THE SUBUNIT MOLECULAR WEIGHTS OF THE α-KETOACID DEHYDROGENASE MULTIENZYME COMPLEXES FROM *E. COLI*

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1. Introduction

The pyruvate dehydrogenase and 2-oxoglutarate dehydrogenase of *E. coli* (Crookes strain) are multienzyme complexes of molecular weight 4×10^6 and 2.3×10^6 respectively [1, 2]. In the course of studies of the structure and activity of these enzymes, we have had occasion to estimate the molecular weights of the constituent polypeptide chains for each enzyme by means of SDS*-gel electrophoresis [3]. An accurate knowledge of the subunit molecular weights is obviously relevant to any speculation about the organization of these multimeric structures, particularly in estimating the number of copies of a particular enzyme in the complexes.

We present evidence here which suggests that degradation of the enzymes can occur on storage, freezing and thawing, and under certain other experimental conditions, which may explain the substantially lower subunit molecular weights previously reported by other workers for certain components of the pyruvate dehydrogenase complex.

2. Materials and methods

Pyruvate dehydrogenase and 2-oxoglutarate dehydrogenase complexes were purified from *E. coli* (Crookes strain) by the method of Reed and Mukherjee [4]. The specific activity of the pyruvate dehydrogenase (in μmoles NADH produced/min/mg enzyme when assayed by the method of Schwartz, Old and Reed [5]) was never less than 30 U/mg and was sometimes higher. The specific activity of the oxoglutarate dehydrogenase was commonly about 20 U/mg. Resolution of the pyruvate dehydrogenase complex into its constituent enzymes by treatment with 4 M urea or ethanolamine phosphate buffer, pH 9.5, on calcium phosphate gel in cellulose was carried out according to Reed and Willms [6].

Proteins were reduced and carboxymethylated in 8 M urea or 6 M guanidine hydrochloride essentially as described elsewhere [7], except that reduction was effected with 2 mM dithiothreitol. Polyacrylamide gel electrophoresis in the presence of SDS was carried out in 5% or 7.5% gels [3]. The gels were fixed overnight in 50% (w/v) trichloroacetic acid before staining for 1.5 hr in 0.1% Coomassie Blue in 50% trichloroacetic acid and then destained by exhaustive washing in 7% (w/v) acetic acid. Bovine serum albumin, glyceraldehyde-3-phosphate dehydrogenase and lysozyme were used as markers for molecular weight measurements in 7.5% gels, with myosin, β-galactosidase and isoleucine-tRNA synthetase as additional markers in 5% gels [8, 19]. (The myosin, β-galactosidase and tRNA synthetase were the generous gifts of Dr. A.G. Weeds, Dr. J.V. Maizel and Dr. P. Berg, respectively). Estimates of molecular weight made by this technique are generally accurate to better than ± 10% [8].

3. Results

SDS-gel electrophoresis of freshly prepared pyruvate dehydrogenase complex gave three bands with molecular weights estimated as 93,000, 81,000 and 59,000 respectively (fig. 1a). An identical banding

* SDS, sodium dodecyl sulphate.

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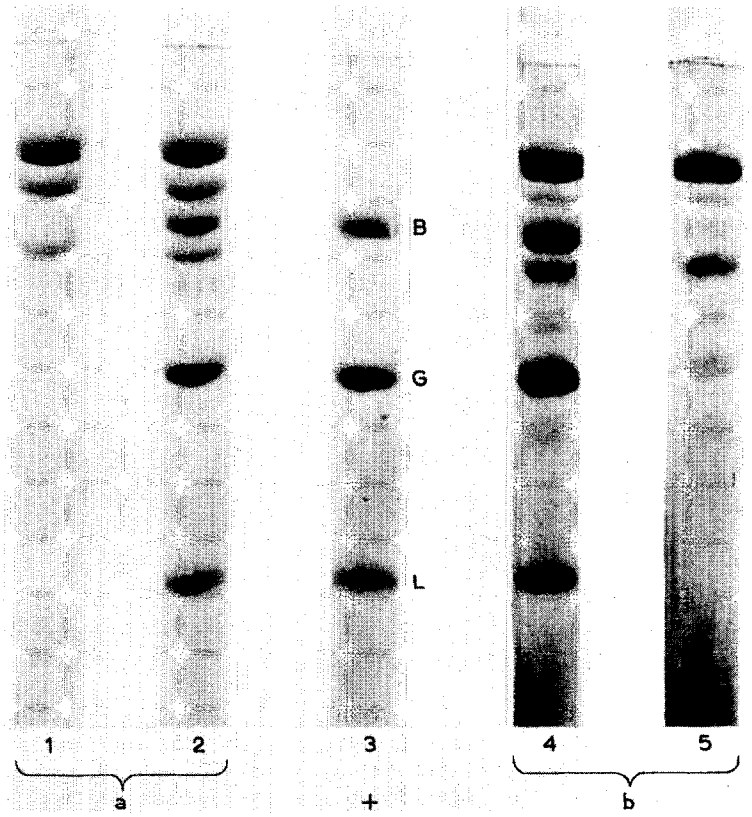

