We have described the simplest case of a single ^2H label in a single histidine residue in a protein. Due to the known limited resolution of proton NMR signals of multiple resonances of a given type of residue in a protein, such as histidines at high and low pH values and tyrosine or methionine residues, one can predict that deuterium resonances of the same residues will not be resolved, particularly in view of the deuterium line widths. However, deuterium labeling and deuterium NMR observation may be a convenient general method for measuring protein mobility and self-association in solution. We are extending our studies to other systems where the deuterium probe may supply unique information.

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Isolation and Characterization of Fourteen Ribosomal Proteins from Small Subunits of Yeast†

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ABSTRACT: A method for preparation of a large amount of ribosomal subunits from *Saccharomyces cerevisiae* by a Ti-15 zonal rotor is described. The proteins of the small subunits (ca. 50 000 A_{260} units) were separated into 22 fractions by chromatography on carboxymethylcellulose columns. Fourteen proteins were then purified from the ten chromatographic fractions by filtration through Sephadex G-100 or Sephacryl S-200. The isolated proteins are YP 6, YP 7, YP 9, YP 12, YP 14′, YP 14″, YP 28, YP 38, YP 45, YP 50, YP 52, YP 58, YP 63, and YP 70. The molecular weights and amino acid compositions of these proteins are presented.

In a previous paper (Otaka & Kobata, 1978), we identified 74 proteins from yeast ribosomes by two-dimensional polyacrylamide gel electrophoresis. A large-scale preparation of these individual ribosomal proteins has become necessary for sequencing studies. Here, we describe, first, a procedure for preparing a large amount of small and large subunits from yeast cells. The ribosomal proteins prepared from the respective subunits were then fractionated by carboxymethylcellulose (CMC)[1] column chromatography. Most of the protein fractions from the first CMC column chromatography contained more than one protein species and were therefore further fractionated by chromatography on columns of Sephadex G-75, Sephadex G-100, Sephacryl S-200, or phosphocellulose. As a result, we have so far succeeded in purifying 14 ribosomal proteins from small subunits and 23 from large subunits in milligram quantity. Recently, one of

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[1] Abbreviations used: CMC, carboxymethylcellulose; TMD-I buffer, 50 mM Tris-HCl (pH 7.4)–10 mM $MgCl_2$–1 mM dithiothreitol; DTT, dithiothreitol; TD buffer, 50 mM Tris-HCl (pH 7.4)–1 mM dithiothreitol; TMD-II buffer, 50 mM Tris-HCl (pH 7.4)–0.1 mM $MgCl_2$–1 mM dithiothreitol; 2-D electrophoresis, two-dimensional electrophoresis; $NaDodSO_4$, sodium dodecyl sulfate.

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these large subunit proteins, YP 44, has been completely sequenced (Itoh & Wittmann-Liebold, 1978). The detailed fractionation procedures for these small subunit proteins and their molecular weights and amino acid compositions are presented in this paper. For the proteins from large subunits, the results will be presented in the next paper of this series.

Materials and Methods

Yeast Cells, Culture Medium, and Growth Conditions. Cells of yeast, *Saccharomyces cerevisiae*, used in the large-scale preparation were provided in a pressed cake form without additives by Dr. I. Hino of Kyowa Hakko Kogyo Co., Ltd. The cells were stored at −70 °C and appeared to be stable for at least 3 months under these conditions.

