An X-ray solution scattering study of the cofactor and activator induced structural changes in yeast pyruvate decarboxylase (PDC)

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Received 14 February 1990; revised version received 24 April 1990

Structure and activation pattern of pyruvate decarboxylase (PDC) from yeast was studied by synchrotron radiation X-ray solution scattering. The results give a direct proof that the reversible deactivation of PDC at pH 8.0 is accompanied by the dissociation of the tetrameric holoenzyme into dimeric halves. The kinetics of this process was followed. At pH 6.5 the dimeric halves reassociate to a tetramer even in the absence of cofactors. The changes of the scattering pattern upon binding of the substrate-like activator pyruvamide indicate that the structure expands in the course of the enzyme activation.

Pyruvate decarboxylase; X-ray solution scattering

1. INTRODUCTION

Pyruvate decarboxylase is an enzyme from the cytosol that catalyzes the decarboxylation of 2-oxoacids to the corresponding aldehydes. The enzyme extracted from yeast is an $\alpha_2\beta_2$ tetramer [1] with a molecular mass of 240 kDa [2]. In physiological conditions around pH 6.0 the two cofactors thiamine pyrophosphate (TPP) and Mg^{2+} are bound in a quasi-irreversible manner. At pH-values above 7.0 the cofactors are released and an apoenzyme is formed. Previous studies [3] suggested that simultaneously the tetrameric enzyme dissociates in two dimeric halves. Recombination of the apoenzyme into the holoenzyme takes place at pH values below 7.0 in the presence of TPP and Mg^{2+}.

The activity of PDC is regulated by the substrate as indicated by a sigmoidal dependence of the rate on substrate concentration [4–7] and a lag phase in the appearance of products corresponding to enzyme activation [8]. Conservation of the various regulatory states of the enzyme by cross-linking [9] and retardation of substrate activation by immobilization of the enzyme on a solid matrix [10] suggest that a structural change occurs during the transition from the inactive to the substrate-activated form. In the present solution X-ray scattering study we have investigated the structural changes during enzyme dissociation and recombination as well as during substrate activation.

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Abbreviations: PDC, pyruvate decarboxylase; TPP, thiamin-pyrophosphate

2. MATERIALS AND METHODS

PDC was extracted from yeast from the brewery Wernesgrün [1]. The enzyme is homogeneous as indicated by SDS-PAGE, with a specific activity of 50–60 units/mg. The activity was determined spectrophotometrically using alcohol dehydrogenase (ADH; Boehringer-Mannheim) and NADH (AWD Dresden) as complementary enzyme system [11]. Pyruvamide was synthesized according to Vogel and Schinz [12]. TPP was obtained from Serva (Heidelberg). All other chemicals were of analytic grade. Protein concentrations were determined at 280 nm using an extinction coefficient of 281 000 $M^{-1} \cdot cm^{-1}$.

The X-ray solution scattering measurements were made at 7°C on the X33 camera [13] of the EMBL in HASYLAB at the storage ring DORIS of the Deutsches Elektronen Synchrotron at Hamburg using the standard data acquisition and evaluation systems [14,15]. Static scattering patterns were recorded during 3 min in the range $1.5 \times 10^{-2} \leq s \leq 2.2 \times 10^{-1}$ nm^{-1}, where $s = 2\sin\theta/\lambda$, λ is the wavelength and 2θ the scattering angle. The dissociation of PDC was followed with a time resolution of 30 s.