Fig. 1. SDS-gel electrophoresis (7.5% gels) of pyruvate dehydrogenase complex from *E. coli.* (a) Enzyme prepared in this laboratory run in the absence (gel 1) and presence (gel 2) of markers; (b) enzyme supplied by Dr. L. Reed run in the presence (gel 4) and absence (gel 5) of markers. Gel 3 shows markers run alone: these were bovine serum albumin (B), rabbit glyceraldehyde-3-phosphate dehydrogenase (G) and lysozyme (L) with assumed molecular weights of 68,000, 36,000 and 14,300 respectively [8].

pattern was obtained for complex that had been reduced and carboxymethylated or had been oxidized with performic acid [9]. It seems reasonable to conclude, therefore, that these values represent the minimum subunit molecular weights for the component enzymes of the pyruvate dehydrogenase complex. Resolution of the complex into its constituent enzymes [6] showed that the band of molecular weight 59,000 arose from the lipoamide dehydrogenase [6] and that of molecular weight 93,000 from pyruvate decarboxylase [10]. The band of molecular weight 81,000 (fig. 1a) is therefore assumed to derive from lipoyl reductase-transacetylase.

A frozen solution of pyruvate dehydrogenase complex prepared from the same strain of *E. coli* was generously given to us by Dr. Lester Reed. When freshly thawed by us it had a specific activity of 20 U/mg; on SDS gels it gave the bands of molecular weight 93,000 and 59,000 present in our preparation but only a weak band of molecular weight 81,000, together with bands at 36,000 (fairly strong doublet), 30,000 and 40,000 (fig. 1b). A few days later the same solution (in 20 mM potassium phosphate, pH 7.0) kept at 2° now showed no trace of the 81,000 molecular weight band. However, a strong band of 35,000 and other weaker ones (68,000, 52,000, 30,000, 11,000) had appeared (fig. 2a). This suggested progressive degradation of the transacetylase (subunit molecular weight 81,000).