For the labeling of cells with isotopes, the following media were used. Preculture: 20 g of glucose, 2 g of KH_2PO_4, 8 g of $(NH_4)_2SO_4$, 16.3 mg of $MgCl_2$, 2 g of yeast extract, 0.2 mg of vitamin B_6, 0.2 mg of nicotinic acid, 0.2 mg of pantothenate, 10 mg of inositol, 0.002 mg of biotin, 0.1 mg of *p*-aminobenzoic acid, and 0.1 mg of vitamin B_2 per L of distilled water. Main culture: 20 g of glucose, 2 g of asparagine, 1.5 g of KH_2PO_4, 0.5 g of $MgSO_4 \cdot 7H_2O$, 2 g of $(NH_4)_2SO_4$, 0.33 g of $CaCl_2 \cdot 2H_2O$, and 0.1 mg of KI per L of distilled water. Ten milliliters of preculture grown at 28 °C for 18 h was transferred into 100 mL of the main-culture medium and incubated at 28 °C for 2 h. One hundred microcuries of [^{3}H]lysine (40 Ci/mmol; The Radiochemical Centre, Amersham, England) or ten microcuries of [^{14}C]lysine (318 mCi/mmol; same source as above) were added to the culture. After 5-h incubation, cells were harvested. To obtain a small amount of nonlabeled ribosomal proteins, we cultured the cells in the medium described in a previous paper (Otaka & Kobata, 1978). The cells were washed once with TMD-I buffer [50 mM Tris-HCl, pH 7.4, 10 mM $MgCl_2$, and 1 mM dithiothreitol (DTT)] and stored at −70 °C until used.

Preparation of Ribosomes. The ribosomal subunits from a small amount of labeled or unlabeled cells were prepared as described previously (Otaka & Kobata, 1978). For large-scale preparation, the above methods were scaled up as follows. All manipulations were carried out at 4 °C except for sucrose gradient centrifugation in a Spinco Ti-15 zonal rotor. The packed cells (500 g) were suspended in 120 mL of TMD-I buffer, mixed with 4000 g of glass beads (0.5-mm diameter), and broken in a Vibrogen-Cell mill run at top speed for 8–10 min. The crude extract obtained by several washings of the vibrated mixture with TMD-I buffer (2000 mL) was centrifuged at 5000 rpm for 5 min in a Sorvall superspeed centrifuge to remove glass beads and cell debris. The supernatant was recentrifuged to remove nuclei and mitochondria in a Spinco 42 rotor at 32 000 rpm for 15 min. The post-mitochondrial supernatant was treated with 1/400 volume of 20% Triton X-100 and centrifuged in a Spinco Ti-15 zonal rotor at 31 000 rpm for 13 h to obtain crude 80S ribosomes. These were resuspended in 260 mL of TMD-I buffer by gentle stirring, and aggregates were removed by centrifugation at 10 000 rpm in a Sorvall centrifuge for 5 min. To the ribosomal suspension, an equal volume of 10% ammonium sulfate containing 30 mM $MgCl_2$ was added and stirred gently for 20 min in an ice bath. This mixture was then overlayered on 6 mL of bentonite-treated 35% sucrose in TMD-I buffer and centrifuged in a Spinco 42 rotor for 6.5 h at 32 000 rpm. The ammonium sulfate washed ribosomal pellet was then resuspended in 100 mL of TMD-I buffer and stirred gently for 30 min, followed by centrifugation for 10 min at 10 000 rpm to remove aggregates. The suspension of the purified 80S ribosomes so obtained was stored at −70 °C until used. The yield was usually 90 000 A_{260} units from 500 g of packed cells.

Preparation of Ribosomal Subunits and Ribosomal Proteins. The purified 80S ribosomes for large-scale preparation were dialyzed against TD buffer (50 mM Tris-HCl, pH 7.4, and 1 mM DTT) for 5 h. The sample (100–170 mL; 100–150 A_{260} units/mL) was loaded on a convex exponential sucrose gradient in a Spinco Ti-15 zonal rotor. The sucrose gradient was composed of 5–32% (w/w) in TMD-II (50 mM Tris-HCl, pH 7.4, 0.1 mM $MgCl_2$, and 1 mM DTT) with 80 mL of 45% sucrose as a cushion and an overlayer of 270 mL of TMD-II buffer. The centrifugation was at 28 000 rpm for 17 h at 10 °C.

