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Lactate Dehydrogenase Isozymes: Dissociation and Recombination of Subunits

Abstract. *Lactate dehydrogenase from beef tissues may be resolved electrophoretically into five isozymes each of which is a tetramer. These tetramers can be dissociated into monomers by freezing in 1M sodium chloride. On thawing, reassociation into functional tetramers occurs. On the basis of charge and amino acid composition there are two kinds of monomers. Lactate dehydrogenase-1 contains one kind of monomer and lactate dehydrogenase-5 the other kind. A mixture of equal quantities of these two isozymes, after dissociation and reassociation, leads to the production of all five isozymes in the expected proportions of 1:4:6:4:1.*

Numerous laboratories have reported that the lactate dehydrogenase (*1*) of many organisms exists in five distinct isozymic forms (*2*). These isozymes have the same molecular weight (about 135,000) but differ in net electrical charge and hence may be separated electrophoretically. Moreover, each isozyme is a tetramer (*3*) since it may be dissociated by urea or guanidine into four polypeptide subunits of equal size. These subunits exist in two distinct electrophoretic varieties, A and B. Assortment of these subunits in all possible combinations of four would yield five isozymes of the following compositions. LDH-5 = A^4B^0, LDH-4 = A^3B^1, LDH-3 = A^2B^2, LDH-2 = A^1B^3, LDH-1 = A^0B^4 (*3*, *4*). This hypothesis of LDH isozyme structure has since been supported by other investigators (*5*) who have presented additional immunochemical evidence; likewise total amino acid analyses of LDH-1, LDH-3, and LDH-5 also support the hypothesis, since, in accordance with prediction, the composition of LDH-3 is approximately the average of the quite distinct compositions of LDH-1 and LDH-5 (*6*).

However, none of this evidence is conclusive. A critical test of the subunit hypothesis must involve the construction of the five isozymes from the subunits. This test has now been completed and the results are shown in Fig. 1. Crystalline LDH was prepared from beef tissues containing all five isozymes. The LDH-1 and LDH-5 were then separated from the mixture of isozymes by DEAE-column chromatography and by electrophoresis through a column of cellulose (*7*). According to our hypothesis each of these two isozymes should consist of only one kind of subunit: A polypeptides in LDH-5 and B polypeptides in LDH-1. These two isozymes were mixed in equal proportions in 1*M* NaCl, frozen overnight and then resolved by starch-gel electrophoresis. The mixture of LDH-1 and LDH-5 generated all five isozymes and in approximately the expected proportions of 1:4:6:4:1 which should be attained at equilibrium if random reassociation of subunits into tetramers occurs (Fig. 1). Identical treatment of LDH-1 or LDH-5 alone produced no change in these isozymes—the dissociated subunits reassembled into the original tetramer only. These results, particularly in view of the corroborative chemical evidence, provide a satisfying demonstration of the validity of the original hypothesis of isozyme structure.

The relative amounts of the various isozymes produced depend upon the proportions in which LDH-1 and LDH-5 are mixed. The distribution may be skewed in accord with the input of subunits to generate the various isozyme patterns described in normal tissue homogenates (*5*, *8*). Since the two subunits, A and B, have different amino acid compositions they are different proteins and probably under the control of separate genes. The isozyme patterns generated in developing tissues can thus be attributed to the different relative amounts of A and B polypeptides synthesized in each cell as a result of differential gene function (*6*, *8*).

In apparent conflict with this hypothesis is the fact that under suitable conditions of electrophoresis a single isozyme may be represented by two, three, or more closely spaced bands. In the reassociation experiments multiple bands commonly appeared (although they are not apparent in Fig. 1) at the location of each of the newly generated isozymes. These multiple bands cannot represent distinct isozymes in terms of protein composition but must be produced by minor changes in molecular migration, perhaps because of combination of the isozyme with different amounts of NAD (*9*). More difficult to fit into the current hypothesis is the

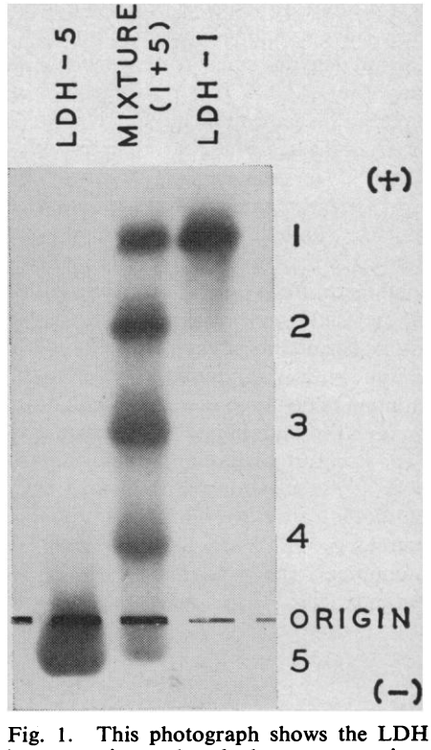

Fig. 1. This photograph shows the LDH isozymes in each of three preparations after electrophoretic resolution in starch gel. On the right is LDH–1, on the left LDH–5, and in the middle are the isozymes resulting from a mixture of equal quantities of these two preparations. All five isozymes were generated in the mixture in the approximate ratio of 1:4:6:4:1, the expected distribution after random reassociation of subunits. The total enzyme activity in the mixture was the sum of the activities of the single isozyme preparations. All three preparations were placed in 1*M* NaCl and frozen overnight before electrophoretic resolution. Electrophoretic and staining procedures have been described previously (*8*).

sixth isozyme in human sperm (*10*). The synthesis of this sixth isozyme must either involve an additional gene or represent a specialized modification of one of the other isozymes.

