DETECTION OF LACTATE DEHYDROGENASE ISOENZYMES IN SINGLE MAMMALIAN OOCYTES DURING CLEAVAGE BY A MICROMODIFICATION OF DISC ELECTROPHORESIS

V. S. Repin, I. M. Akimova, and V. B. Terovskii

UDC 591.315-99:577.158.347.087.4:543.545

A micromodification of the method of disc electrophoresis in glass capillary tubes is described. It can be used to study the protein composition and activity of lactate dehydrogenase isoenzymes in single rat and mouse oocytes during cleavage.

KEY WORDS: lactate dehydrogenase – isoenzymes; mammalian oocytes; disc microelectrophoresis of proteins.

The development of the techniques of ultramicrobiochemical investigations has made possible the systematic study of the metabolism of mammalian embryos at the preimplantation stages of development. Various micromethods for investigating the synthesis of nucleic acids [6, 7, 9, 11] and proteins [8] and for determining the activity of enzymes [3, 6] and isoenzymes [2, 10] have been described. Considerable attention is being paid to the study of certain enzymes of respiratory and glycolytic phosphorylation, for it has been found that certain metabolites of the tricarboxylic cycle are factors controlling normal cleavage of the fertilized oocyte [3, 4, 6].

Methods of manipulation of very small samples that have been developed, together with the high sensitivity of the methods of determination used, have made it possible to carry out such investigations on the scale of hundreds or, in some cases, even of tens of zygotes. The production of so many fertilized oocytes simultaneously is achieved by inducing superovulation in females by preliminary administration of hormones [3, 6]. To study the initial cytodifferentiation of the embryos in the preimplantation period, it is absolutely necessary to develop micromethods enabling tests to be carried out on the single-cell scale.

This paper describes a modification of the method of disc microelectrophoresis in polyacrylamide gel in capillary tubes, capable of detecting the protein composition, activity, and isoenzyme spectrum of lactate dehydrogenase (LDH) in single rat and mouse oocytes during cleavage.

EXPERIMENTAL METHOD

Noninbred albino rats and CBA and C57BL mice were used. The day on which spermatoza were discovered in the vaginal smears of the rats was taken as the first day of pregnancy. The first day of pregnancy in mice was determined by the discovery of a vaginal plug.

Cleaving oocytes at the given period of development were obtained by washing out the isolated uterine cornua (fifth day of pregnancy in rats, fourth day of pregnancy in mice) or the oviduct (2nd-3rd day of pregnancy) with warm medium No. 199 made up from Hanks' solution with the aid of a micropipet. This procedure and the subsequent manipulation with the oocytes were carried out under the control of the MBS-2 stereoscopic loupe under a magnification of 60 ×. For the morphological study and photography of the embryos a type MBI-6 microscope with phase-contrast attachment was used. To remove the zona pellucida

Institute of Experimental Medicine, Academy of Medical Sciences of the USSR, Leningrad. (Presented by Academician of the Academy of Medical Sciences of the USSR V. S. Il'in.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 78, No. 7, pp. 50-52, July, 1974. Original article submitted August 2, 1973.

the oocytes were placed in a 0.5% solution of pronase (Sigma, West Germany), made up in 0.06 M Tris-HCl, pH 7.3 [5]. After enzymic destruction of the zona pellucida the oocytes were quickly transferred into 1 ml of warm medium No. 199 and again into fresh medium No. 199 to remove all the pronase. Next, by means of a microsyringe, one embryo was drawn into a specially shaped micropipet with an internal diameter of 150 μ and a volume of 0.05-0.1 μl and transferred into 2 or 3 volumes of medium to produce lysis of the oocyte (2% Triton X-100, 0.2% bromphenol blue, 0.015 M Tris-HCl, pH 7.3). The completeness of destruction of the oocytes in the detergent solution was verified under the loupe. The lysate of the oocyte was then quickly tranferred to a capillary tube in which electrophoresis was carried out.