The small- and large-subunit fractions indicated with brackets in Figure 1 were pooled. Seven-tenths volume of cold ethanol was then added to both fractions in the presence of 10 mM $MgCl_2$. After overnight storage at −20 °C, the suspension was centrifuged for 10 min at 7500 rpm. The pelleted ribosomal subunits were redissolved in 0.05 M sodium acetate buffer (pH 5.6) containing 6 M urea.

Ribosomal proteins were extracted with acetic acid as described by Hardy et al. (1969), dialyzed against cold 5% acetic acid, and then lyophilized.

Column Chromatography. CMC (CM-52, Whatman) column chromatography of protein samples doubly labeled with [^{3}H]- and [^{14}C]lysine was done essentially as described by Otaka et al. (1968). The elution gradient was from 0.05 to 0.65 M sodium acetate buffer containing 1 mM DTT.

The chromatographic profile of isotopically labeled proteins with 50 mg of carrier proteins was monitored both by counting radioactivity in a 50-μL sample taken from each fraction and by measuring absorption at 750 nm of a 50-μL sample by the method of Lowry et al. (1951). The remainder was kept at −20 °C until used for two-dimensional (2-D) electrophoresis.

For the large-scale chromatography, ribosomal proteins from approximately 50 000 A_{260} units of small subunits were loaded on a CMC column (2.5 × 63 cm) and eluted with a linearly increasing concentration of sodium acetate buffer (pH 5.6), 0.05 to 0.65 M, containing 6 M urea and 1 mM DTT in a total volume of 8 L at a rate of about 15 (mL/20 min)/tube at 4 °C. The column was then washed with 0.65 M sodium acetate buffer (pH 5.6) containing 1 M NaCl, 6 M urea, and 1 mM DTT. The protein elution was monitored by the absorption at either 230 or 280 nm. Fractions were separately pooled, lyophilized after dialysis, and kept at −70 °C until used.

Sephadex G-100 and Sephacryl S-200 obtained from Pharmacia Fine Chemicals AB (Uppsala, Sweden) were used for further separation of proteins when necessary. The lyophilized samples were redissolved in 0.05 M NaH_2PO_4 buffer (pH 6.5 by methylamine) containing 6 mM 2-mercaptoethanol, 0.3 M KCl, and 6 M urea. Detailed conditions for filtrations are listed in the legend of Figure 5. Fractions containing the purified proteins were pooled, dialyzed exhaustively against cold 2% acetic acid, lyophilized, and kept at −70 °C for further studies. Other fractions containing more than one protein species were kept at −70 °C for further fractionation.

Electrophoresis. Polyacrylamide disc electrophoresis at pH 4.5 was carried out as described by Leboy et al. (1964).

The 2-D electrophoretic method originally described by Mets & Bogorad (1974) was performed according to our modification described in a previous paper (Otaka & Kobata, 1978). To identify the isolated protein and to examine its purity, we separately analyzed two samples on gel slabs with and without 25 μg of total 80S ribosomal proteins.

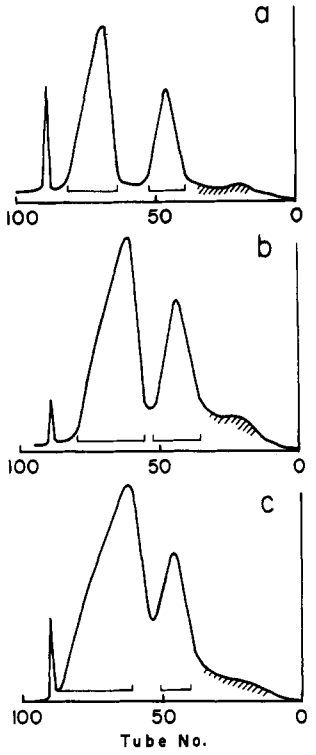

FIGURE 1: Separation of yeast ribosomal subunits by a Ti-15 zonal rotor. (a) 10000 A_{260} units. (b) 17000 A_{260} units. (c) 25000 A_{260} units. The ordinate is A_{260} in arbitrary units.

