Dagmar Heinova et al., J Chromatogr Sep Tech 2017, 8:4(Suppl)
DOI: 10.4172/2157-7064-C1-028

Isoelectric focusing technique as a tool for separation of bird lactate dehydrogenase isoenzymes

Dagmar Heinova and **Zuzana Kostecka**
University of Veterinary Medicine in Kosice, Slovakia

Statement of the Problem: Lactate dehydrogenase (EC 1.1.1.27, LDH) is an enzyme ubiquitously distributed in cells of vertebrates, plants, and bacteria. Structurally, it is a tetramer of four units which in animals exists in five electrophoretically distinguishable forms known as isoenzymes. Electrophoretic techniques routinely applied in separating mammalian LDH isoenzymes use a buffer system of pH 8.6 at which their electrophoretic migration depends on the migration of the two pure types, i.e., H4 and M4 forms. The more the two homotetramers differ in charge, the more separable are the hybrids by electrophoresis. In the case of bird LDHs, the two pure types migrate close together towards the anode at pH 8.6 producing only one diffuse enzymatic zone.

Methodology & Theoretical Orientation: For the separation of bird LDHs, we chose isoelectric focusing technique in a gradient of pH 3 to 9. Separation conditions were used as follows: 2000 V, 2.5 mA, 3.5 W, 15°C, 20 min separation time. Gels were stained with nitro blue tetrazolium technique in 0.1 M Gly-NaCl-NaOH buffer pH 8.3 at 37°C for 30 min.

Findings: Using above described methodology we achieved good and clear resolution of all five forms of the enzyme of bird origin with their localization being in the pH region of 6.2 to 8.1 (chicken LDH isoenzymes), 5.4 to 7.7 (pheasant LDHs), and 5.3 to 8.3 (turkey lactate dehydrogenase isoenzymes). Mammalian molecules of LDH were more acidic and widespread with the pI values being in the range of 4.5 to 9.0.

Conclusion & Significance: Using IEF technique it was possible to compare the pattern of LDH isoenzymes in serum and tissues of mammals and birds as well as to observe the pattern of the enzymes in some tissues of chicken embryo.

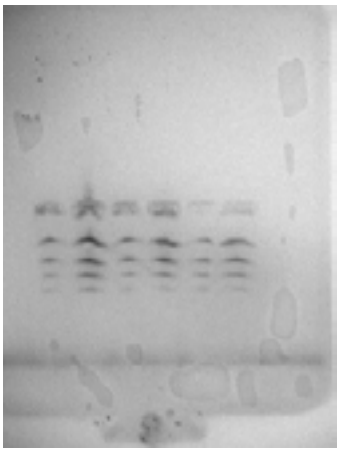

Figure 1: An electrophoretic pattern of chicken embryo breast muscle LDH isoenzymes.

Biography

Dagmar Heinova has her experience in enzymology with a special focus to proteolytic enzymes and lactate dehydrogenase isoenzymes. She developed a colorimetric method for determination of pepsin activity which was patented. She succeeded in separating bird LDH isoenzymes based on experience in her research. Results of her research are applied in the education of students at the University of Veterinary Medicine and Pharmacy in Kosice in the field of Biochemistry.

dagmar.heinova@uvlf.sk