Amino acid composition of α- and β-chains of yeast and wheat germ pyruvate decarboxylase

Hartmut Zehender and Johannes Ullrich*

Biochemisches Institut der Universitat Freiburg, Hermann-Herder-Str 7, D-7800 Freiburg, FRG

Received 8 November 1984

Pyruvate decarboxylase, electrophoretically isolated from brewer's yeast and wheat germ, was resolved into α- and β-chains by SDS electrophoresis on polyacrylamide gel gradients The chains were analysed for amino acid compostion by routine methods Tryptophan was assayed without chain separation using the 280/288 nm absorbance in 6 M guanidine HCl Considerable differences found between α- and β-chains rule out the hypothesis that β-chains originate from α-chains by proteolytic removal of a short piece of chain Thus, the recently isolated structural gene for yeast PDC cannot suffice for coding both chains

Pyruvate decarboxylase *Subunit structure* *Amino acid composition* *Thiamin diphosphate* *Brewer's yeast* *Wheat germ*

1 INTRODUCTION

Upon removal of its cofactors thiamin diphosphate and Mg^{2+} at pH > 8, tetrameric holo-PDC reversibly dissociates into mostly dimeric apo-PDC [1,2]. In high-resolution SDS–polyacrylamide gel electrophoresis (PAGE) of PDC from brewer's yeast [3,4] and wheat germ [5], two types of polypeptide chains were separated which differ slightly in size ($M_r(\alpha)$ = 63000–65000; $M_r(\beta)$ = 61000–62000) and also in dyeing intensity ($\alpha > \beta$) with Coomassie brilliant blue, and thence possibly in their quantity. This led to the assumption [6] that β-chains may be produced from α-chains by limited proteolysis, either physiologically or as preparation artifacts. Further support for this view came from DNA experiments [7,8] demonstrating in a haploid yeast the existence of – so far – only one structural PDC gene of ~2.0 kb length, which is about the size necessary for coding one of the PDC chains. To prove the hypothesis, amino acid analyses were run on the PDC chains from both sources.

* To whom correspondence should be addressed

Abbreviation PDC, pyruvate decarboxylase (2-oxo-acid carboxy-lyase, EC 4 1 1 1)

2. MATERIALS AND METHODS

Brewer's yeast (*Saccharomyces cerevisiae* var. *carlsbergensis*), washed and pressed, was obtained from Feldschlosschen-Brauerei AG, Rheinfelden. Fresh (unheated) wheat germ (*Triticum aestivum*) of West European origin (mixture from various areas) were purchased from Dr Ritter GmbH, Koln-Deutz. All chemicals were of analytical grade unless otherwise stated.

PDC was extensively purified from fresh yeast [9] and from wheat germ ([5], cf.[10]). The final purification step was native electrophoresis on concave polyacrylamide gel gradients [11,12], followed by activity-staining with dianilinoethane [13]. The PDC-containing gel slices were crushed and extracted with 1% SDS [14]. Electrophoresis of the concentrated extracts on SDS–polyacrylamide gel gradients [11] resulted in chain separation, detected by Coomassie brilliant blue staining [11]. Extra gel slices containing α- and β-chains of PDC prepared from a similar yeast (VEB.Brauerei Wernesgrun, DDR) were obtained from Dr M

Published by Elsevier Science Publishers B V (Biomedical Division)
00145793/85/$3 30