Sodium dodecyl sulfate ($NaDodSO_4$) disc electrophoresis for molecular weight estimation was done by the procedure of Weber & Osborn (1969) with some modifications. The spacer gel (1 cm length) containing 5% acrylamide was stacked on the separation gel (10 cm length) containing 10% acrylamide. The protein standards used were bovine serum albumin (M_r 68000), ovalbumin (M_r 45000), chymotrypsinogen A (M_r 25000), and lysozyme (M_r 14400), all obtained from Schwarz/Mann (New York).

Amino Acid Analysis. The purified proteins (20–40 μg) were hydrolyzed for 20 h in 6 N HCl with 0.02% 2-mercaptoethanol at 110 °C in evacuated sealed tubes. Amino acid analysis was performed using a Beckman 121M amino acid analyzer. No corrections for incomplete hydrolysis or for decomposition were made; tryptophan and cysteine were not determined.

Results and Discussion

Preparation of Ribosomal Subunits and Ribosomal Proteins. When the ammonium sulfate washed 80S ribosomes were dialyzed against TD buffer, they dissociated into subunits (Otaka & Kobata, 1978). A sample of 100 mL containing 10000 A_{260} units of the dialyzed ribosomes (about 900 mg) was loaded on the convex exponential sucrose gradient in a Ti-15 zonal rotor and centrifuged. Figure 1a shows that the ribosomes were completely separated into large and small subunits. In the case of yeast ribosomes, only smaller quantities (about 150 mg) have hitherto been successfully separated in a zonal rotor (van der Zeijst & Bult, 1972; van der Zeijst et al., 1972; Grankowski et al., 1976), while a separation procedure of up to 1.4 g of rat liver or muscle ribosomes has been reported by Wool and his co-workers (Sherton & Wool, 1972; Sherton et al., 1974). As shown in Figure 1c, the amount up to about 25000 A_{260} units (about 2 g) could be loaded without significant disturbance of resolution. The fraction indicated with oblique lines appears to be degradation products of small subunits, since it contained mainly small-subunit proteins when examined by 2-D electrophoresis. The yield of small subunits was approximately 15% of the original ribosomes loaded and that of the large subunits was 40%, while the overall recovery of A_{260} was about 75%.

Both subunit fractions were pooled and precipitated with ethanol after addition of $MgCl_2$. The ribosomal pellets were redissolved in 0.005 M sodium acetate buffer (pH 5.6) containing 6 M urea (500 A_{260} units/mL). The use of 6 M urea in this step gave a good recovery (98–100%) of ribosomal proteins after 67% acetic acid treatment, probably because of better solubility of pelleted ribosomes as compared with the buffer without urea.

Fractionation of Ribosomal Proteins by Chromatography on Carboxymethylcellulose Columns. A mixture of the [^{14}C]lysine-labeled small-subunit proteins and [^{3}H]lysine-labeled large-subunit proteins (about 2 and 3 mg, respectively) was chromatographed on a CMC column (0.8 × 40 cm). The recovery of radioactivity after chromatography was almost 100%, and most peaks of small-subunit proteins did not coincide with those of the large-subunit proteins (Figure 2).

In Figure 3 is shown a CMC column chromatography (0.8 × 40 cm) of 50-mg small-subunit proteins plus [^{3}H]lysine-labeled proteins. The radioactivity profile was almost the same as that of the [^{14}C]lysine-labeled proteins shown in Figure 2, indicating that this amount of protein can be chromatographed with good resolution.

