Possible Presence of Two Protein Components in O-Acetylserine·O-Acetylhomoserine Sulfhydrylase of the Yeast *Saccharomyces cerevisiae*; Electrophoretic investigations

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Summary

A purified preparation of cysteine and homocysteine synthetase (O-acetyl-L-serine and O-acetyl-L-homoserine sulfhydrylase) of the yeast *Saccharomyces cerevisiae* was subjected to polyacrylamide gel disc electrophoresis under various conditions in order to obtain information about its molecular status. Although a purified preparation of the enzyme retained constant catalytic activities even after repeated freezing and thawing, polyacrylamide gel electrophoresis sometimes showed a broad protein band over the main protein band for such "old" preparations, suggesting polymerization of the enzyme molecule into various sizes. No such broad band was observed when electrophoresis was carried out on the "old" preparation in the presence of a high concentration of pyridoxal 5'-phosphate (5 mM) nor when a borohydride-reduced preparation was subjected to the electrophoresis.

Electrophoresis carried out at pH 9.4 using a small quantity of the purified preparation (5-20μg of protein) showed two protein bands which were located very close to each other. But at more acidic pHs (pH 8.0 and pH 2.3), no separation of proteins occurred. Polyacrylamide gel electrophoresis carried out in the presence of sodium dodecyl sulfate also showed two protein components, slightly different in their molecular weight. The polypeptide composition of the enzyme is also discussed.

Introduction

In order to get information about the purity of a given protein preparation obtained from an organism, polyacrylamide gel disc electrophoresis has been most widely employed because of its simplicity in handling, its aharpness in separating proteins, and its economical cost. The procedure has also been applied to find out the subunit composition of many purified proteins by introducing a proper protein-denaturing agent

Abbreviations : OAS, O-acetyl-L-serine ; OAH, O-acetyl-L-homoserine ; PLP, pyridoxal 5'-phosphate ; SDS, sodium dodecyl sulfate.

(such as sodium dodecyl sulfate (SDS)) into the system of electrophoresis.

The yeast *Saccharomyces cerevisiae* possesses a unique enzyme catalyzing biosynthesis of both cysteine and homocysteine from O-acetyl-L-serine (OAS) and O-acetyl-L-homoserine (OAH) by replacing their acetate ions with sulfhydryl groups of hydrogen sulfide. This enzyme has tentatively been called OAS-OAH sulfhydrylase (*1*). Purified preparations of the enzyme have also been subjected to polyacrylamide gel electrophoresis both with and without SDS. Native gel electrophoresis (without SDS) of the final enzyme preparation obtained through DEAE-cellulose column chromatography (specific activity of about 30 units/mg of protein or higher) gave a single protein band, except for minor contaminating proteins, even when 100μg of protein or more was used (*2*). However, when a purified preparation was used after repeated freezing and thawing the same type of electrophoresis sometimes gave a broad band of stained proteins in the top region of the gels.

Tryptophanase of *Aeromonas liquefaciens* has been reported to polymerize after freezing at −20°C and show bands of polymers on a polyacrylamide gel of electrophoresis (*3*). Such an interesting phenomenon moved me to do electrophoretic investigation of OAS-OAH sulfhydrylase under various conditions. This paper advances the possibility that the enzyme is composed of two types of polypeptide which behave differently at different pHs.

Experimental Procedure

Purification of the enzyme —— OAS-OAH sulfhydrylase was purified from pressed bakers' yeast *Saccharomyces cerevisiae* as described previously (*2*), except that the enzyme was extracted by subjecting the cell suspension to agitation in a Dyno Mill (W. A. Bachofen Maschienenfabrik, type KDL), as described previously (*4*).

Assay of enzyme activity —— This was carried out as described previously (*5*) with OAS or OAH as the primary substrate at a final concentration of 5 mM and with hydrogen sulfide as the co-substrate at a final concentration of 1 mM. Protein was determined by the method of Lowry *et al.* (*6*) with bovine serum albumin as a standard.

