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IMMOBILIZED HYBRIDS OF GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE

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Summary

Yeast glyceraldehyde-3-phosphate dehydrogenase (glyceraldehyde-3-phosphate:NAD^+ oxidoreductase (phosphorylating), EC 1.2.1.12) immobilized on CNBr-activated Sepharose 4-B has been subjected to dissociation to obtain matrix-bound dimeric species of the enzyme. Hybridization was then performed using soluble glyceraldehyde-3-phosphate dehydrogenase isolated from rat skeletal muscle. Immobilized hybrid tetramers thus obtained were demonstrated to exhibit two distinct pH-optima of activity characteristic of the yeast and muscle enzymes, respectively. The results indicate that under appropriate conditions the activity of each of the dimers composing the immobilized hybrid tetramer can be studied separately.

Studies on the hybridization of oligomeric enzymes have proved to be of considerable value in investigating subunit structure and function. This approach is very attractive for studying subunit interactions and molecular mechanisms of cooperative phenomena. Hybridization of glyceraldehyde-3-phosphate dehydrogenase, a tetrameric enzyme composed of four chemically identical subunits, was studied in several laboratories. The formation of hybrids between the enzymes of different origin was demonstrated [1—4], and the procedure of isolation of a hybrid composed of the yeast and rabbit muscle dehydrogenases was described [5]. It seems, however, that the studies of the properties of the hybrid molecules obtained with soluble forms of enzymes should be somewhat complicated due to the existence of equilibrium between the hybrids and the component enzymes [3—5].

Another approach to the hybridization studies is the use of an immobilized oligomer, which may be dissociated under appropriate conditions, followed by reassociation of matrix-bound subunits with soluble subunits of the partner enzyme. The possibility of obtaining the matrix-bound dimeric

form of glyceraldehyde-3-phosphate dehydrogenase capable of reassociating with the soluble subunits has been demonstrated in our previous study [6]. In this communication we describe the preparation and some properties of an immobilized hybrid tetramer composed of the yeast and muscle enzyme subunits, respectively.

Glyceraldehyde-3-phosphate dehydrogenase was isolated from rat skeletal muscle [7] and from baker's yeast [8]. Immobilization of the yeast enzyme on Sepharose 4-B was performed as described previously [6] using 10 mg CNBr/ml packed gel. The amount of Sepharose-bound protein was determined spectrophotometrically in a solution of polyethylene glycol, as described elsewhere.

The immobilized enzyme (120 μg protein/ml packed gel) retained 80% of activity of the soluble dehydrogenase. The dissociation was performed under the conditions described by Stancel et al. [9]. The suspension of matrix-bound protein was extensively washed at 4°C with 0.15 M NaCl/5 mM EDTA/4 mM 2-mercaptoethanol (pH 7.6). 1 ml 0.1 M ATP/0.15 M NaCl was then passed through 1 ml packed gel. Another 1 ml same solution of ATP was added and the suspension was incubated for 2 h at 4°C.

The immobilized enzyme was then washed with 0.1 M sodium phosphate (pH 8.3)/5 mM EDTA/4 mM 2-mercaptoethanol to completely remove ATP and dissociated protein. The above treatment results in a 55% decrease of the amount of matrix-bound protein and in a 60% drop of the catalytic activity. This suggests the dissociation of the tetrameric enzyme into dimers to have occurred. The following experiments on reassociation substantiate the conclusion that the loss of the immobilized enzyme content is due to the splitting of the non-covalently bound protein.

To 1 ml suspension of immobilized dimeric species of the yeast enzyme (0.5 ml packed Sepharose gel plus 0.5 ml 0.1 M sodium phosphate (pH 8.3)) was added 0.1 ml crude preparation of rat skeletal muscle glyceraldehyde-3-phosphate dehydrogenase (4.1 mg/ml in 0.1 M sodium phosphate (pH 8.3)). After 16 h incubation at 4°C under constant stirring, the suspension was washed until no dehydrogenase activity was detectable in the washings.