Aliquots of the fractions in Figure 3 were individually subjected to 2-D electrophoresis to analyze their components. The protein numbering system used in our previous paper

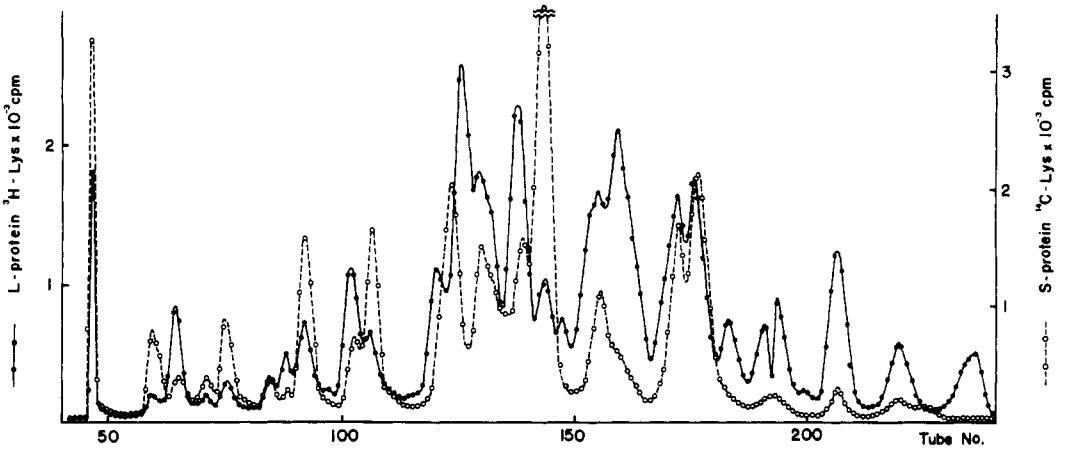

FIGURE 2: Carboxymethylcellulose chromatography of isotopically labeled small- and large-subunit proteins.

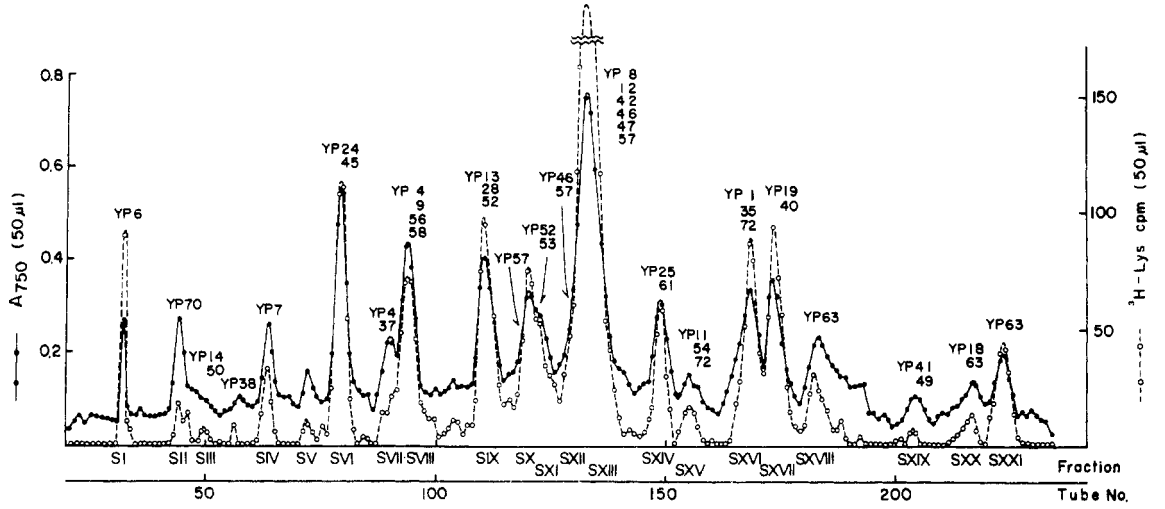

FIGURE 3: Carboxymethylcellulose chromatography of isotopically labeled small-subunit proteins with 50 mg of carrier proteins.

