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Limited proteolysis of bovine muscle and heart lactate dehydrogenase is inhibited by phospholipid liposome interaction

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Limited proteolysis of phospholipid complexes of heart and muscle bovine lactate dehydrogenase by trypsin and chymotrypsin has been studied under nondenaturing condition at pH 7.5. Chymotrypsin cleaves the polypeptide chain of heart and muscle lactate dehydrogenase into two principal fragments and LDH subunits were protected by lipids towards the proteinase attack. Enzymatic activity of heart and muscle lactate dehydrogenase was abolished by limited proteolytic cleavage. In complexes, both isoenzymes were protected against proteinases attack by lipids.

A number of reports have proved that the lactate dehydrogenase isozymes should be included in the class of glycolytic enzymes which have been found to have the ability of reversible association with subcellular membrane structures [1–3]. Because of their capacity to distribute between two locations (cytosol and membranes) Wilson [4] called the enzymes 'ambiquitious'. Some recent studies [5,6] have suggested that interaction between a macromolecule and membrane may well influence the degree of exposure of portions of the molecule which are sensitive to proteolysis and affect protection in this may. On the other hand, membrane binding of a protein may with equal facility be visualized as acting to enhance its rate of degradation. There is an increasing number of examples in the literature where membrane association of a protein appears to precede an involvement with lysosomal proteolysis, and recent theories on the mechanism of control of protein degradation rely heavily on the concepts of interactions between proteolysis enzymes, their inhibitors, and cellular structure [7]. This behaviour raises interesting possibilities of metabolic rechanneling, and points to the desirability of research studies in the field of interaction closely investigating the possibility that biphasic interactions contribute not only to the short-term metabolic response but also to the longer-term adjustments to variations in the physiological environment.

In our previous papers [8,9] the interaction of two types of bovine lactate dehydrogenase (muscle and heart) with erythrocyte lipid liposomes as a model membrane was investigated by ultracentrifugation. In the presence of erythrocyte lipid liposomes bovine muscle and heart lactate dehydrogenase form two kinds of complexes: lactate dehydrogenase adsorbed to liposomes and soluble molecular lactate dehydrogenase-phospholipid complexes.

The two complexes reveal different dependence of their stability on pH values. The soluble muscle lactate dehydrogenase complexes become unstable when the pH values decrease, and heart lactate dehydrogenase complexes become unstable with increasing pH values.

In the present work we have investigated the limited proteolysis of muscle and heart lactate dehydrogenases and their insoluble phospholipid complexes. Trypsin (crystallized, bovine type XI) and trypsin inhibitor (ovumucoid egg type II-O) were purchased from Sigma, α-chymotrypsin was purchased from Worthington and phenylmethylsulfonyl fluoride was purchased from Calbiochem. Bovine heart lactate dehydrogenase (LDH-1) was purchased from POCH (Gliwice). Bovine muscle lactate dehydrogenase (LDH-5) was prepared from bovine muscle according to Pesce et al. [10]. The muscle and heart preparation had a specific activity of 300 U/mg and gave one band during polyacrylamide gel electrophoresis. To prepare the enzyme for experiments the lactate dehydrogenase suspension was centrifuged down and the pellet was dissolved in 50 mM Tris-HCl (pH 7.5) and dialysed for 48 h against the same buffer at 5 °C. This procedure produced the apo-form of the enzyme. The amount of lactate dehydrogenase in suspension was determined by the enzyme activity assay according to the method of Bergmeyer et al. [11].

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Lipids were extracted from bovine erythrocyte with *n*-butanol according to the method described by Zahler et al. [12]. The butanol from the lipids was evaporated to dryness under a nitrogen stream. To the thin lipid film obtained the appropriate amount of buffer solution was added and the liposome suspension was produced by mechanical shaking with glass beads for 30 min at room temperature. The concentration of lipids was calculated indirectly by phosphorus determination according to Bartlett [13]. Preparations of liposomes were not contaminated by trypsin and chymotrypsin inhibitors. Addition of increasing amounts of the liposomes to the solution of trypsin or chymotrypsin does not change their enzymatic activity which was determined according to Hummel [14] and Schwert et al. [15].

Limited proteolysis has been studied under nondenaturing conditions at pH 7.5. The reaction mixture included 5 mg protein/ml of LDH or enzyme-phospholipid complexes and 0.05 mg/ml proteinase (trypsin or chymotrypsin) in 50 mM Tris-HCl buffer (pH 7.5) and 10 mM $MgCl_2$. The reaction mixtures were held at 19–20 °C and after various time intervals 5-μl samples were withdrawn from each mixture and introduced both into the sample buffer containing 2% SDS for gel electrophoresis and into a stop solution for subsequent assay of residual LDH activity. The stop solution contained trypsin inhibitor at a concentration of 0.065 mg/ml for trypsin, and phenylmethylsulfonyl fluoride at a final concentration of 1 mM for chymotrypsin experiments; 0.1 M NaCl was added to dissociate the complexes.