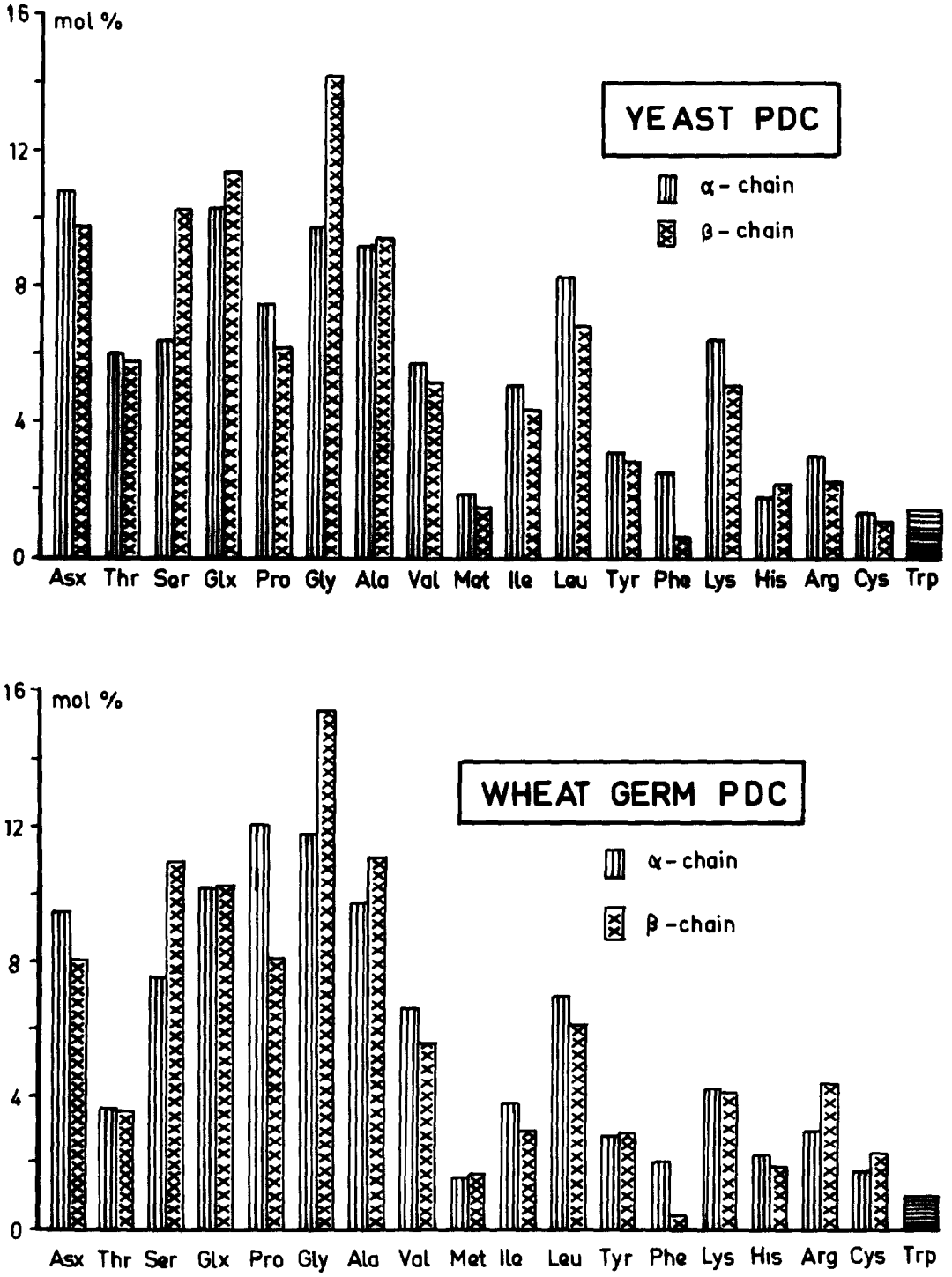

Fig 1 Amino acid composition (mol%) of α- and β-chains of pyruvate decarboxylase from brewer's yeast and wheat germ. Trp contents are only given for the natural mixture of chains.

Sieber and Professor Dr A. Schellenberger, University of Halle.

Hydrolysis of the PDC-chain containing gel slices [15] failed to allow reliable analyses of the basic amino acids. Thus, the minced gel slices were extracted with SDS–$NaHCO_3$ solution [16] and the extracts freeze-dried. Hydrolysis of the extracts was performed under exclusion of oxygen with 6 M HCl containing 1% thioglycollic acid [15] at 115°C for 24, 48, and 72 h. No time-dependent losses of Ser, Thr, Tyr, His, Met, or Arg were observed, but Trp was fully destroyed, and Cys was barely detectable For exact Cys determinations, aliquots of the samples were oxidised with performic acid prior to hydrolysis [17]. For amino acid analyses, a Biotronik LC-6000E with 6 × 220 mm Durrum DC-6A column was used. Quantitation was performed by post-column derivatisation either with ninhydrin and colorimetry or with *o*-phthalaldehyde and fluorimetry. Attempts at saving Trp by hydrolysis with mercaptoethanesulfonic acid [18] led to deteriorated separations. Therefore, Trp was only assayed by photometry of the unhydrolysed natural mixture of chains at 280/288 nm in 6 M guanidine·HCl [19], using hen egg white lysozyme as standard with 6 Trp/mol. Additional amino acid analyses were run on holo-PDC samples from both sources without previous chain separation.

