A Multienzyme Aggregate with Glycolytic Activity from *Escherichia coli*

DIANE M. GORRINGE* and V. MOSES

*Department of Plant Biology and Microbiology, Queen Mary College, London E1 4NS, U.K.*

The degree of intermolecular organization among the component enzymes of glycolysis is a controversial matter. In spite of attempts to resolve the disagreements (Green et al., 1965; Hubscher et al., 1971), direct evidence for a glycolytic multienzyme complex for long remained unconvincing. It was therefore of great interest when data were provided that suggested that interactions between glycolytic enzymes is a real possibility (Clarke & Masters, 1973, 1974; Mowbray & Moses, 1976). Conventional methods of cell disruption are probably not conducive to the preservation of the structural and functional integrity of delicate enzyme macro-structures. *Escherichia coli* was therefore

* Present address: School of Biological Sciences, University of East Anglia, Norwich NR4 7TJ, U.K.

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![Graph](image_url)

**Fig. 1.** *Elution profile for a concentrated sample from the supernatant of a lysed sphaeroplast preparation*  
After centrifugation of the lysate at 1000g, for 15 min, it was applied to an agarose A-1.5M gel column and eluted with 0.1 M-potassium phosphate buffer, pH 7.4, containing 0.125 M-NaCl, 1 mM-MgSO₄ and 1 mM-mercaptoethanol. Fractions were collected and assayed for enzyme activity: ■, phosphofructokinase; ◊, phosphoglucose isomerase; ●, glyceraldehyde 3-phosphate dehydrogenase; ▲, triose phosphate isomerase. One unit is 1 μmol of substrate converted/min.
chosen as the organism from which to attempt the isolation of a glycolytic multienzyme particle, since, by first forming stable sphaeroplasts, the cells may be gently ruptured.

A glycolytic complex containing one of each of the appropriate enzymes would have mol. wt. about $1.4 \times 10^6$. Such an aggregate particle could be readily separated from the individual glycolytic enzymes by a molecular-sieve technique, provided that it did not dissociate too rapidly on dilution.

The soluble fraction obtained at $1000 \text{ g}_{av.}$ from a lysed lysozyme–EDTA sphaeroplast preparation was concentrated by ultrafiltration and applied to an Agarose A-1.5M Bio-Gel column. The elution profile (Fig. 1) demonstrates that two species of glycolytic enzymes were eluted from the column. Most of the enzyme activity appeared in the low-molecular-weight region, the enzymes being eluted as individual entities in order of their molecular weights. However, a proportion of each enzyme activity was found in the high-molecular-weight region of the eluate, with those activities showing a high degree of co-chromatography. By using a column calibrated with a series of marker proteins of known molecular weight, the peak of activity for the high-molecular-weight species corresponded to a mol. wt. of $1.15 \times 10^6$. This compares favourably with the predicted molecular weight for a multienzyme complex.

It was decided to determine whether the individual glycolytic enzymes of low molecular weight could reassociate to form the high-molecular-weight species. The fractions of the eluate encompassing the individual enzymes were therefore pooled and concentrated by ultrafiltration. A sample applied to an agarose A-1.5M column yielded an elution profile essentially similar to that obtained previously, showing that some degree of aggregation had taken place on concentration.

If the high-molecular-weight species represents the glycolytic enzymes associated in complex-formation, one might expect the enzymes there to be so arranged that intermediates are passed directly from one catalytic site to the next in an organized sequence. Such an aggregate of enzymes in a totally cell-free system, with each enzyme in close proximity and correct spatial relationship to the others, could be recognized only if it

![Fig. 2. Production of alanine from glucose by high-molecular-weight material](image)

A sample of the high-molecular-weight species, isolated from concentrated individual glycolytic enzymes via an agarose A-1.5M gel column, was incubated with $[^{14}\text{C}_6]\text{glucose}$ and the appropriate cofactors. The $[^{14}\text{C}]\text{alanine}$ formed was separated from the residual $[^{14}\text{C}_6]\text{glucose}$ by paper chromatography and assayed for $^{14}\text{C}$ content. Various dilutions of the high-molecular-weight material were used: ■, undiluted; ○, diluted 1:2 with buffer; ●, diluted 1:4 with buffer.
were reasonably stable and contained all the relevant enzyme activities. It must of necessity be capable of catalysing the complete integrated sequence of reactions.

The high-molecular-weight species originating from the concentrated individual enzymes was used to demonstrate throughput activity of the glycolysis pathway. Because such a preparation was obtained by concentration of lower-molecular-weight material, it would contain less adventitious inactive protein than would high-molecular-weight material isolated directly from the supernatant of lysed sphaeroplasts. A sample was incubated with [14C]glucose and the appropriate cofactors. The addition of alanine aminotransferase together with an excess of glutamic acid ensured the conversion into alanine of any pyruvate formed. The [14C]alanine produced in this way was separated from residual [14C]glucose and glutamic acid by paper chromatography and assayed for 14C content. When glucose was absent from the reaction vessel no alanine could be detected on the chromatograms by ninhydrin. It was therefore concluded that all the alanine produced by the complete system was derived by sequential steps from the substrate glucose. Fig. 2 shows the amount of [14C]alanine produced with time. Even at a very low concentration of total protein in the reaction vessel (as in the 1:4 dilution sample), [14C]alanine was still produced, although the specific radioactivity was clearly much decreased. Inclusion in the reaction mixture of unlabelled glycolytic intermediates decreased the incorporation of 14C into alanine to a much smaller extent than that expected if free mixing of intermediates had taken place.

Although these results do not prove unequivocally that the high-molecular-weight species represents a glycolytic multienzyme complex, one in which the enzymes are organized into a structure able to maintain its integrity even during passage through a gel column, some organization must exist for throughput activity to have been demonstrated in the cell-free system in the presence of excess quantities of unlabelled metabolites; that is, a direct handing-over of intermediates occurred through sequential steps. This is exactly the property expected of an organized multienzyme system. The structure we observed seems to be fairly stable, so that aggregation of the glycolytic enzymes in vitro is unlikely to be random and may well reflect the true state of the enzymes in vivo.

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Specificity of Nuclear Protein Kinases in Differentiating Chick Skeletal-Muscle Cells

NGUYEN THI MAN, GLENN E. MORRIS and ROBIN J. COLE

School of Biological Sciences, University of Sussex, Falmer, Brighton BN1 9QG, U.K.

There is evidence to suggest that proteins entering nuclei can alter the pattern of gene transcription (De Robertis & Gurdon, 1977) and other evidence that such regulatory proteins exist within the phosphoprotein fraction of non-histone proteins extractable from isolated chromatin (Stein et al., 1974; Kleinsmith, 1975). In the present report, we describe preliminary observations on the analysis of both nuclear protein kinases and the non-histone proteins that they phosphorylate in differentiating skeletal-muscle cell cultures as they undergo changes in their pattern of gene expression.

Embryonic-chick skeletal-muscle cells were prepared by mechanical dissociation, grown as previously described (Morris et al., 1976) and harvested on the third day when