INTRACELLULAR LOCALIZATION OF GLYCOLYTIC ENZYMES IN CROSS-STRIATED MUSCLES OF LOCUSTA MIGRATORIA*

D. Pette and H. Brandau
Physiologisch-Chemisches Institut, Universität Marburg
Deutschland

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In rabbit skeletal muscle up to 2/3 of the total myogen may be accounted for by the protein of the hitherto crystallized enzymes in the EMBDEN-MEYERHOF chain (EMC) [ENGELHARDT, 1941; c.f. CZOK and BÜCHER, 1960]. The extramitochondrial (e.m.) location of these enzymes leads to the conclusion that the essential moiety of the e.m. myogen fraction is represented by the protein of the glycolytic enzymes. From the comparison of different cross-striated muscles in locusta migratoria evidence was found indicating that the e.m. myogen may be located predominantly within the isotropic zones of the myofibril apparatus [VOGELL et al., 1959]. Electronmicroscopically controlled extractions of the flight muscle in locusta migratoria have shown that pronounced substantial defects within the interfilamental spaces, but not within the interfibrillar sarcoplasm or endoplasmic reticulum, result from the extraction of the e.m. myogen [PETTE, BROSEMER and VOGELL, 1962]. However, in the same muscle the histochemical demonstration of glyceraldehyde-3P dehydrogenase (GAPDH) and lactate dehydrogenase (LDH) by means of the diaphorase-coupled reduction of tetrazolium salts [c.f. PEARSE, 1961] results in an intra- (i.m.) or perimitochondrial formazan deposition [HESS and PEARSE, 1961; PETTE and BRANDAU, 1962]. Obviously, this finding does not indicate the true intracellular location of the two dehydrogenases, but rather is due to the i.m. site of the diaphorase, since in this coupled reaction of dehydrogenase and diaphorase the reduction of the tetrazolium salt takes

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place at the site of the diaphorase [FARBER et al., 1956].

This disadvantage has been overcome in a newly developed method for the histochemical demonstration of pyridine-nucleotide linked dehydrogenases at the site of their action [PETTE and BRANDAU, 1962]. This method proves to be independent of the histotopical and intracellular distribution of the diaphorase activity, as the staining reaction is caused by contact of the tissue slice with a thin and plane film of agar-gel which contains, in homogeneously dispersed form, the substrates of the reaction [c.f. DAOUST, 1957, 1961] as well as the tetrazolium salt and purified DPN-specific diaphorase, or instead of the latter phenazine methosulfate which is able to replace the diaphorase since it acts as a redox-mediator between the reduced form of DPN or TPN and the oxidized form of the dye [SINGER and KEARNEY, 1954; ALLEN, 1961]. Thus, by the contact of the tissue slice with the gel layer the heterogeneous distribution of the diaphorase activity within the tissue is compensated and the reduction of the tetrazolium salt is rendered possible directly at the site of the dehydrogenase activity.

This technique has been applied to the intracellular localization of GAPDH and LDH in the flight muscle and the two leg muscles (m.extensor tibiae, m.flexor tibiae) of locusta migratoria. The specific staining of the two dehydrogenases results in a corresponding pattern of the intracellular formazan deposition in these muscles. In addition to a moderately positive reaction of the mitochondria within the interfibrillar spaces, a distinct staining of the myofibrils is observed. In the relaxed fibers the formazan is deposited in a pattern of cross-striations, which can be identified by analysis in the polarization microscope as the isotropic zones of the myofibrils.

The results obtained are in agreement with the conclusions drawn from fractionated extractions of these muscles [VOGELL et al., 1959; PETTE, BROSEMER and VOGELL, 1962; RHODE, 1962]. Furthermore, they seem to be supported by the finding of EMMART et al. (1962). By means of the fluorescent antibody

technique these authors have localized GAPDH within the isotropic zones of the myofibrils in the wing and leg muscles of periplaneta americana although this finding was interpreted as indicating an i.m. location of the enzyme. As mentioned above, a positive staining reaction of the mitochondria is also observed when the activities of GAPDH and LDH are demonstrated in the wing and leg muscles of locusta migratoria by means of the gel-film technique. However, an i.m. location of these two enzymes can be deduced neither from the results obtained by the fractionated extraction of these muscles nor from the enzyme pattern of isolated flight muscle mitochondria [DELBRÜCK et al., 1959; VOGELL et al., 1959; PETTE, BROSEMER and VOGELL, 1962; RHODE, 1962]. Therefore, it seems more probable that the positive staining of the mitochondria results from the perimitochondrial location of a certain part of the activities of GAPDH and LDH, although it is possible that this phenomenon is due to diffusion and to an interference of the i.m. diaphorase activity. Nevertheless, the essential part of the activity of these two enzymes is demonstrable within the isotropic zones of the myofibrils and therefore proves a type of location, which has already been found to be representative for the intracellular distribution of glycogen in these muscles [SIESS and PETTE, 1960].

Besides the fact that the total activity of the complete sequence of the glycolytic enzymes is extractable from the e.m. compartment, a surprising congruence of the individual extraction kinetics is found for these enzymes in the three muscles examined [PETTE, BROSEMER and VOGELL, 1962; RHODE, 1962]. This finding favours the assumption of an identical aggregate condition and intracellular location for these enzymes within the e.m. compartment of the muscle cell. Therefore, by combining the findings from the fractionated extraction with the results obtained from the direct intracellular staining of GAPDH and LDH, it may be concluded that an essential part of the activity of the glycolytic enzymes in these three muscles is located within the isotropic zones of the myofibrils.

From the moiety of the e.m. myogen and from the space available within the interfilamental interstices in the isotropic zones as estimated from the electron micrographs [VOGELL et al., 1959; PETTE, BROSEMER and VOGELL, 1962], the concentration of the myogen within the isotropic zones can be approximately calculated. This calculation has been made for the indirect flight muscle of locusta migratoria. Assuming that 50 % of the e.m. myogen (i.e. 17 mg of protein per gram of fresh weight) is located within the isotropic zones, and that the space available in the interfilamental interstices amounts to 8 % of the cellular volume, a concentration is found which corresponds to a 20 % protein solution.

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