SELF-ASSOCIATION OF THE PYRUVATE DEHYDROGENASE COMPLEX FROM *AZOTOBACTER VINELANDII* IN THE PRESENCE OF POLYETHYLENE GLYCOL

Hans J. BOSMA, Gerrit VOORDOUW, Arie DE KOK and Cees VEEGER
Biochemisch Laboratorium, Landbouwhogeschool, De Dreijen 11, 6703 BC Wageningen, The Netherlands

Received 22 August 1980

1. Introduction

Bacterial pyruvate dehydrogenase complexes are composed of multiple copies of three different enzymes, i.e., pyruvate dehydrogenase (E_1, EC 1.2.4.1.), lipoate acetyltransferase (E_2, EC 2.3.1.12) and dihydrolipoamide dehydrogenase (E_3, EC 1.6.4.3). These three enzymes catalyse the overall reaction:

$$\text{Pyruvate} + \text{NAD}^+ + \text{CoASH} \xrightarrow[Mg^{2+}]{TPP} \text{acetyl-SCoA} + \text{NADH} + \text{H}^+ + \text{CO}_2$$

The best-studied pyruvate dehydrogenase complex is that of *Escherichia coli:* 24 chains of E_2 form a core of octahedral symmetry to which 12–24 E_1 dimers and 6–12 E_3 dimers are bound [1,2]. The exact stoichiometry of the complex is still subject to discussion [2–7]. Consequently reports on the M_r values of the *E. coli* complex show variability, M_r values 3–6.1 × 10^6 have been reported [4–9]. Despite these discrepancies, which are probably caused by use of different determination methods, there is general agreement that the pyruvate dehydrogenase complex from *E. coli* is a well-defined multienzyme complex of high M_r.

The pyruvate dehydrogenase complex isolated from *Azotobacter vinelandii* is much smaller than that from *E. coli* [10]. Its M_r has been estimated as 0.8–1.2 × 10^6. Its sedimentation coefficient is 17–20 S, that of the *E. coli* complex 53–63 S [4,8, 9,11]. However a 17–20 S particle has been observed as a minor component in some *E. coli* pyruvate dehydrogenase complex preparations [9,11]. The presence of a 53–63 S form of the *A. vinelandii* complex has not yet been reported. This form can be observed under properly chosen solution conditions as will be demonstrated in this article.

Abbreviations: TTP, thiamine pyrophosphate; PEG, polyethylene glycol

2. Materials and methods

Frozen *A. vinelandii* cells (ATCC 478) were obtained from Diosynth BV (Oss) and were used as starting material in all enzyme purifications. Polyethylene glycol 6000 was obtained from Baker chemicals (Deventer).

2.1. *Purification of pyruvate dehydrogenase complex*

Pyruvate dehydrogenase complex was isolated from *A. vinelandii* cells as in [10] with some slight modifications. Cells were ruptured with a French press and the isoelectric precipitation step was omitted since hardly any 2-ketoglutarate dehydrogenase activity could be detected after protamine sulphate precipitation. Gel chromatography on a Sepharose 6B column (2.5 × 80 cm) was used as the final purification step, which resulted in preparations with spec. act. 15-18 μmol NADH . min^{-1} . mg $protein^{-1}$, when tested according to [10].

2.2. *Ultracentrifugation*

Sedimentation velocity experiments were performed with a MSE analytical ultracentrifuge using Schlieren optics for detection. Appropriate viscosity and density measurements were made for correction of the sedimentation coefficient to standard conditions.

2.3. *Electron microscopy*

The two-step droplet method was used to prepare

the specimens on carbon-coated grids. A 1% (w/v) solution of uranyl acetate was used as negative stain. Micrographs were obtained with a Philips EM 300 electron microscope operating at 80 kV accelerating voltage on Kodak 4463 sheets with an electron optical magnification of 45 000 times.