Polyacrylamide gel disc electrophoresis —— Electrophoresis was carried out according to the method of Ornstein and Davis (*7*), using 7.5% cross-linked gels. Samples were mixed with equivolumes of 50% (w/w) sucrose solutions and placed on stacking gels without using sample gels. An electric current of 4 mA per gel was applied for 45-60 min at pH 9.4. Where indicated, pyridoxal 5'-phosphate (PLP) was added to both the gel and the electrode buffer at a concentration of 0.5 or 5.0 mM. Electrophoresis was also carried out at pH 8.0 with the electric current set at 1.25 mA per gel at the beginning of the electrophoresis, which continued for 105 min. A constant electric current of 2.5 mA per gel was applied when the electrophoresis was performed at pH 2.3.

The method of Weber and Osborn (*8*) was employed for electrophoresis in the presence of SDS. The enzyme protein was treated with 1% SDS and 5% 2-mercaptoethanol at 100°C for 5 min. In all cases the protein was stained with Amido black 10B and gels were destained electrophoretically in 7% acetic acid.

Materials —— Pressed bakers' yeast, *Saccharomyces cerevisiae,* was obtained from Oriental Yeast Company, Osaka. O-Acetyl-L-serine and O-acetyl-L-homoserine were synthesized according to the method of Sakami and Toennies (*9*). A molecular weight marker set (bovine serum albumin, ovalbumin, bovine pancreas chymotrypsinogen A, and horse heart cytochrome *c*) was purchased from Boehringer Mannheim and DEAE-cellulose (DE-52) from Whatman. Other reagents were commercial products of the highest quality.

Results and Discussion

It has been ascertained that a purified preparation of OAS-OAH sulfhydrylase of *S. cerevisiae* can be kept at −20°C without any loss of catalytic activity for at least one year. Nor has loss of activity been observed for the enzyme preparation when it was subjected to repeated freezing at −20°C and thawing at 30°C (designated in this paper as "old" preparation). However, as noted in the introduction, such an "old" preparation sometimes showed different behavior in polyacrylamide gel disc electrophoresis (pH 9.4) than that shown by the "fresh" preparation of the enzyme, that is, one not subjected to freezing and thawing. Figure 1 compares two representative examples of the results of the electrophoresis. Both gels were supplied with 100μg of protein. The "fresh" preparation at the left shows one clear protein band, except for faint staining which seems to be due to minor contaminating protein. On the other hand, the "old" preparation at the right has very dark and broad staining over the main band. It is natural to consider that the substance (s) composing the broad band is the same as or a derivative (s) of that composing the main band, based on the fact that both the "fresh" and "old" preparations showed the same catalytic activities. Cowell and DeMoss reported in 1973 that *Aeromonas liquefaciens* tryptophanase had a tendency to

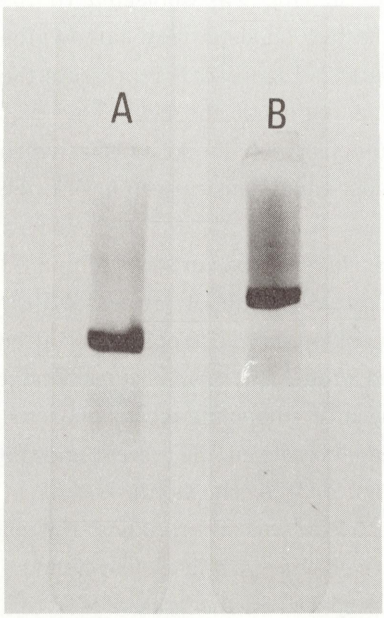

Fig. 1. Electrophoresis of 100μg of protein on polyacrylamide gel (pH 9.4). A final preparation obtained through a purification procedure (*2*) was subjected to electrophoresis. A constant current of 4 mA per gel was applied at pH 9.4 for 60 min. Protein was stained with Amido black. Gel (A) was supplied with a "fresh" enzyme preparation and gel (B) with an "old" preparation. Other conditions are described in "Experimental Procedure". Gel (A) is the same as that presented in a previous paper (*2*).

