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Multi-enzyme aggregates: New evidence for an association of glycolytic components

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SUMMARY

Sedimentation studies on a cytosol fraction of rat muscle have provided evidence in support of the occurrence of a multi-enzyme aggregate of glycolytic components under physiological conditions of pH and ionic strength.

Reasons for previous failures to detect such associations in mammalian tissues are discussed.

Modern techniques of subcellular fractionation have afforded a satisfying understanding of the disposition of macromolecular components between the various subcellular organelles, but have left in some doubt the question of whether the individual enzymes in the cytosol associate amongst themselves or with the membraneous surfaces in the cell[1]. In relation to the sequence of enzymes involved in glycolysis, for example, although these components are generally identified as constituents of the "soluble" fraction, the possible existence of a glycolytic complex and the inherent metabolic advantages of such an assembly have been widely canvassed[2–4]. To this date, though, specific tests for an association of glycolytic enzymes in the cytosol by sedimentation techniques have failed to detect any significant presence of multi-enzyme aggregates[1,5]. In the light of recent studies with mammalian tissues which have provided indications of macromolecular interactions involving important glycolytic enzymes[6,7], however, it was decided to reinvestigate the possibility of multi-enzyme association under conditions which it was considered would more closely simulate physiological situations. As a consequence of these studies, the present communication is able to provide new and proemial evidence of a significant degree of association between some of the main glycolytic enzymes in a cytosol fraction of rat skeletal muscle.

For these investigations, a myogen preparation was produced from the hind leg muscles of adult rats by the procedure of Beisenherz *et al.*[8] as described by Arnold and Pette[9]. Essentially, this methodology involved a buffered extraction of muscle mince, centrifugation at 100 000 x *g* for 1 h, and ammonium sulphate fractionation between 1.5 M and 3.2 M. The one variation of the cited methodology was that 0.1 M phosphate buffer, pH 7.5, was used for extraction of the muscle mince. The ammonium sulphate suspension of myogen resulting from this procedure was centrifuged, and the pellet resuspended in 0.005 M imidazole, 0.002 M mercaptoethanol, 0.15 M KCl; pH 7.0, and then extensively dialysed against this buffer solution. After dialysis any precipitate was removed by centrifugation at 11 000 x *g* for 15 min in an International PR-6 Refrigerated Centrifuge. Examination of this fraction with the electron microscope confirmed the absence of any residual membrane fragments. The dialysed myogen preparation, adjusted to the desired protein concentration, was then centrifuged at 45 000 rev./min for 90 min in special 2 ml adaptors in the SW50L rotor of a Beckman L2-65B ultracentrifuge at 4 °C. Following centrifugation, 25–30 fractions were collected in sequence from the bottom of the tubes and assayed for enzyme activity. Assays of each fraction were carried out in triplicate. Control centrifugations were carried out under identical conditions using commercially pure samples of each of the enzymes; these enzymes being obtained from Sigma Chemical Co., St. Louis, Mo. Aldolase was assayed by a procedure essentially the same as that described by Blostein and Rutter[10]; lactate dehydrogenase activity by the procedure of Wroblewski and La Due[11]; pyruvate kinase according to the method of Bucher and Pfleiderer[12]; and, phosphofructokinase by the method of Racker[13]. All assays were carried out in 0.05 M Tris–HCl, pH 7.5, (except phosphofructokinase which was assayed at pH 8.2) in a final volume of 1.0 ml with a Unicam SP800 recording spectrophotometer at 30 °C. Protein was assayed by the method of Lowry *et al.*[14].

Typical sedimentation profiles of aldolase, lactate dehydrogenase and pyruvate kinase activities obtained after centrifugation of rat muscle myogen preparations are presented in Fig. 1, where the ratio of the activity in each fraction to total activity in the original myogen sample is plotted against radial distance. It will be noted from these profiles that each of these enzymes exhibited two distinct boundaries. The initial incomplete boundary at the top of the tube was shown by control centrifugations of the pure enzymes to correspond to the sedimentation of the free enzyme. Additionally prolonged sedimentation runs on the myogen preparation resulted in the formation of complete boundaries which yielded S values corresponding to those expected for these enzymes, as shown by comparable runs with the purified enzymes. The major point of interest in the sedimentation profiles, however, was the presence of a significant second boundary for each of the three enzymes tested; this boundary sedimenting at a much faster rate than that expected for the free enzyme. No such boundary was detected in the control centrifugations of the purified enzymes. The results presented in Fig. 1 demonstrate that these boundaries occurred in parallel with the sedimentation profile for each of the three enzymes, and an uncorrected sedimentation coefficient of 23 S was estimated for this second boundary.