Fig. 1 displays, by means of polyacrylamine gel electrophoresis in the presence of SDS, the products of

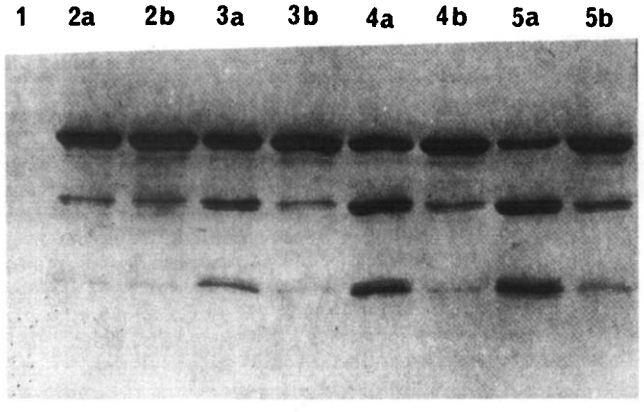

Fig. 1. Limited proteolysis of H-LDH and its insoluble complexes by chymotrypsin, shown by SDS-polyacrylamide electrophoresis of digestion products. The products of greatest mobility run towards the bottom of the gel. Samples are in pairs, with those labeled b identical to the corresponding sample labeled a, except that the digestion mixture of b contains insoluble complexes of H-LDH. Samples are: 1, chymotrypsin control (nothing visible); 2, H-LDH digested for 1 min; 3, H-LDH digested for 30 min; 4, H-LDH for 60 min; 5, H-LDH digested for 120 min.

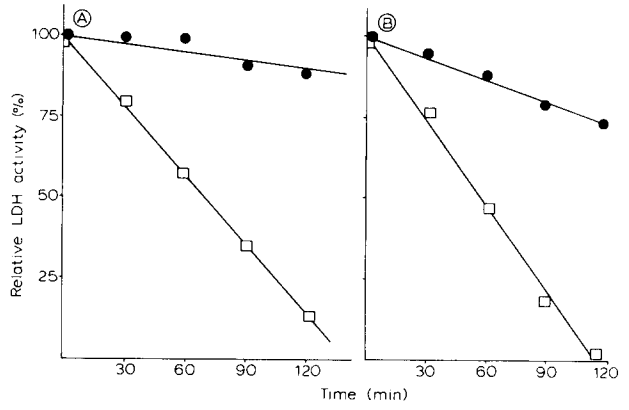

Fig. 2. The effect of phospholipid liposome interaction on H-LDH activity as a function of time after the initiation of (A) tryptic and (B) chymotryptic digestion of H-LDH. ○, insoluble complexes of H-LDH; ●, H-LDH.

chymotrypsin digestion of heart lactate dehydrogenase and its insoluble phospholipid complex. Slot 2 contains a sample of LDH after the initiation of digestion. As the time of digestion increases (slots 3 to 5) the amount of protein in the subunit band decreases. Very similar results were obtained for muscle results were obtained for muscle lactate dehydrogenase and its insoluble phospholipid complexes (data not shown). It may be see, that lactate dehydrogenase subunits were protected by phospholipids agents attack by chymotrypsin.

Enzymatic activity is abolished by limited proteolytic cleavage as is shown in Fig. 2. After about 2 h of proteolysis with either trypsin or chymotrypsin at 19 °C, the activity has been lost completely. The residual activity at a given time after the initiation of digestion by chymotrypsin is qualitatively proportional to the amount of material remaining in the LDH subunit band found in electrophoresis. This suggests that the cleavage which produces two fragment bands is also responsible for the enzymatic activity loss. In phospholipid-LDH com-

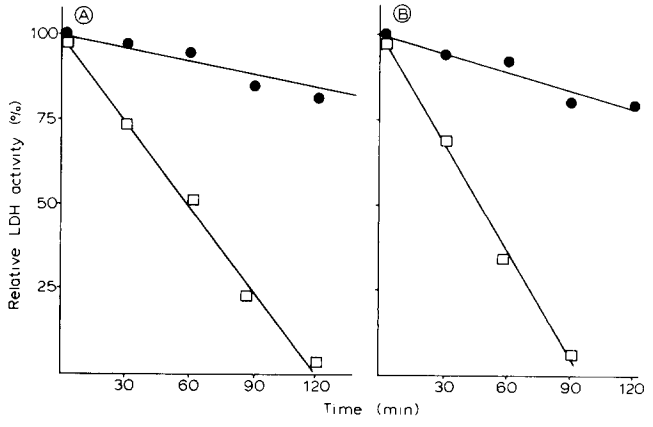

Fig. 3. The effect of phospholipid liposome interaction on M-LDH activity as a function of time after the initiation of (A) tryptic and (B) chymotryptic digestion of M-LDH. ○, insoluble complexes of M-LDH; ●, M-LDH.

plexes, the activity of the enzyme was retained during incubation. It may be concluded, that phospholipids showed a protective effect on the enzyme against proteolysis. Very similar results were obtained for muscle isozyme and its complexes (Fig. 3). The assumption can be made that the peptide susceptible to proteolysis may be located in, or close to, the phospholipid binding site of the enzyme. An alternative interpretation of the data would be that proteinases can adsorb at another site of the phospholipid liposomes than LDH isozymes. This adsorption does not reduce the catalytic activity of the proteinases but makes the contact between proteinase and LDH isozymes more difficult.

The question of whether the phenomenon of proteinase inactivation of lactate dehydrogenase isozymes and their complexes is physiologically relevant needs further investigation.

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