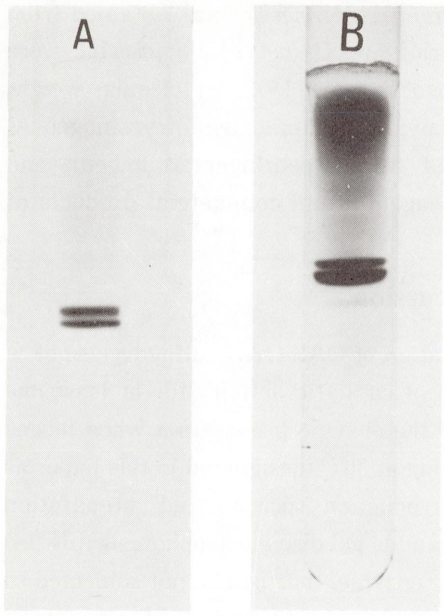

Fig. 2. Electrophoresis of 20μg of protein at pH 9.4 (A), "fresh" preparation ; (B), "old" preparation. Conditions were the same as those for Fig. 1.

polymerize into various sizes without losing any catalytic activity after the purified preparation was kept at −20℃ (*3*). Similar polymerization might have also occurred in the case of OAS-OAH sulfhydrylase, since both enzymes have some common characteristics (PLP requirement, similar molecular weight, tetrameric structure, *etc*). (See also the later discussion).

Other information was acquired when a small quantity of enzyme protein (5-20μg) was subjected to electrophoresis under the same conditions as above : the main band of the enzyme was composed of two closely located bands which were not observed when the large amount (100μg of protein or more) of the enzyme was subjected to the electrophoresis (Fig. 2, A). Since the space between the two bands is very narrow, the two components seem to have very similar characteristics. Figure 2, B represents the "old" preparation. Note the broad staining in the top region of the gel. This was probably derived from the upper component, since the density of the upper relative to that of the lower was very low compared with the almost equal density seen in 2-A, the result obtained for the "fresh" preparation.

Since the PLP-binding force of the enzyme has been shown to be very low compared with that of other enzymes (*2*), the polymerization may be a result of release of PLP from the subunit, particularly from the upper component of the electrophoresis, after freezing and thawing. If so, holo-enzyme or a reduced product of holo-enzyme would produce clear protein bands without the broad staining after the same electrophoresis. Also, under conditions of high PLP concentration, no apo-enzyme can be present and the broad band in the top region would disappear. Figure 3, A shows the result of electrophoresis of the reduced holo-enzyme. When 5 mM PLP was added to both the gel and the electrode buffer, the electrophoresis of the native enzyme gave one clear band on the gel (Fig. 3. B). (As a stacking gel could not be prepared in the presence of this

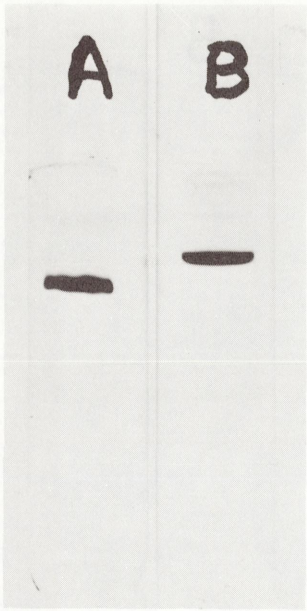

Fig. 3. Electrophoresis (pH 9.4) of a reduced product of holo-enzyme and of native enzyme in the presence of 5 mM PLP. (A), A purified "old" preparation of the enzyme (1 ml) was dialyzed overnight at 4°C against 1,000 ml of 0.02 M Tris-HCl buffer (pH 7.8) supplemented with 1 mM EDTA and 0.2 mM PLP. The dialyzate (65μg of protein in 0.1 ml) was reduced with 0.02 ml of 0.01% sodium borohydride solution (in 1 mM NaOH) and an aliquote containing 20μg of protein was subjected to electrophoresis for 50 min as described in "Experimental Procedure". (B), PLP was added at a concentration of 5 mM to both the gel and the upper electrode buffer (cathodic). The "old" preparation (20μg) was subjected to the electrophoresis for 45 min. Other conditions were the same as above.

concentration of PLP, a sample was placed directly on a separation gel.) A similar result was obtained with PLP present at a concentration of 0.5 mM, but the clear-band effect of PLP was slightly less. As preparing of a stacking gel was possible in this case, it can be said that formation of a clear band was essentially due to the presence of PLP, not to the omission of the stacking gel.