The proteins of the lysed oocytes were fractionated by the technique of vertical microelectrophoresis in capillary tubes as developed by Sandakhchiev et al. [1]. Calibrated glass capillary tubes 460-470 μ in diameter and 30 mm long were used. The inner surface of the capillary tubes was rinsed with chromate mixture, water, and absolute ethanol and then treated with the water repellant 0.3% dimethyldichlorosilane made up in freshly distilled benzene. Electrophoresis was carried out in 7% polyacrylamide gel, previously equilibrated with 0.38 M Tris-HCl, pH 9.2. The gel was polymerized actually in the capillary tubes. The capillary tube with gel was placed in a special cell and a layer of buffer was drawn above the meniscus of the gel by means of a micropipet. The micropipet was fixed to a micromanipulator and connected to the microsyringe. The surface of the gel was covered with 0.1 μl concentrating buffer (0.06 M Tris-HCl, pH 6.7, 20% sucrose), above which the lysate of the oocyte was applied in a volume of 0.1-0.3 μl, and above this the electrode buffer (0.005 M Tris, 0.038 M glycine, pH 8.3). During concentration of the sample electrophoresis was carried out with a voltage of 150 V. When the anionic front reached the beginning of the gel (bromphenol blue was used as the indicator) the voltage was increased to 300 V and the anionic front was passed to within 3-5 mm (measured with the ocular micrometer) of the meniscus. After disconnection of the current the gel was expelled from the glass capillary tube by means of a steel needle into a 0.1% solution of Amido Black in 7% acetic acid in order to stain it for protein. To determine LDH activity the gel was placed in 7 ml of incubation medium of the following composition: 2 ml 0.5 M K-phosphate buffer, pH 7.4; 1 ml 0.05 M $MgCl_2$; 1 ml 0.01 M lactate, neutralized with Na_2CO_3 to pH 7.4; 4 mg NAD; 4 mg nitro-blue tetrazolium; 0.4 mg phenazine metasulfate in 3 ml H_2O. The gel was incubated in the medium for 30 min at 37°C. This was followed by fixation in 7% acetic acid. To remove the bromphenol blue the gel was kept for a short time in a solution of glacial acetic acid. The stained gel was then drawn from the fixing fluid into the capillary tube, the ends of the capillary tube were sealed with plasticine, and densitometry was carried out with a two-color microphotometer.

EXPERIMENTAL RESULTS

A typical picture of fractionation of the solubilized proteins of a rat embryo at the two-blastomere and blastocyst stage is shown in Fig. 1. Control experiments with pure serum albumin showed that the method

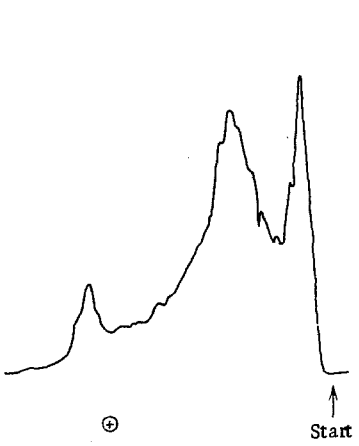

Fig. 1

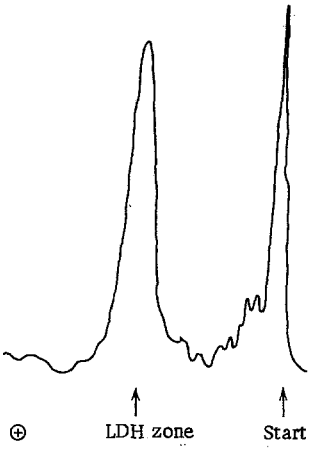

Fig. 2

Fig. 1. Densitogram of solubilized proteins of a rat blastocyst fractionated by disc microelectrophoresis (13 embryos used).

Fig. 2. Detection of LDH activity in a single rat embryo at the two-blastomere stage.

used is capable of reliably determining 10^{-9} g protein. According to Brinster [6], a single cleaving mouse oocyte may contain about 26 ng protein. Consequently the method can be used to study the protein spectra of single rat and mouse oocytes.

The method of capillary disc microelectrophoresis described above was used to study LDH isoenzymes in cleaving oocytes of noninbred albino rats and of CBA and C57BL mice from the two-blastomere to the blastocyst stage. The results showed that in this period of development rat and mouse embryos contain only one isoenzyme with relatively low mobility (Fig. 2). All the embryos evidently contain the identical isoenzyme, for when many oocytes were investigated no additional bands could be found. It is not clear whether the LDH of the oocytes corresponds to one of the LDH isoenzymes of the differentiated cells, as some workers have postulated [2, 10].

LITERATURE CITED

1. L. P. Naumova and L. S. Sandakhchiev, in: Ultramicroanalysis of Nucleic Acids [in Russian], Moscow (1973).
2. S. Auerbach and R. L. Brinster, Exp. Cell Res., 46, 89 (1967).
3. J. D. Biggers, J. Reprod. Fertil., 14, Suppl. 41 (1971).
4. J. D. Biggers, in: R. J. Blandau (editor), The Biology of the Blastocyst, Chicago (1971), p. 319.
5. P. Bowman and A. McLaren, J. Embryol. Exp. Morph., 24, 331 (1970).
6. R. L. Brinster, in: Mammalian Reproduction, Second Colloquim of the Society for Biological Chemistry, New York (1970), p. 229.
7. J. Knowland and C. Graham, J. Embryol. Exp. Morph., 27, 167 (1972).
8. C. Manes and J. Daniel, Exp. Cell Res., 55, 261 (1969).
9. L. Piko, Develop. Biol., 21, 257 (1970).
10. J. Rapola and O. Koskimies, Science, 157, 1311 (1967).
11. H. R. Woodland and C. F. Graham, Nature, 221, 327 (1969).