To investigate this we examined samples of our

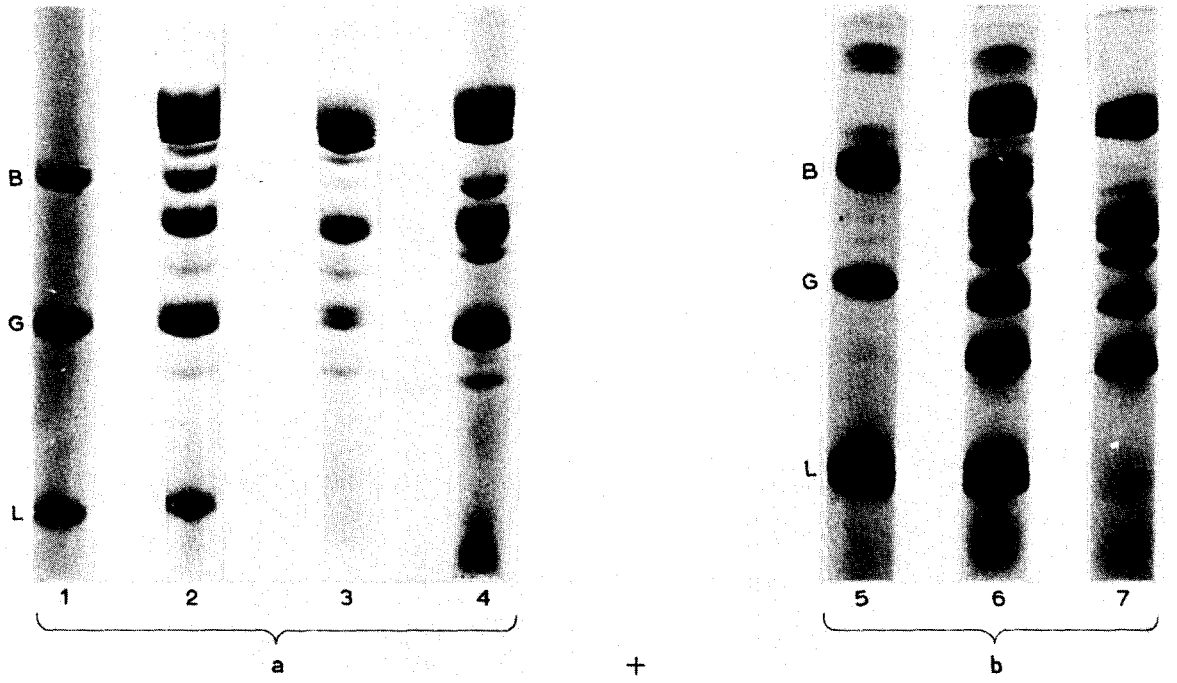

Fig. 2. SDS-gel electrophoresis (7.5% gels) of degraded pyruvate dehydrogenase complex from *E. coli.* (a) Enzyme supplied by Dr. L. Reed, run in the presence (gel 2) and absence (gel 3) of markers, and (gel 4) after several days in solution at 2° (see text) run in the absence of markers; (b) enzyme prepared in this laboratory and stored for several months (see text), run in the presence (gel 6) and absence (gel 7) of markers. Markers (see fig. 1) were run alone on gels 1 and 5.

own pyruvate dehydrogenase complex which were being stored in frozen solution with very occasional brief thawing. Fresh complex gave the clean band pattern described above (fig. 1a) but after 3–4 months bands of molecular weight 68,000 and 40,000 had appeared. After 12 months the band of molecular weight 93,000 was weaker, that of 81,000 had disappeared and only the dihydrolipoyl dehydrogenase (59,000) seemed unaffected; additional bands were present corresponding to molecular weights of 47,000, 35,000, 27,000 and (traces) 11,000–13,000 (fig. 2b). It was remarkable that the sample still retained considerable pyruvate dehydrogenase activity when assayed by NAD^+ reduction [5].

When freshly prepared oxoglutarate dehydrogenase complex was subjected to SDS-gel electrophoresis, three bands were observed with molecular weights of 95,000, 59,000 and 51,000 (fig. 3). These probably correspond to 2-oxoglutarate decarboxylase, lipoamide dehydrogenase and lipoyl reductase-transsuccinylase respectively. A preparation of the same complex also generously given to us by Dr. Lester Reed behaved identically. However, the oxoglutarate, like the pyruvate, complex underwent degradation when stored as described above. The bands of molecular weight 95,000 and 51,000 were progressively lost, leaving ultimately only the lipoamide dehydrogenase and a spectrum of lower molecular weight components. Not surprisingly, perhaps, such extensively degraded material was totally inactive in the NAD^+ reduction assay.

4. Discussion

Subunit molecular weights previously reported [1, 2, 14] for the pyruvate dehydrogenase complex are 45,000 (decarboxylase), 36–40,000 (transacetylase) and 56,000 (lipoamide dehydrogenase). However, there has been a recent revision of the subunit