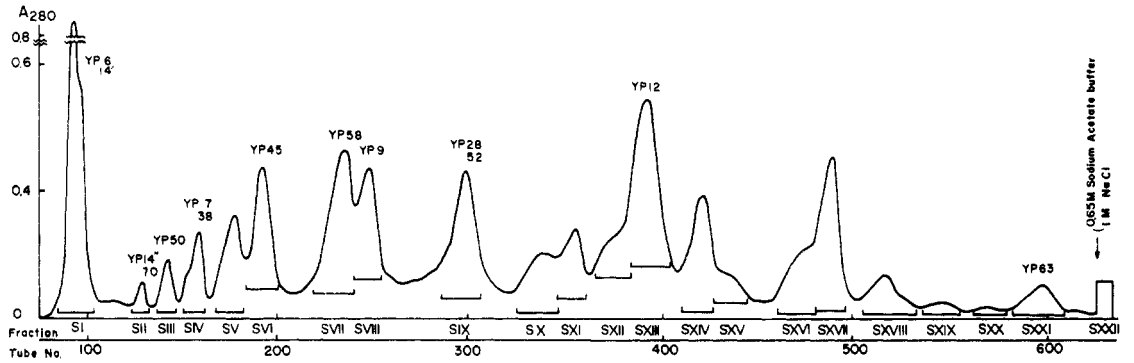

FIGURE 4: Preparative carboxymethylcellulose chromatography of small-subunit proteins. The inserted YP numbers indicate the protein species isolated from the fractions.

Table I: Amino Acid Composition[a] and Molecular Weight of Proteins Isolated from Yeast Small Ribosomal Subunits

protein:	YP 6	YP 7	YP 9	YP 12	YP 14′	YP 14″	YP 28	YP 38	YP 45	YP 50	YP 52	YP 58	YP 63	YP 70
M_r ($\times 10^{-3}$):	37.5	29.0	28.9	32.0	25.7	26.5	24.5	16.5	15.0	14.5	15.0	14.5	13.0	8.0
Asp	11.9	7.3	7.7	10.6	18.2	16.2	8.2	9.7	9.6	10.0	6.5	10.2	7.7	9.7
Thr	6.6	5.7	6.7	5.1	3.3	3.8	3.5	7.6	1.9	8.4	4.2	5.4	5.7	8.1
Ser	7.6	4.6	4.1	3.1	6.2	6.7	5.0	5.8	5.9	5.8	6.1	6.6	4.2	5.3
Glu	9.9	12.1	10.6	7.0	9.0	9.6	11.6	16.6	13.0	12.6	9.2	11.0	3.9	11.1
Pro	3.4	4.6	6.0	4.9	6.7	5.3	5.2	4.4	4.1	3.8	3.9	3.4	3.0	3.1
Gly	8.8	8.2	11.6	9.6	7.3	8.9	5.6	6.7	10.5	6.6	8.7	9.8	7.8	7.8
Ala	9.0	11.0	8.5	6.3	5.7	6.7	10.2	5.7	9.6	7.2	9.7	7.5	7.5	3.4
Val	7.8	9.3	8.5	7.7	8.1	7.9	6.3	10.2	7.9	10.1	9.4	8.1	10.7	13.0
Met	1.3	1.8	0.5	0.5	0.1	0.4	0.3	0.4	1.2	0.3	0.4	0.5	0.0	1.5
Ile	5.5	5.2	6.3	5.7	4.3	4.7	4.5	7.2	6.7	4.3	5.6	7.1	1.6	4.2
Leu	9.5	8.6	8.9	10.0	8.1	7.9	10.4	6.7	6.1	6.7	8.2	7.9	6.9	9.3
Tyr	2.1	2.4	1.8	2.9	3.1	3.3	2.9	1.2	3.7	3.8	3.9	1.8	5.1	0.2
Phe	3.6	3.5	2.8	3.5	5.1	4.5	3.7	1.9	2.1	3.7	3.9	3.3	1.6	1.7
His	2.3	1.2	1.4	3.5	1.5	1.9	2.0	1.7	1.5	1.4	2.1	2.5	5.5	0.1
Lys	6.8	7.6	7.9	10.6	9.5	8.4	9.5	9.3	8.0	10.2	10.7	8.6	23.0	6.3
Arg	4.2	7.1	6.8	9.2	4.0	4.1	11.3	5.0	8.3	4.3	7.6	6.4	5.9	15.2

[a] The values are in units of mole percent.