3. RESULTS AND DISCUSSION

The obtained amino acid compositions of α- and β-chains of yeast and wheat germ PDC are shown in fig.1. No difference was found between PDCs from the two yeasts. The values found for holo-PDCs agreed with those of $(\alpha+\beta)/2$. For yeast PDC, they confirmed earlier values [6,20] of Asp, Ala, Tyr, Lys, His, Arg, and Trp within the limits of error, although these analyses had been done on less pure and partially degraded PDC, but showed considerable differences for Ser, Pro, Gly, and Phe, and small differences for the other amino acid residues.

The observed differences between α- and β-chains of both PDCs tend to have the same sign, but not always the same magnitude. For several residues their sign and magnitude are far from being compatible with the hypothesis that mere proteolytic removal of a short piece of chain could produce β from α (see Ser, Pro, Gly, Ala, Arg, and particularly Phe). Therefore, the two chains must be coded by different structural genes having at least partially different DNA sequences. These genes may well be located on allelic chromosomes in the diploid yeast and the hexaploid wheat which served as sources for the PDCs investigated here, but further search appears necessary in order to find and elucidate a second structural gene for PDC even in haploid yeasts.

ACKNOWLEDGEMENTS

Our thanks go to the Feldschlosschen-Brauerei for generous gifts of yeast, to Dr Sieber and Professor Schellenberger for sending α- and β-chains of yeast PDC, to Frau D. Trescher for technical assistance in the routine preparations of crude PDCs, to Frl D. Gierschner and Herrn H. Johner for performing the amino acid analyses, and to the Deutsche Forschungsgemeinschaft, D-5300 Bonn-Bad Godesberg, for financial support (U1 21/9-2).

REFERENCES

[1] Gounaris, A D., Turkenkopf, I., Buckwald, S. and Young, A. (1971) J Biol. Chem 246, 1302–1309.
[2] Gounaris, A D , Turkenkopf, I., Civerchia, L.D and Greenlie, J (1975) Biochim. Biophys. Acta 405, 492–498.
[3] Ullrich, J. and Freisler, H (1977) Hoppe-Seyler's Z Physiol Chem. 358, 318.
[4] Hopmann, R.F W (1980) Eur. J. Biochem. 110, 311–318
[5] Zehender, H (1984) Doctoral Thesis, Fak. f Biologie d. Univ Freiburg
[6] Ullrich, J. (1982) Ann NY Acad Sci 378, 287–305
[7] Schmitt, H D. and Zimmermann, F.K (1982) J. Bact 151, 1146–1152.
[8] Schmitt, H.D. and Zimmermann, F K (1983) Mol Gen Genet. 192, 247–252
[9] Sieber, M , Konig, S , Hubner, G and Schellenberger, A. (1983) Biomed Biochim. Acta 42, 343–349.
[10] Singer, T P. and Pensky, J (1952) J Biol. Chem 196, 375–388
[11] Gorg, A., Postel, W , Westermeier, R , Righetti, P G and Ek, K (1981) LKB Appl Note 320, LKB Produkter AB, S-16126 Bromma

[12] Hames, B.D. (1981) in. Gel Electrophoresis of Proteins: A Practical Approach (Hames, B.D and Rickwood, D. eds) pp.1–92, IRL Press Ltd, Oxford, Washington.
[13] Zehender, H., Trescher, D and Ullrich, J. (1983) Anal. Biochem 135, 16–21.
[14] Drescher, D.G. and Lee, K.S. (1978) Anal. Biochem. 84, 559–569.
[15] Houston, C.C. (1971) Anal. Biochem. 44, 81–88.
[16] Sreekrishna, K , Jones, C.E., Guetzow, K.A , Prasad, M R and Joshi, V C. (1980) Anal Biochem. 103, 55–57
[17] Schram, E., Moore, S and Bigwood, E J (1954) Biochem J. 57, 33–37
[18] Penke, B., Ferenczi, H and Kovács, K (1974) Anal. Biochem. 60, 45–50
[19] Edelhoch, H (1967) Biochemistry 6, 1948–1954
[20] Ullrich, J , Wittorf, J.H. and Gubler, C.J. (1969) FEBS Lett 4, 275–277