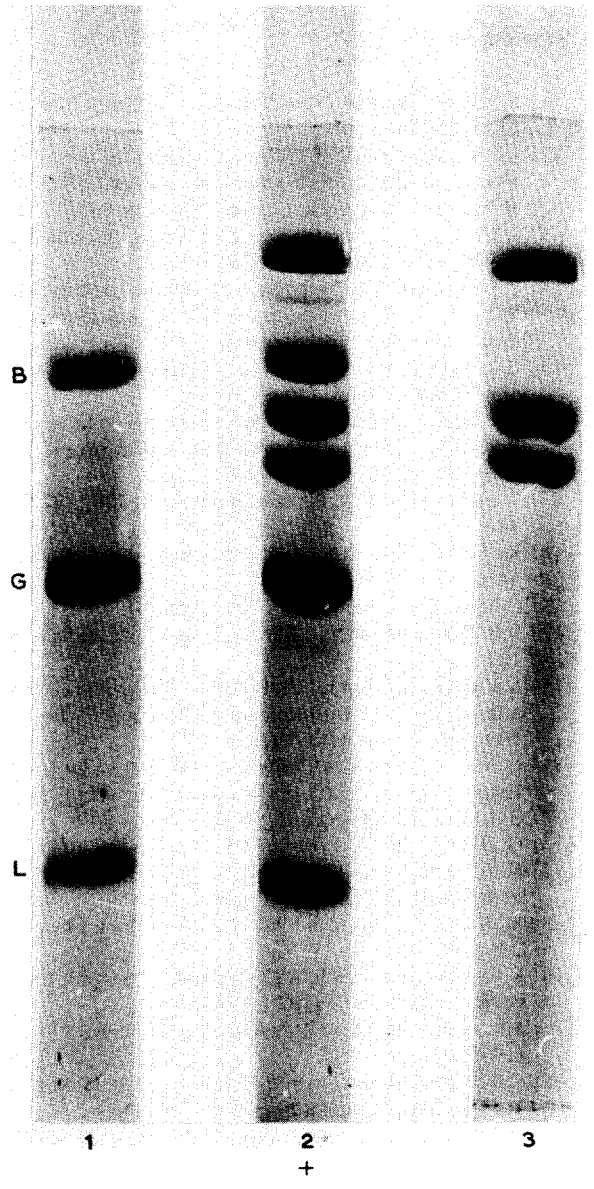

Fig. 3. SDS-gel electrophoresis (7.5% gels) of 2-oxoglutarate dehydrogenase complex. Gel 1, markers (see fig. 1); gel 2, complex plus markers; gel 3, complex alone.

molecular weight of the decarboxylase to 90,000 [11], which agrees with the value (93,000) reported here, and it has been suggested that the pyruvate decarboxylase of another strain (K12) of *E. coli* also has a subunit molecular weight of 90,000 [12, 13]. It seems likely that the lower value obtained previously could be the result of proteolytic degradation of the type observed in these experiments. A similar explanation could also account for the lower value (36–40,000) given for the subunit molecular weight of the transacetylase [1, 2, 14]. Cleavage of the polypeptide chain (molecular weight 81,000) somewhere near the middle could result in the appearance of chains with an apparent molecular weight of 36,000 when examined in the ultracentrifuge [14]. Chemical evidence (end-group analysis, peptide mapping etc.) that the polypeptide chains of molecular weight 36,000 are probably identical [14] is harder to accommodate. Though unlikely, it is not inconceivable that the transacetylase polypeptide chain comprises two identical polypeptide regions, each corresponding to a molecular weight of approximately 36,000, thereby also accounting for the presence of 1 mole of covalently bound lipoic acid per 30–35,000 g of protein [15]. This would imply a gene duplication and fusion to give single polypeptide chains with 2 identical binding sites, comparable with that recently postulated for human transferrin [16].

It is likely that the increasing degradation of the α-ketoacid dehydrogenase complexes on storage may be due to the copurification of a proteolytic enzyme. A similar situation has recently been reported for yeast hexokinase [17] where contamination with proteolytic activity fortuitously reduces the subunit molecular weight from 51,000 to 25,000. It is interesting that in the pyruvate dehydrogenase complex, the transacetylase seems to be the component most sensitive to the degradation, since it is thought to be the 'inner' basic structural unit of the complex with binding sites for both the decarboxylase and lipoamide dehydrogenase [1, 2].

The lipoyl reductase-transacetylase forms a self-assembling subcomplex with a molecular weight of approximately 1×10^6 [14, 18]. On the basis of elegant electron micrographs, Reed and coworkers postulate a model of the transacetylase comprising 24 copies of the transacetylase chain (molecular weight 36,000) arranged in groups of three chains at each of the 8 vertices of a cube [1, 2, 18]. If, however, we adopt our value of 81,000 for the molecular weight of the transacetylase chain, while re-

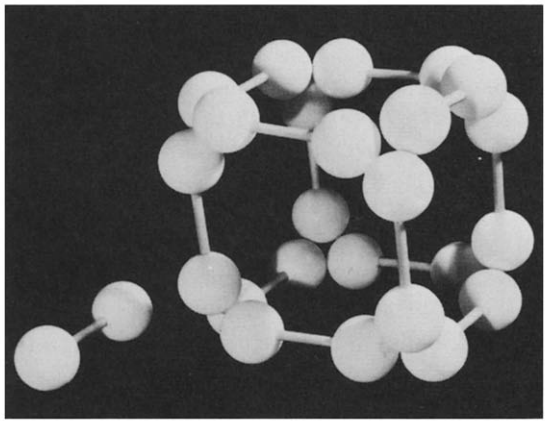

Fig. 4. A possible model (not to scale) for the transacetylase component of the pyruvate dehydrogenase complex of *E. coli*, composed of 12 polypeptide chains (molecular weight 93,000) disposed on the edges of a cube (for further details, see text). A monomer is shown alongside.

taining the value of 1×10^6 for the assembled transacetylase subcomplex, it becomes impossible to dispose the resultant 12 chains in the same trimer clusters at the vertices of a cube. On the other hand, an alternative geometrical arrangement of 12 chains, based on equivalent (or pseudoequivalent) positions on the 12 edges of a cube could account equally well for the concentration of mass at the vertices indicated by the electron micrographs and the requirements of cubic symmetry. Such an arrrangement of molecules with dumbell shape is shown in fig. 4, where the chemical subunit is twice the packing unit.

Acknowledgements

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