Both gels 3-A and 3-B show another band in a region where a broad band must in fact be present. This can be safely ascribed to a contaminating protein, because the density of this band is negligible compared with that of the main band. These results support the idea that the broad band observed over the main band is the result of polymerization of apo-type subunits, which occupy the upper position on the main band.

A difference between *S. cerevisiae* enzyme and *A. liquefaciens* enzyme with respect to polymerization must here be noted. In the case of *A. liquefaciens (3),* the polymerizing unit is the native enzyme (both holo-and apo-types), which is composed of four identical subunits and has a molecular weight of about 216,000. Bands corresponding to its dimer, trimer, and tetramer are clearly seen on a gel after staining. On the other hand, *S. cerevisiae* enzyme seems to behave in a considerably different way. First, the polymers produce a broad band in which the individual polymers are indistinguishable. In this case, the polymerizing unit must be smaller, probably the subunit of the enzyme having a molecular weight of about 50,000 (*10*), so that polymers of continuous size can be formed. Only apo-type subunits will participate in it. Both the enzymes look not to require disulfide bonding to polymerize (*3, 10*). Two closely located bands are not detectable in either photo in Fig. 3, although only 20μg of protein was electrophoresed. This may be due to fusion of the two components, probably caused by diminished physicochemical difference between them under unphysiological conditions. We must remember the presence of as many as 27 lysines in the subunit of the enzyme (*10*). Some of them might

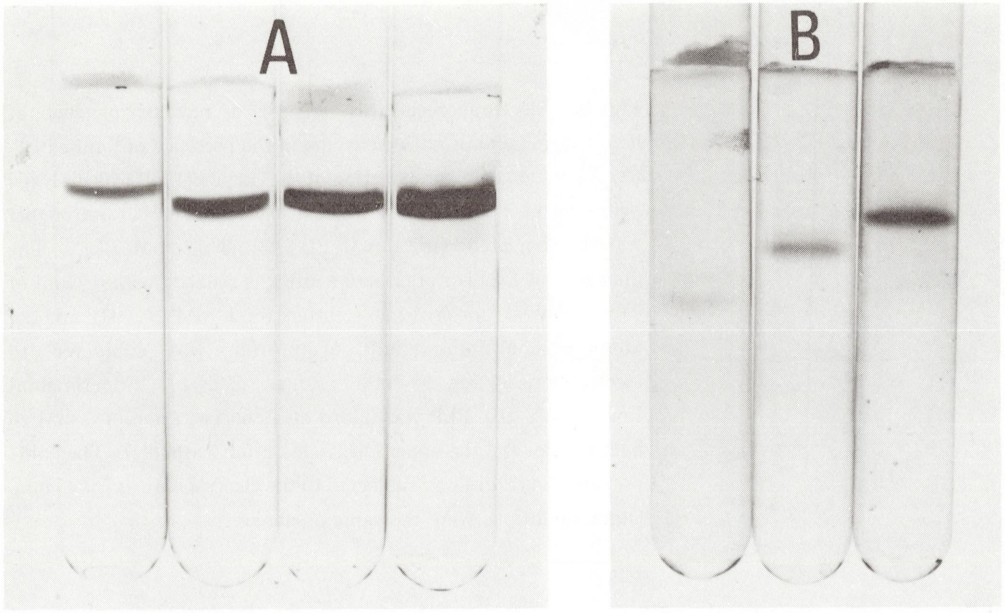

Fig. 4. Purity of the enzyme preparation. Various quantities of enzyme preparation were subjected to electrophoresis at pH 8.0 (A) and at pH 2.3 (B). Four gels of (A) were supplied with 6, 12, 24, and 48μg of protein, and electrophoresis was continued for 105 min under the conditions described in "Experimental Procedure". Three gels (B) were supplied with 45, 90, and 150μg of protein in 0.03, 0.06, and 0.1 ml of solution, respectively. Electrophoresis was carried out for 150 min. Other details are in "Experimental Procedure".

react unphysiologically with PLP at such high concentrations.

