Vol. 93, No. 3, 1980 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
April 14, 1980 Pages 709-712

The structure of the Escherichia Coli pyruvate dehydrogenase complex is probably not unique

Bernard SCHMITT and René COHEN

Institut de Recherche en Biologie Moléculaire
C.N.R.S. - PARIS VII
Laboratoire de Physique Biologique
2, place Jussieu 75221. PARIS Cédex 05 France

Received February 18,1980

Summary

Measurements of apparent diffusion coefficients of the pyruvate dehydrogenase complex from E. coli, with the use of the active enzyme centrifugation method at different angular velocities, show that the complex is heterogeneous although the heterogeneity is limited. Conditions of extraction and conditions of centrifugation in which the complex would show a monodisperse behaviour have not been found.

MATERIALS AND METHODS

Strain, conditions of growth as in (1). Bacteria were broken in a French press at low pressure so that the 17 S component was not produced (see (1)). The complex was purified from about 30 g of cells as in (2) except that the isoelectric precipitation was omitted. The purification factor obtained was about 40. In Active Enzyme Centrifugation experiments (3-5), two identical cells were used for each run. The substrate solution was as in (1) ; the phosphate concentration was 100 mM, pH 7,2 or pH 6,7 as indicated in the text. The pyruvate dehydrogenase complex initial concentration of the band could be increased by replacing pyruvate by α-cetobutyrate and/or NAD^+ by acetylpyridine dinucleotide ; this procedure maintained the absorption of the cell in the same range. Apparent sedimentation and diffusion coefficients were obtained from the difference curves by a simple procedure : the differences curves can be analysed without modification by the Vinograd's method (6) which was developed for band sedimentation and this method yields correct values for both sedimentation and diffusion coefficients ; this last point was established by sophisticated computer simulations (J. M. Claverie (7) and personal communication).

Abbreviations :

PDH complex : Pyruvate dehydrogenase (EC 1.2.4.1.) + dihydrolipoamide transacetylase (EC 2.3.1.12) + dihydrolipoamide dehydrogenase (EC 1.6.4.3).

AEC : Active Enzyme Centrifugation

0006-291X/80/070709-04$01.00/0

INTRODUCTION

Measurements of the E. coli pyruvate dehydrogenase complex molecular weights have been made in different laboratories for about twenty years and are not in good agreement (8-13). It seems that the differences between these measurements are significant and do not reflect only their great difficulty. Of course, these measurements were founded upon the usual explicit hypothesis of a perfectly homogeneous sample and also upon the implicit hypothesis of the synthesis by E. coli of a perfectly defined complex.

The PDH complex isolated from E. coli is made of a few tens polypeptide chains linked by non covalent bonds. There exists three species of chains which can be separated on SDS polyacrylamide gels. The complex may also be dissociated into distinct enzymes which can catalyse specific reactions. Following the proton's path, one finds successively : the pyruvate dehydrogenase, the dihydrolipoyl transacetylase, the dihydrolipoyl dehydrogenase. Each of these enzymes have only one kind of polypeptide chain. The transacetylase binds the other two types of chains ; curiously, if the previously separated enzymes are mixed, one observes that all the association sites between enzymatic species cannot be simultaneously occupied (14). This observation by itself leads one to doubt the PDH complex has a unique composition. On the other hand, the measurements in different laboratories of the stoicheiometry of the three polypeptide species of the native complex are not in agreement.

RESULTS AND DISCUSSION

Our own observations on the hydrodynamic behaviour of the complex in crude extracts as well as of a purified sample are new arguments in favor of the PDH complex not being unique. The apparent sedimentation and diffusion coefficients of the active PDH in a crude extract at different angular velocities are presented in the Table 1. The pH of the substrate solution was 7, 2 ; the s_{app} et D_{app} values are given at 20°C, the experiments temperature. Another set of experiments at pH 6, 7 has yielded similar results ; moreover a partially purified extract shows the same qualitative behaviour. One can see that the sedimentation coefficient does not vary with angular velocity (this is in contradistinction with a preceeding paper (1) where only one sample gave a non constant sedimentation coefficient ; this purified sample was from another laboratory and may have not sustained the trip well). One may attempt to explain the variable apparent diffusion coefficient by an association dissociation equilibrium : however this coefficient does not change significantly if the complex initial

Table 1

rpm x 10^{-3}		12	16	20	24	28
s_{app} in S	cell 1	52,6	53,6	54,7	55,1	54,6
	cell 2	nd	53,9	53,6	54,6	54,5
D_{app} in $cm^2/s \times 10^7$	cell 1	1,9	2,6	3,2	3,4	5,2
	cell 2	nd	2,5	3,3	3,8	5,5

Apparent sedimentation and diffusion coefficients of the PDH complex in a crude extract obtained by the active enzyme centrifugation method. At each angular velocity two identical cells were used. The s and D values are given at 20°C uncorrected for viscosity and density of the substrate solution which contains : phosphate buffer 100 mM pH 7.2 ; pyruvate 2 mM ; coenzyme A 0.5 mM ; NAD^+ 2 mM ; thiamine PP 0.2 mM ; $MgCl_2$ 1 mM ; mercaptoethanol 10 mM.

concentration changes (between 1 µg/ml and a few tens µg/ml) ; therefore one is left with the simple hypothesis of a limited sample heterogeneity.

Until recently (13) this limited "60 S" heterogeneity was not observed (or it has been neglected) probably because the diffusion coefficients of particles differing only slightly in molecular weights are much too close ; but one must point out that the diffusion as measured in the AEC method is extremely sensitive to even a narrow s dispersion of the particles.

Besides this heterogeneity of the "60 S" complexes, light active complexes have been found in several laboratories (1, 13, 15). In our laboratory, the 17 S complex seems to result from the shearing of the 60 S complex, whereas in Perham's laboratory (13), the 20 S complex is in reversible equilibrium with the 60 S complex.

Taken together these results show that, in crude extracts, the pyruvate dehydrogenase reaction is not catalysed by a unique particle of strictly determined composition and suggest that it is also true in the in vivo conditions. The physiology behind this finding is not clear. However, it seems important to stress that the bacterium could do well with a relative indeterminism in a limited number of its structures.

Acknowledgements : The authors thank Mrs Annick Larousse for technical assistance.

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