sensitive residue. No change is noted in K_m because, for this enzyme, K_m nearly approximates the dissociation constant for the enzyme–substrate complex and inactivation by *N*-ethylmaleimide or serine apparently does not affect the binding of substrate.

This work was supported in part by a research grant from the National Institutes of Health (AM 11619).

Department of Biochemistry, A. T. PHILLIPS
The Pennsylvania State University,
University Park, Pa. (U.S.A.)

1 E. CHARGAFF AND D. B. SPRINSON, *J. Biol. Chem.*, 148 (1943) 249.
2 E. CHARGAFF AND D. B. SPRINSON, *J. Biol. Chem.*, 151 (1943) 273.
3 E. F. GALE AND M. STEVENSON, *Biochem. J.*, 32 (1938) 392.
4 L. GOLDSTEIN, W. E. KNOX AND E. J. BEHRMAN, *J. Biol. Chem.*, 237 (1962) 2855.
5 J. S. NISHIMURA AND D. M. GREENBERG, *J. Biol. Chem.*, 236 (1961) 2684.
6 W. A. WOOD AND I. C. GUNSALUS, *J. Biol. Chem.*, 181 (1949) 171.
7 A. T. PHILLIPS AND W. A. WOOD, *J. Biol. Chem.*, 240 (1965) 4703.
8 C. F. BREWER AND J. P. RIEHM, *Anal. Biochem.*, 18 (1967) 248.

BBA 63291

Homogenous crystalline phosphoglycerate phosphomutase of high activity. A simple method for lysis of yeast

It was shown in 1957[1] that the crystalline yeast phosphoglycerate phosphomutase (D-phosphoglycerate 2,3-phosphomutase, EC 5.4.2.1), by most criteria pure, showed 5 distinct peaks on electrophoresis and these peaks were considered to represent different amounts of bound phosphate[2]; others[3] suggested this effect to be due to different lysine contents resulting from proteolysis. By varying the length of fermentation, all the electrophoretically different forms of the mutase were crystallized and showed to have specific activities ranging from 200 to 4100, the latter being nearly twice the value first described by us[1]. The procedure of CHIBA, SUGIMOTO AND KITO[3] yields small amounts of the mutase and we have not been able to repeat it. During the last 10 years, we have found that while our method is very reproducible the specific activity of the crystals changes, presumably reflecting the activity of the contaminant proteolytic enzyme(s). Thus, a method which would consistently yield preparations of the highest specific activity would be welcome, particularly in view of the new interest being taken in this enzyme[4]. The present paper presents a simplified procedure, together with a very simple technique for lysing yeast.

3-*P*-glycerate, $(NH_4)_2SO_4$ (enzyme grade) and Lab-trol were obtained from Schwarz, Mann and Dade, respectively. Crystalline phosphoglycerate phosphomutase and phosphopyruvate hydratase (2-phospho-D-glycerate hydro-lyase, EC 4.2.1.11), free of mutase, were prepared as previously described[1,5]. The enolase–mutase coupled assay[1] was used. Specific activity is defined as the enzyme units/mg of protein[6] with the use of Lab-trol as a standard. Disc electrophoresis was performed as previously

described[7]. Solutions of $(NH_4)_2SO_4$ saturated at 25° and neutralized to pH 7 with conc. NH_4OH were used. The pH was determined with the glass electrode in a 1:20 dilution.

Unless otherwise indicated, all operations were carried out at 0–3° and centrifugations were for 15 min at approx. 8000 × *g* with the International centrifuge (Model B 20). All volumes refer to the initial volume for a particular step. All reagents were measured and added at 0–3° except the acetone which was measured at −20° and mixed in at about −60° (*i.e.*, kept in dry ice bath). The preparation of dry yeast has been already described[5]. The dry yeast can be kept in the cold room for over 5 years.

Mix 200 g dry yeast with 400 ml of 1 M NH_4OH. Keep the mixture for 16–18 h at room temperature. Then add 24 ml of 0.5 M Na_4EDTA, 890 ml distilled water and 280 g $(NH_4)_2SO_4$. Heat at 70° ± 1° in a water bath for 5 min. Cool, centrifuge and discard the precipitate. To each 100 ml of the supernatant fluid (Fraction 1) add 30 g of $(NH_4)_2SO_4$. After the $(NH_4)_2SO_4$ is dissolved, centrifuge. Discard the supernatant fluid and take the precipitate in water to about one-half the starting volume of Fraction 1. This is Fraction 2. Mix Fraction 2 rapidly into 1 vol. of acetone. After a few min standing, the bulk of the clear supernatant fluid is poured off and the rest centrifuged at about 3000 × *g* for 5 min. Discard the supernatant fluid and make a paste (with the aid of an homogenizer) with a volume of water equal to the volume of the precipitate. The well-suspended mixture is centrifuged for 5 min. The supernatant is kept and the precipitate is extracted again with a volume of water equal to the amount used in the first extraction. Centrifuge and discard the precipitate. Mix both supernatant fluids and bring to one-half the volume of Fraction 2. This is Fraction 3. Mix Fraction 3 with 1.5 vol. of $(NH_4)_2SO_4$ solution, centrifuge, discard the precipitate, and add 1.5 vol. of $(NH_4)_2SO_4$ to the supernatant fluid and centrifuge. The precipitate containing the enzyme is extracted successively with 30 ml of $(NH_4)_2SO_4$ mixtures of decreasing salt concentration as follows: 20 ml of the satd. $(NH_4)_2SO_4$ solution and 10 ml of water; 19 ml of $(NH_4)_2SO_4$ and 11 ml of water; 18 ml of $(NH_4)_2SO_4$ and 12 ml of water; 17 ml of $(NH_4)_2SO_4$ and 13 ml of water. The first, second, third, fourth (Fraction 4 in Table I) and the fifth extractions yielded 800, 11 000, 140 000, 475 000, and 120 000 units, respectively. Fraction 4 becomes turbid in about 1–2 h and is centrifuged. The precipitate is discarded, and the supernatant fluid is kept in a beaker (covered with filter paper) in the refrigerator. Crystals appear overnight. The next day it is stirred, left standing in the cold for a couple of hours, and then centrifuged. A summary of the purification is shown in Table I.