3. Results

Previous ultracentrifugation studies on the pyruvate dehydrogenase complex of *A. vinelandii* showed that all of the sample sedimented as a single peak with a sedimentation coefficient of 17–20 S [10]. The pyruvate dehydrogenase complex preparations described here were isolated with a slightly different procedure (see section 2). The main difference with previous work from our laboratory is that the fourth component is absent from the final purified preparations, without making use of blue dextran–Sepharose chromatography as in [12]. The resulting three-component complex sediments as a single peak with $s_{20,w}$ = 17–20 S (fig.1A). Addition of Mg^{2+} and/or TPP does not affect the ultracentrifugation patterns as shown in fig.1A. However low concentrations of PEG 6000, which do not cause precipitation of the complex, have a pronounced effect (fig.1B). In the presence of 3% (w/v) PEG 6000 and 10 mM $MgCl_2$ a sedimentation pattern showing two peaks is observed. The slower peak has $s_{20,w}$ = 18–26 S, the faster has $s_{20,w}$ = 51–58 S. Under the conditions indicated in the legend to fig.1B 50–90% of the material is rapidly sedimenting (depending on the preparation). When $MgCl_2$ is omitted from the solution the relative amount of faster sedimenting material decreases, lowering the PEG concentration has the same effect.

The value of the sedimentation coefficient of the faster sedimenting peak, as well as its sharpness are not affected by these variations. These results indicate that the addition of PEG causes self-association of the *A. vinelandii* pyruvate dehydrogenase complex to a particle similar in size to the complex from *E. coli*. The distinct nature of this self-association follows from the fact that little material with intermediate sedimentation coefficient (30 S$<s_{20,w}<$50 S) is observed.