3. RESULTS AND DISCUSSION

3.1. *Solution X-ray scattering patterns*

Solution X-ray scattering patterns of PDC were obtained in 0.1 M sodium citrate buffer, pH 6.2, in the protein concentration range 1–7.2 mg PDC/ml. In this range the forward scattering is proportional to the pro-

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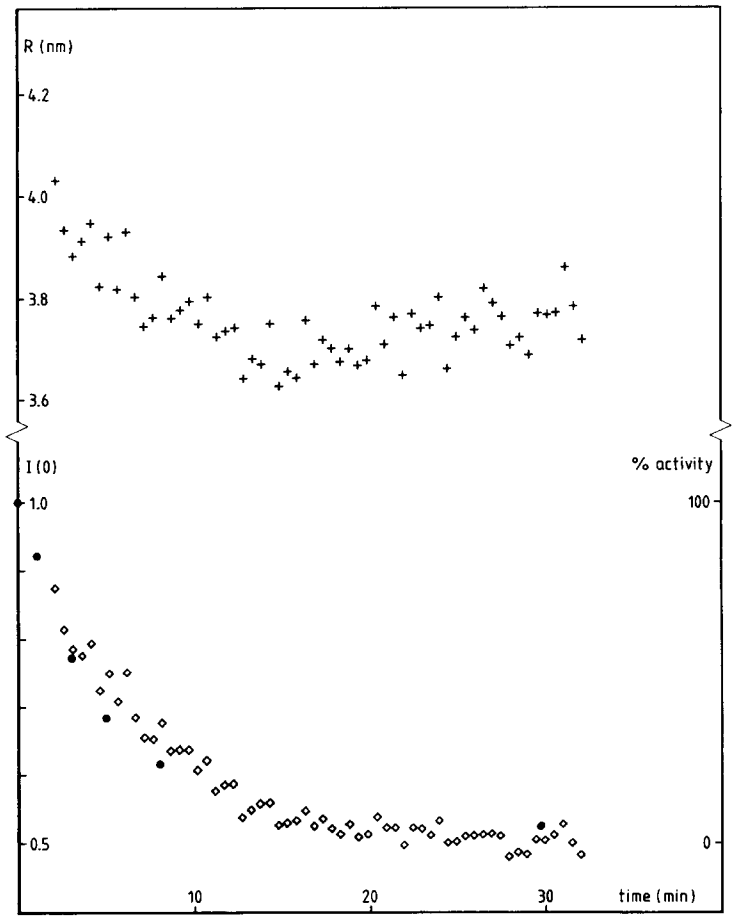

Fig. 1. Time dependence of the forward scattering (●), the enzymatic activity (◇) and the apparent radius of gyration during the dissociation of PDC in glycine/phosphate buffer pH 8.0. [PDC] = 7.2 mg/ml.

tein concentration and the radius of gyration has a constant value of 4.1 ± 0.1 nm, in agreement with previous observations [16]. A concentration-dependent aggregation of PDC can thus be excluded. Even after exposures of 30 min corresponding approximately to a dose of 0.5 Mrad no loss of enzymic activity could be detected.

3.2. *Dissociation of holo-PDC into apoenzyme and recombination of the apoenzyme with the cofactors*

Release of TPP and Mg^{2+} from holo-PDC was initiated by changing the pH from 6.2 to 8.0. The kinetics of this process can be monitored by the decrease in activity (Fig. 1) which has a rate constant of 6.7×10^{-4} s^{-1}. The decrease in activity is paralleled by a reduction of 50% of the forward scattering and of the radius of gyration by about 0.45 nm which occur at the same rate. This indicates that under the conditions used the formation of apo-PDC is accompanied by a change in quaternary structure. The reduction of the forward scattering corresponds to the dissociation of the holoenzyme in two dimeric halves as previously inferred [3]. The distance between the centres of the dimers in the tetramer calculated from the radii of gyration using the parallel axis theorem is 3.7 nm in agreement with previous observations [16].

After dissociation, adjusting the pH to 6.5 results in a rapid recovery of the forward scattering to its initial level, although the sample does not present measurable enzymic activity. Reactivation only occurs after addition of excess TPP and Mg^{2+}. The most significant changes in the scattering curves occur at $s > 0.1$ nm^{-1}. Thus, at pH 6.5 the dimeric halves reassociate to a tetramer even in the absence of cofactors. This tetramer then recombines with the cofactors to give the enzymatically active holoenzyme.