TABLE I

SUMMARY OF PURIFICATION

The conditions of assay were as described in the text. Purification was from 200 g of dry yeast.

Fraction No.	*Vol. (ml)*	*Total units*	*Total protein*	*Specific activity*	*Yield (%)*
1	1060	1908 000	17 808	108	100
2	500	1860 000	7 000	266	98
3	250	1597 500	1 250	1278	84
4*	2	295 000	70	4200	15

* Crystalline.

Similar results have been obtained with dry yeast preparations from a week to 5 years old. There was less than 20% variation in some 20 preparations. This method can also be applied to (and to lyse) fresh yeast (taking into consideration the water present in the yeast) as follows: 1 lb of yeast (about equal to 130 g dry yeast) is mixed with 270 ml of NH_4OH and stirred slowly for 18–20 h. 250 ml of water, 180 g of $(NH_4)_2SO_4$ and 16 ml of 0.5 M EDTA are then added. From here on the procedure is as described above for the dry yeast.

Fast or slow drying or storage of the fresh yeast does not greatly influence the preparations; cakes of fresh yeast (2 days old, according to the manufacturer) were kept in the cold room and immediately after arrival and at 9, 20, 29, and 40 days, several cakes were dried as described above, and a number of cakes were dried quickly (immediately after arrival) by passing the yeast through several wire screens with decreasing size of holes, useing a fan, as well as spreading in a very thin layer. 2 g of the dried yeast samples were mixed with 4 ml of 1 M NH_4OH and left standing overnight. 4 ml of H_2O were then added and the samples centrifuged. The supernatant fluids were assayed. The quickly dried sample had 3800 units/ml (unstored yeast). The slowly dried samples from yeast stored for 0, 9, 20, 29, and 40 days showed 3800, 4100, 4200 3700, and 3500 units/ml, respectively.

Optimum extractibility is obtained in about 15 h and at 22–30° with either fresh or dry yeast. Also, it makes little difference whether 0.35–1.0 M NH_4OH is used for the extraction. Below 0.35 M or above 1.0 M, NH_4OH is less effective.

In agreement with the results obtained by others[3], the enzyme with a specific activity of over 4000 has the same crystalline appearance, *i.e.*, rhombic plates as previously described for the enzyme with specific activity of about 2000 (ref. 1). Also, disc electrophoresis and electrophoresis in cellulose acetate strips show essentially one band with either kind of preparation. Indeed, it is of interest that the gel electrophoresis does not differentiate between preparations having a 5-fold difference in specific activities.

This study was supported by grants No. 63-G-10, American Heart Association, and AM 01855 and AI 03505, U.S. Public Health Service. E.M. was a Juan March Foundation Fellow, and I.S. was a Fullbright Scholar.

Department of Biochemistry,
University of Kansas
Medical Center,
Kansas City, Kan. (U.S.A.)

ENRIQUE DE LA MORENA
ILDEU SANTOS
SANTIAGO GRISOLIA

1 V. W. RODWELL, J. C. TOWNE AND S. GRISOLIA, *Biochim. Biophys. Acta*, 20 (1956) 394.
2 H. EDELHOCH, V. W. RODWELL AND S. GRISOLIA, *J. Biol. Chem.*, 228 (1957) 891.
3 H. CHIBA, E. SUGIMOTO AND M. KITO, *Bull. Chem. Soc. Japan*, 24 (1960) 418.
4 S. GRISOLIA, *Colloque sur L'Evolution Biochimique*, Paris, 1967, in the press.
5 S. GRISOLIA, D. B. BOGART AND A. TORRALBA, *Biochim. Biophys. Acta*, 151 (1968) 298.
6 L. C. MOKRASCH, W. D. DAVIDSON AND R. W. MCGILVERY, *J. Biol. Chem.*, 222 (1956) 179.
7 S. H. BISHOP AND S. GRISOLIA, *Biochim. Biophys. Acta*, 139 (1967) 344.

Received November 9th, 1967