Previous efforts to reassemble functional LDH molecules after dissociation in urea, guanidine, or at acid *p*H were of doubtful success (*11*). These dissociation procedures rupture hydrogen bonds and also destroy the tertiary and secondary (helical) structure of the monomers. Such denatured monomers apparently do not recombine into tetramers nor do they exhibit enzymatic activity. Since the monomers that were dissociated by NaCl behave differently, they must have retained much of their original structure.

Enzyme activity was not greatly reduced by treatment with NaCl, and this result suggests that the monomers may retain enzyme activity. If so, the overall conformation of the monomers must have remained essentially intact. Lactate dehydrogenase with a molecular weight of 72,000 has previously been reported as enzymatically active (*12*) which indicates that the dimer is functional. Measurements of the degree of polymerization and concomitant enzyme activity under identical conditions should soon allow us to describe with confidence the minimal functional unit of the LDH molecule.

The formation of enzymatically active tetramers by random association of monomers demonstrates that the conformational requirements for the quaternary structure of LDH are readily available in the structure of the monomers. However, after the disruption of the secondary and tertiary structure of the monomers (as with urea) they apparently lose their ability to become functional LDH molecules again even though the primary structure (linear sequence of amino acids) remains intact. We may conclude therefore that the simple arrangement of the primary structure of the monomers is translated with difficulty, if at all, into protein structures of greater complexity. Apparently the physical-chemical environment in the cell at the site of protein synthesis, at least in the case of LDH, is of decisive importance in determining the higher states of molecular configuration (*13*).

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References and Notes

1. The following abbreviations are used: LDH, lactate dehydrogenase; NAD, nicotinamide adenine dinucleotide; DEAE, diethylaminoethyl-cellulose.
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Phase Transformation at High Temperatures in Hafnia and Zirconia

Abstract. *Phase transformation curves for HfO_2 and ZrO_2 have been made. Transformation hysteresis is discussed. The transformation of monoclinic to tetragonal as temperature increases occurs over the range 1000° to 1200°C in ZrO_2, and 1500° to 1600°C in HfO_2. With decreasing temperature the transition back to monoclinic occurs from 970° to 750°C in ZrO_2 and 1550° to 1450°C in HfO_2. These transformations have been visually observed in ZrO_2, but not in HfO_2.*

In spite of the wide differences in their atomic weights and densities, the elements zirconium and hafnium have very similar chemical properties. Therefore, the oxides ZrO_2 and HfO_2 should also have quite similar properties such as molecular volume, melting temperature, and crystal form. Both are monoclinic at room temperature and transform to tetragonal at high temperatures. In this work, phase transformations in ZrO_2 and HfO_2 were studied with a high-temperature x-ray diffractometer assembly (*1*).

Figure 1 illustrates the phase transformation characteristics of spectrographically pure (*2*) ZrO_2. With increasing temperature, the stable monoclinic form begins to transform to the tetragonal structure at 1000°C and the transformation is complete at 1190° to 1200°C. The tetragonal form is now stable to lower temperatures when the temperature is decreased and remains tetragonal to about 970°C; it is completely transformed to monoclinic by about 750°C. The temperature is plotted against a ratio of the intensity of the strongest line (111) of the tetragonal phase and the strongest line of the monoclinic phase (111). Since these curves resemble somewhat an electrical hysteresis curve, they will be referred to as the phase transformation hysteresis curve. Impurities or additions influence the range of transition especially with decreasing temperature where the transformation temperature may extend as low as 450°C in very impure samples.

In the high-temperature diffractometer specimen mount used in this work, the sample may be viewed visually through the port where optical pyrometer measurements are made. During heating and cooling through phase-transformation points, the sample can actually be seen to change phase as a result of individual particle movement caused probably by sharp discontinuities in the thermal expansion. The transformation with decreasing temperature is easily observed, while the transformation from monoclinic to tetragonal with increasing temperature is more difficult to observe. The temperature at which greatest sample movement is noted is the point on the transformation curve where the rate of change from one phase to the other is large. These points are noted on the curves in Fig. 1.

The monoclinic phase in HfO_2 is stable to a much higher temperature than this phase in ZrO_2 as seen in the transformation curves shown in Fig. 2. The transformation occurs over a narrower range of about 100°C and the temperature difference across the hysteresis loop is only about 50°C. Visual evidence of the HfO_2 phase change has not been observed.

Phase-change temperatures shown in Fig. 2 are considerably lower and the range is narrower than previously reported (*3*). The range reported here is for fresh samples which have not