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The domain structure of transketolase from baker's yeast

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Transketolase from baker's yeast was shown by electron microscopy to have the form of a rectilinear tetrahedron and to consist of 4 structural units with a molecular mass of 30–40 kDa.

Domain structure; Electron microscopy; Transketolase

1. INTRODUCTION

Transketolase (sedoheptulose-7-phosphate: D-glyceraldehyde-3-phosphateglycolaldehydetransferase, EC 2.2.1.1) is a thiamine enzyme catalysing the transfer of a two-carbon fragment (active glycolaldehyde) from keto sugars (donor substrates) to aldo sugars (acceptor substrates). The enzyme has a molecular mass of 158–159 kDa [1,2] and is composed of 2 subunits of equal molecular mass [1–4].

The present paper provides experimental data on the domain structure of a transketolase molecule.

2. MATERIALS AND METHODS

Crystalline preparations of transketolase from baker's yeast were obtained by the method of Racker et al. [5] with minor modifications [6] and by immunoaffinity chromatography [7]. According to SDS PAGE data both preparations were devoid of contaminant proteins. Their specific activity was 15–23 E/mg.

The protein was determined spectrophotometrically, assuming $A_{280}^{0.1\%} = 1.45$ [8].

Transketolase, taken at a concentration of 50 μg/ml in 50 mM MOPS, 1 mM EDTA buffer, pH 7.6, was applied onto thin collodion film substrates and contrasted with 1% uranyl acetate. Micrographs were recorded on a Philips EM-400 electron microscope (magnification 50 000 at 80 kV).

3. RESULTS AND DISCUSSION

The results of electron microscopy studies of the transketolase three-dimensional structure are given in Fig. 1. The structure of enzyme preparations, obtained by two different methods, was found to be identical. This observation is supported by the presence of two characteristic projections of particles (Fig. 1a, see facing page). One of the most frequent projections (Fig. 1b) has the form close to an equilateral triangle with sides of 8–9 nm. The other projection of the particles (Fig. 1c) has the form of a rectangle or a square with sides of 7.5–8.5 nm. This projection contains 4 areas of electron density, an indication of the presence of 4, or divisible by 4, structural units in the enzyme. The triangular projection proves that the transketolase molecule is not flat. The observed projections enable us to conclude that the enzyme consists of 4 identical or morphologically similar units with the symmetry 222 (D_2). Thus, the form of the transketolase molecule is close to a rectilinear tetrahedron with sides of 8–9 nm. The volume of the structural unit corresponds to a molecular mass of 30–40 kDa.

The molecular mass of transketolase is 158–159 kDa [1,2]; this enzyme is a dimer with subunits of equal molecular mass [1–4]. Thus, it may be suggested that the subunits, in their turn, consist of two domains, similar in mass, each corresponding to one structural unit observed by electron microscopy.

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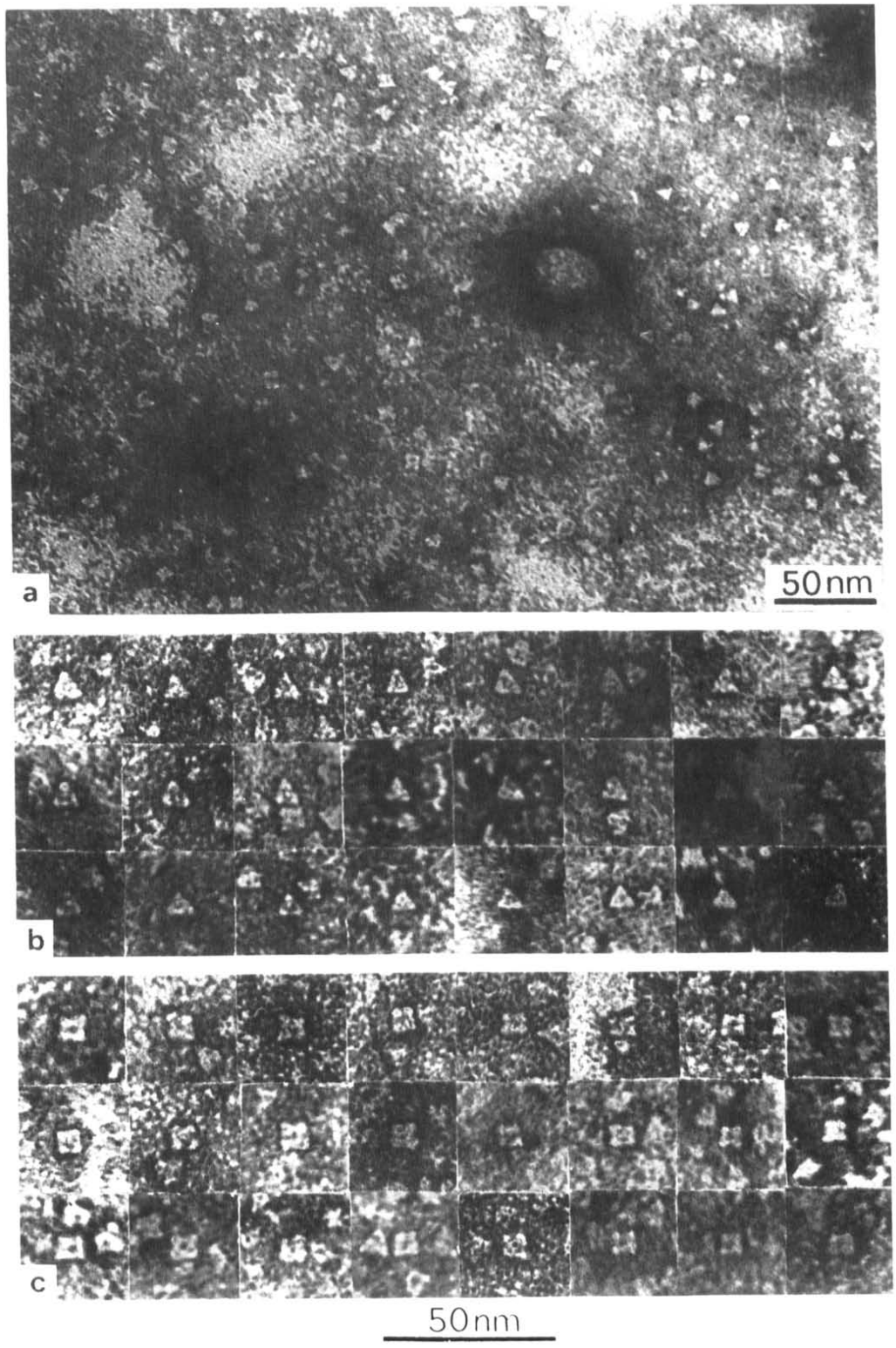

Fig. 1. Electron microscopy of transketolase from baker's yeast, obtained by immunoaffinity chromatography. Negative staining with uranyl acetate:
(a) general view of transketolase preparation;
(b) triangular projection of a transketolase molecule;
(c) rectangular projection.