The association of the matrix-bound yeast enzyme subunits with the soluble subunits of the rat muscle dehydrogenase is evidenced by the change of the pH vs. activity profile of the immobilized enzyme. Fig. 1 shows that the immobilized tetrameric and dimeric species of the yeast dehydrogenase exhibit a similar pH-dependence of activity, which is analogous to that of the free form of the enzyme. The pH optimum of activity in 50 mM Tris·HCl is shifted to the left as compared with that observed in sodium pyrophosphate of the same concentration. Previously, we have demonstrated that this shift is caused by the change of the ionic strength of the buffer [10].

Unlike the yeast enzyme, glyceraldehyde-3-phosphate dehydrogenase isolated from rabbit and rat skeletal muscle reveal maximum activity at pH 8.7—9.2 in buffers of different ionic strengths [10, 11]. Immobilization of the rat muscle enzyme on Sepharose 4-B causes a shift of pH optimum of activity to 9.9—10.2 [11]. It is to be expected therefore that association of the muscle type subunits with the immobilized yeast subunits should bring about a change in the pH dependence of activity.

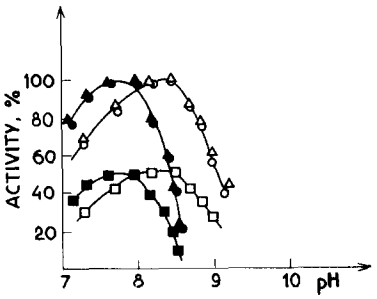

Fig. 1. Activity of free and immobilized yeast glyceraldehyde-3-phosphate dehydrogenase at different pH values. The assay mixture contained 5 mM EDTA/5 mM sodium arsenate/1.5 mM glyceraldehyde-3-phosphate/1.2 mM NAD^+/50 mM Tris · HCl (closed symbols) or 50 mM sodium pyrophosphate (open symbols) at 20° C and indicated pH values. ▲,△: free enzyme; ●,○: immobilized tetrameric enzyme; ■,□: immobilized dimeric form of the enzyme (% of activity of the tetrameric form).

As seen in Fig. 2, that was what we observed. Immobilized hybrid molecules exhibit in 50 mM sodium pyrophosphate a broad pH optimum of activity, suggesting the superposition of pH optima of the yeast and muscle species to have occurred. If 50 mM Tris · HCl was used instead of pyrophosphate, the activity of the yeast dehydrogenase could be revealed at more acid pH values, and two distinct pH optima of activity of the immobilized enzyme became apparent (Fig. 3).

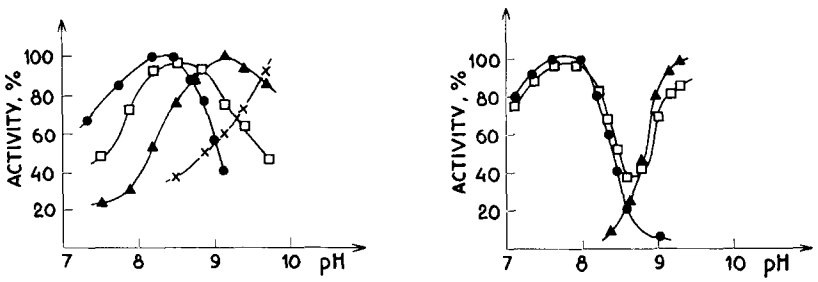

Fig. 2. pH vs. activity profiles of yeast and rat skeletal muscle glyceraldehyde-3-phosphate dehydrogenases, as well as of hybrids of these enzymes in 50 mM sodium pyrophosphate. Conditions are indicated in the legend to Fig. 1. X,▲: enzyme from rat skeletal muscle immobilized and free form, respectively; ●: immobilized dimeric form of the yeast enzyme; □: hybrid form.

Fig. 3. pH vs. activity profiles of yeast and rat skeletal muscle glyceraldehyde-3-phosphate dehydrogenases, as well as of the hybrids of these enzymes in 50 mM Tris · HCl. Conditions are indicated in the legend to Fig. 1. ▲: free rat skeletal muscle enzyme; ●: immobilized dimeric form of the yeast enzyme; □: hybrid form.

These results indicate that hybridization of the matrix-bound dimers of the yeast dehydrogenase with soluble muscle enzyme has really occurred. We believe that the ability to follow separately the catalytic activity of the yeast and muscle subunits within the native hybrid molecule should be useful in studies on subunit interactions in glyceraldehyde-3-phosphate dehydrogenase.

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