These results are in agreement with previous observations which had shown that the recombination rate is independent of the apoenzyme concentration and that protein association processes play no role in the recombination [10]. Further, it had been shown that at pH 6.2 the apoenzyme bound to a solid matrix can be recombined with the cofactors to the active form. This would not be expected if the enzyme existed under the form of dimeric halves at this pH.

Whereas, as illustrated in Fig. 2, the scattering for $s < 0.1$ nm^{-1} hardly changes after recombination with

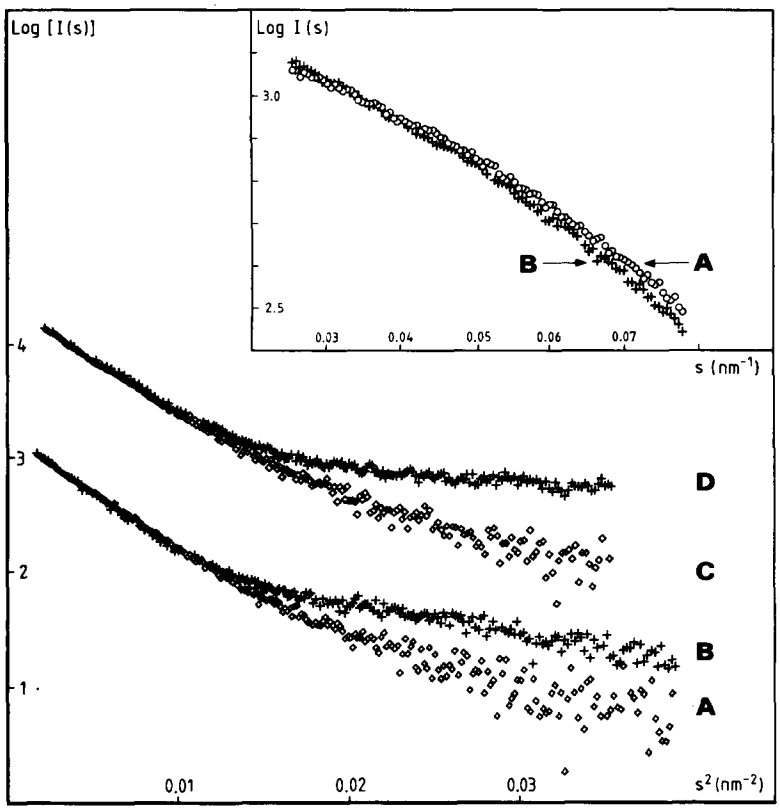

Fig. 2. Guinier plot of the scattering patterns of PDC (A and C), after addition of 50 mM pyruvamide (B) or 10 mM TPP + 10 mM Mg^{2+}, respectively (D). The two sets of curves are displaced by one logarithmic unit for better visualization. [PDC] = 7.2 mg/ml. Inset: plot of log $I(s)$ vs s for A and B.

the cofactors, there are significant differences between the pattern of apo- and holo-PDC at larger angles. These differences indicate, in agreement with circular dichroism studies [17], that upon binding of the cofactors, conformational changes take place in the subunits and/or their interfaces.

3.3. *Activation of PDC with the substrate-like activator pyruvamide*

Comparison of the scattering pattern of PDC in the presence and absence of pyruvamide (Fig. 2) indicates that significant changes occur at larger angles. The intensity in the low angle region is not affected by addition of pyruvamide, but there is an increase of the radius of gyration of PDC from 4.1 ± 0.1 nm to 4.3 ± 0.05 nm in the pyruvamide-activated enzyme. This indication of a structural change upon activation corresponding to an expansion of the enzyme correlates well with the accelerated $^1H/^3H$ exchange in the presence of pyruvate [18] as well as the enhanced reactivity of SH-groups of activated PDC with SH-reagents [19].

The observation of structural changes upon activation is also in line with previous studies indicating that fixation of PDC to a solid matrix strongly delays activation by the substrate [10] but that an active conformation can be conserved by appropriate cross-linking in the presence of substrate [19].

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