(Otaka & Kobata, 1978) was redesignated here with the prefix YP (yeast protein). The protein numbers with the prefix YP are inserted in Figure 3 to indicate the protein species found in each fractions. A few fractions contained one or two proteins, while the others contained several. The proteins, YP 65, YP 66, and YP 71, which were found mainly in small subunits in the previous studies (Otaka & Kobata, 1978), could not be detected here. These proteins might be included in ill-defined chromatographic peaks, which were not examined by 2-D electrophoresis.

For preparative chromatography, the proteins from 50 000 A_{260} units of small subunits were chromatographed on the CMC column (2.5 × 63 cm). The elution profile (Figure 4) was comparable with that of Figure 3.

Isolation of Proteins. Refractionation of proteins in the respective fractions derived from the preparative chromatography (Figure 4) was carried out as follows. The fraction S-I was filtrated through a Sephadex G-100 column (Figure 5a). The fractions indicated with brackets produced a single band by disc electrophoresis at pH 4.5 and by $NaDodSO_4$ disc electrophoresis. 2-D electrophoresis of these fractions indicated that the first fraction with brackets contained exclusively YP

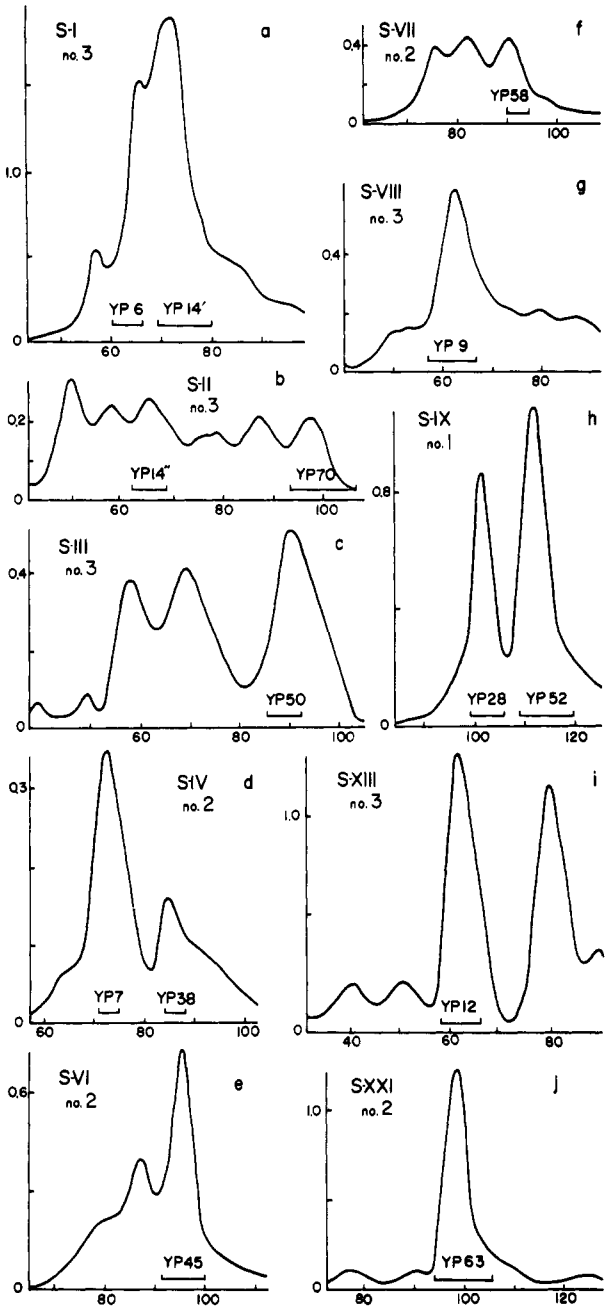

FIGURE 5: Refractionation of proteins. The systems used for filtration were as follows: no. 1 Sephacryl S-200 (2.5 × 140 cm), 3 (mL/h)/cm^2, 3 mL/tube; no. 2 Sephacryl S-200 (1.5 × 150 cm), 3 (mL/h)/cm^2, 1.5 mL/tube; no. 3 Sephadex G-100 (2.5 × 140 cm), 2 (mL/h)/cm^2, 3 mL/tube. Ordinate: protein concentration by absorption at 230 nm in Figure 5b and at 280 nm in the rest. Abscissa: fraction (tube number).

6 (yield, 12 mg by dry weight). The second fraction appeared as a single spot slightly to the left of YP 14. However, this position did not correspond to any of the spots in the standard 2-D pattern of 80S ribosomal proteins. This protein was designated as YP 14′ (yield, 110 mg).