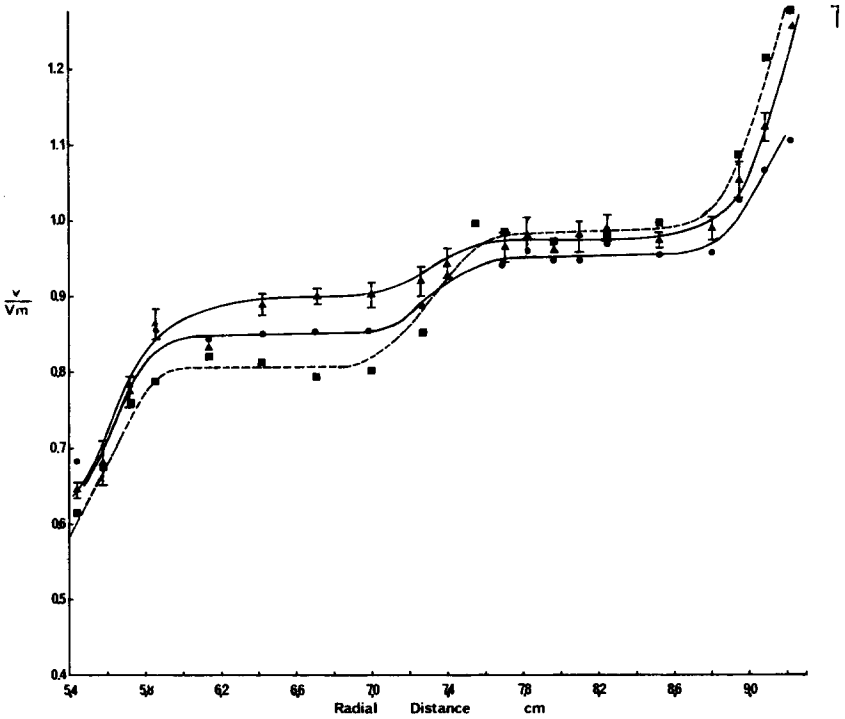

Fig. 1. Enzyme sedimentation in myogen preparation. •, aldolase; ▲, lactate dehydrogenase; ■, pyruvate kinase. Individual values are given as the means of six determinations. For the purposes of clarity, the standard errors of the mean are represented for lactate dehydrogenase only. Conditions of centrifugation as described in text. Myogen protein concentration, 19.6 mg/ml. v/V_m is the ratio of the activity in each fraction to that in the original myogen sample.

In addition to these three enzymes, an endeavour was made to study the sedimentation characteristics of phosphofructokinase in the rat muscle myogen preparation, an attempt which was prompted by the known importance of this enzyme in the regulation of glycolysis[15] and its well-characterized aggregation properties[16,17]. The phosphofructokinase was found to be extremely labile, however, and little or no activity survived the dialysis procedure and remained available for measurement in the centrifugation studies. Other authors have also reported that the phosphofructokinase of rat muscle is an unstable enzyme[18]. Subsequent studies in concentrated myogen preparations from other species using modified buffer conditions, however, have allowed the preservation of phosphofructokinase activity and the demonstration of a boundary for this enzyme which also sediments in parallel with the second boundary previously described for the three other glycolytic enzymes.

The present data, then, may be taken as providing the first definitive evidence from sedimentation studies in support of a multi-enzyme aggregate of these important glycolytic components, occurring in a cytosol fraction of mammalian tissues under physiological conditions of pH and ionic strength.

The extent of contribution from other components towards the association of these enzymes remains to be fully defined. It may be noted, however, that up to 70% of the protein in such myogen preparations has been estimated to be contributed by the glycolytic enzymes[19], and consequently the occurrence of a similar second sedimentation boundary in the case of measurements of total protein concentration was not unexpected. The dimensions of the inflexion in this protein curve were generally less than those displayed by the individual glycolytic enzymes, indicating a degree of specificity in the association. It is also significant that the present studies point to this complex of glycolytic components being very sensitive to factors such as pH, ionic strength, and the concentrations of proteins and metabolites. When considered in conjunction with the results of other lines of investigation affecting the interactions of tissue components[6,20,21], these data may be considered to provide valuable new insight into the reasons why the existence of such complexes have not been substantiated previously[1,5], and emphasize the need for a continuing serious consideration of the potential influence such enzyme aggregates may wield upon the efficiency and control of glycolysis in the cellular situation.

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