The possibility that a significant contaminating protein in the purified preparation yielded the two bands on the gel could not be overlooked. Results of electrophoresis of the preparation carried out at different pHs are shown in Fig. 4,A and 4,B (pH 8.0 and 2.3, respectively). As seen in the legends, various quantities of protein were subjected to the procedure in order to find two adjacent bands, if present. In the 4-B series the migrating distance in each case differed with the sample volume applied to the gels, but all cases presented single bands. The fact that the preparation behaved as a single band over a broad pH range strongly supports the conclusion that the preparation was pure. On the basis of the combined results of electrophoreses carried out under three different conditions with respect to pH value, it can be said that the enzyme protein is composed of two kinds of subunits which are only very slightly different from each other, and that the enzyme dissociates into an individual or some complex of the same subunits under the conditions of the lst electrophoresis (pH 9.4) but no dissociation occurrs at more acidic pHs.

Electrophoresis of the enzyme in the presence of SDS also revealed that the enzyme is composed of two kinds of polypeptides (Fig. 5). Molecular weights of the two components were estimated to be about 53,000 and 51,000 by subjecting standard proteins (bovine serum albumin, ovalbumin, bovine pancreas chymotrypsinogen A, and horse

Fig. 5. SDS-Polyacrylamide gel electrophoresis. The purified preparation was treated with SDS and 2-mercaptoethanol as described in "Experimental Procedure" and an aliquote (3μg in 0.15 ml of the reaction mixture) was subjected to electrophoresis. A constant current of 8 mA per gel was applied for 4.5 hrs. The concentration of acrylamide in the gel was 10%.

heart cytochrome *c*) to the same electrophoresis. Separation of the two subunits was attempted by subjecting it to DEAE-cellulose column chromatography using 0.1 M Tris-HCl buffer (pH 9.4), but the result was unsuccessful. Therefore, the two subunits might be charge isomers.

Previously obtained results from the same process of electrophoresis (*2*) showed that the enzyme was composed of only one kind of polypeptide. The discrepancy between the two results must be explained. Enzyme extraction in the previous study was carried out by subjecting once-dried cells to autolysis at pH 10.0 overnight. Moreover, the purified preparation was dialyzed against 1% pyridine overnight in order to make material for amino acid analysis. SDS-Polyacrylamide gel electrophoresis was carried out with this preparation as the material. Therefore, the enzyme might have suffered denaturation at some step of purification in this previous case.

The enzyme preparation employed in this study was pure enough to investigate its molecular status, and the protein appears to have two kinds of polypeptide chain, the molecular weights of which are slightly different from each other. One seems to bind PLP through a weaker binding force than the other, so that PLP is easily liberated and the chain itself subsequently polymerized. Polymers thus formed will remain behind the main band of the native enzyme in the electrophoreses. It is also likely that the enzyme dissociates into two kinds of subunits which behave differently under electrophoresis carried out at pH 9.4 but the enzyme keeps its structure intact at more acidic pHs. An attempt to distinguish the two components is now going on in my laboratory. Preliminary results show that the upper component of the main band is more sensitive to a protease than the lower. Complete results will be published elsewhere.

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References

(1) Yamagata, S., Takeshima, K., & Naiki, N. (1974) *J. Biochem.* **75,** 1221-1229
(2) Yamagata, S. & Takeshima, K. (1976) *J. Biochem.* **80,** 777-785
(3) Cowell, J.L. & DeMoss, R.D. (1973) *J. Biol. Chem.* **248,** 6262-6269
(4) Yamagata, S. (1980) *J. Biochem.* **88,** 1419-1423
(5) Yamagata, S. (1971) *J. Biochem.* **70,** 1035-1045
(6) Lowry, O.H., Rosebrough, N.J., Farr, A.L., & Randall, R.J. (1951) *J. Biol. Chem.* **193,** 265-275
(7) Ornstein, L. & Davis, B. J. (1964) *Ann. New York Acad. Sci.* **121,** 321-349
(8) Weber, K. & Osborn, M. (1969) *J. Biol. Chem.* **244,** 4406-4412
(9) Sakami, W. & Toennies, G. (1942) *J. Biol. Chem.* **144,** 203-217
(10) Yamagata, S. (1976) *J. Biochem.* **80,** 787-797