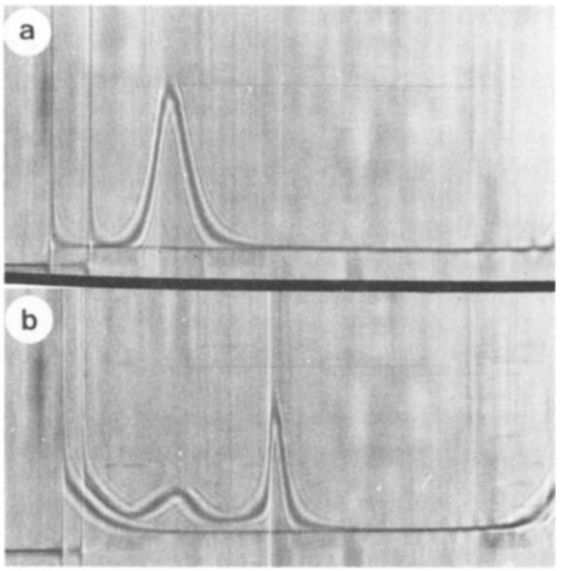
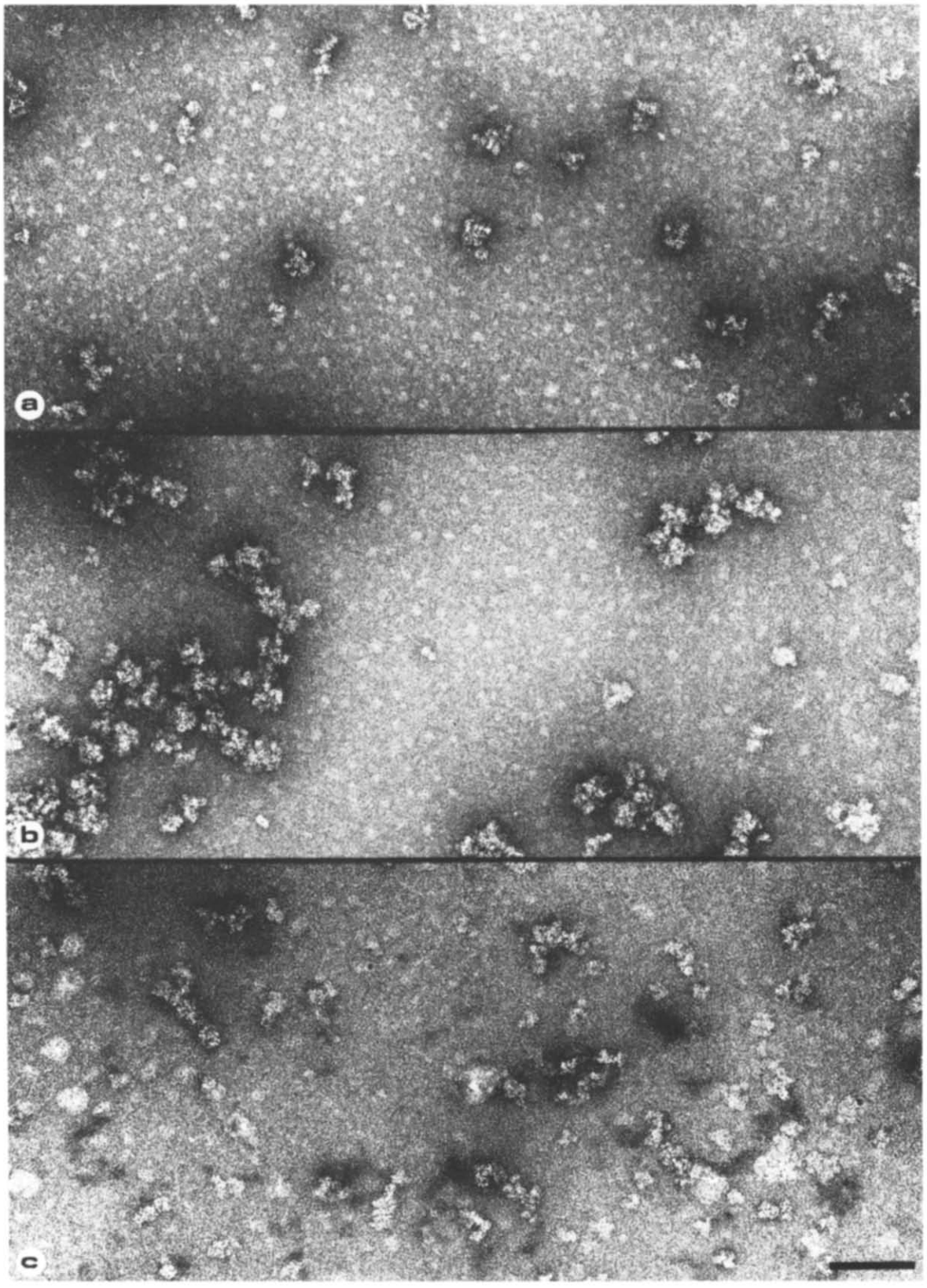


Fig.1. Sedimentation velocity patterns of the pyruvate dehydrogenase complex from *A. vinelandii*. Conditions: temp. 20°C; [protein] 3 mg/ml in 50 mM potassium phosphate buffer (pH 7.0). (A) Without PEG, 1300 after reaching 30 400 rev./min. (B) With 3% (w/v) PEG 6000 and 10 mM $MgCl_2$, 1800 s after reaching 30 300 rev./min. Sedimentation is from left to right.

These results are confirmed by electron microscopy. Crosslinking of the complex in the presence of 3% (w/v) PEG 6000 gives rise to a rather uniform population of spherical protein particles with a diameter of 30–40 nm (fig.2A). The overall dimensions are in the same range as those observed for the 53–63 S pyruvate dehydrogenase complex particles from *E. coli* [1]. The substructure of the *A. vinelandii* complex appears less well defined from our electron micrographs (fig.2A). The crosslinking procedure (see legend to fig.2) leads also to intermolecular crosslinking of these distinct 30–40 nm protein particles (fig.2B). In the absence of PEG 6000 no distinct complexes can be seen although some crosslinked protein aggregates can be observed (fig.2C).

