genetic variation using DNA (e.g., RAPDs, ISSRs) may be more appropriate. Hopefully, this short explanation has helped clear up the mystery surrounding isozymes, allowing the researcher to make a more informed decision.

Literature Cited:

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