Fig.2. Electron micrograph of the pyruvate dehydrogenase complex from *A. vinelandii*. Complex (5 mg/ml) in 50 mM potassium phosphate (pH 7.0) with (A,B) or without 3% (w/v) PEG 6000 (C) was incubated with 2% (w/v) glutaraldehyde for 15 min at room temperature. The solution was then diluted 50-fold with distilled water and the samples were mounted for electron microscopy as in section 2. The bar represents 100 nm.


a
b
c

4. Discussion

It is clear from the results presented in this article that PEG induces association of the 17–20 S pyruvate dehydrogenase complex of *A. vinelandii* to a 51–58 S complex which resembles that usually observed for *E. coli*. The exact reasons for the association-stimulating properties of PEG are not clear at present. Not answered here is the question whether the 51–58 S particle is a defined complex of independently functioning 17–20 S particles or a particle in which the E_2 subunits are redistributed to form an interacting system like that of the *E. coli* complex. Preliminary experiments done in collaboration with R. N. Perham and coworkers (University of Cambridge) have indicated that the number of E_2 subunits that can be acetylated by any one E_1 dimer increases with the increase in particle size (in preparation). This would favor the second proposition.

A 17 S and 19.8 S species of the *E. coli* pyruvate dehydrogenase complex have been reported in [11] and [9] respectively and it was shown that the smaller species is enzymatically active. In [9] the smaller and larger species were shown to be in slow equilibrium and that their polypeptide chain ratios are practically identical. The pyruvate dehydrogenase complexes of *A. vinelandii* and *E. coli* are therefore likely to have a certain degree of resemblance. The main difference is in the greater tendency of the *A. vinelandii* complex to dissociate into a smaller 17–20 S unit.

Acknowledgements

We are indebted to Mr J. F. L. van Breemen and Professor E. F. J. van Bruggen for useful discussions and help with the electron microscopy, to Mrs J. C. Toppenberg-Fang for typing the manuscript, to Mr D. J. F. Middendorp for printing fig.1 and to Mr K. Gilissen for printing the electron micrographs of fig.2. The present investigation was supported by the Netherlands Foundation for Chemical Research (SON) with financial aid from the Netherlands Organization for the Advancement of Pure Research (ZWO).

References

[1] Reed, L. J. and Oliver, R. M. (1968) Brookhaven symp. Biol. 21, 397–412.
[2] Bates, D. L., Harrison, R. A. and Perham, R. N. (1975) FEBS Lett. 60, 427–430.
[3] Reed, L. J., Pettit, F. H., Eley, M. H., Hamilton, L., Collins, J. H. and Oliver, R. M. (1975) Proc. Natl. Acad. Sci. USA 72, 3068–3072.
[4] Koike, M., Reed, L. J. and Carroll, W. R. (1963) J. Biol. Chem. 238, 30–39.
[5] Angelides, K. J., Akiyama, S. K. and Hammes, G. G. (1979) Proc. Natl. Acad. Sci. USA 76, 3279–3283.
[6] Vogel, O., Hoehn, B. and Henning, U. (1972) Proc. Natl. Acad. Sci. USA 69, 1615–1619.
[7] Eley, M. H., Namihira, G., Hamilton, L., Munk, P. and Reed, L. J. (1972) Arch. Biochem. Biophys. 152, 655–669.
[8] Dennert, G. and Höglund, S. (1970) Eur. J. Biochem. 12, 502–507.
[9] Danson, M. J., Hale, G., Johnson, P., Perham, R. N., Smith, J. and Spragg, P. (1979) J. Mol. Biol. 129, 603–617.
[10] Bresters, T. W., de Abreu, R. A., de Kok, A., Visser, J. and Veeger, C. (1975) Eur. J. Biochem. 59, 335–345.
[11] Schmitt, B. (1976) Biochimie 58, 1405–1407.
[12] De Abreu, R. A., De Kok, A. and Veeger, C. (1977) FEBS Lett. 82, 89–92.