The protein YP 70 was isolated from S-II by filtration through a Sephadex G-100 column (Figure 5b) (yield, 2.0 mg). An additional protein, YP 14″, which appeared left of YP 14 spot, was obtained (yield, 2.0 mg). YP 14′ and YP 14″ were located at the same position on the 2-D electrophoretogram, and their amino acid compositions are quite similar (see Amino Acid Composition and Molecular Weight). It should be noted that small-scale preparations of ribosomes from exponentially growing cells did not contain a detectable amount of YP 14′

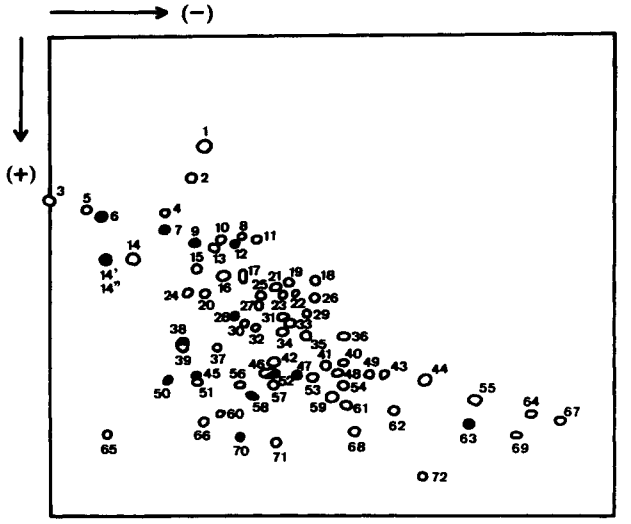

FIGURE 6: Schematic diagram of a two-dimensional polyacrylamide gel electrophoretogram of 80S ribosomal proteins. Isolated protein species are indicated with filled circles.

or YP 14″ [see Otaka & Kobata (1978)], whereas large-scale preparations contained appreciable amounts of these proteins. It is not clear whether this is due to the difference of the growth stage or to the modified procedures used for large-scale preparations.

The following proteins were also purified from respective fractions by the methods indicated: YP 50 from S-III through Sephadex G-100 (yield, 3 mg); YP 7 (yield, 1.8 mg) and YP 38 (yield, 1.3 mg) from S-IV through Sephacryl S-200; YP 45 (yield, 13.0 mg) from S-VI through Sephacryl S-200; YP 58 (yield, 5.0 mg) from S-VII through Sephacryl S-200; YP 9 (yield, 6.6 mg) from S-VIII through Sephadex G-100; YP 28 (yield, 11.0 mg) and YP 52 (yield, 14.0 mg) from S-IX through Sephacryl S-200; YP 12 (yield, 24 mg) from S-XIII through Sephadex G-100; YP 63 (yield, 5.0 mg) from S-XXI through Sephacryl S-200 (Figure 5). The 2-D position of YP 45 was close to that of YP 51. However, oxidation of these proteins results in a selective translocation of YP 51 without effecting YP 45 (Otaka & Kobata, 1978). The protein isolated here was identified as YP 45 in this way.

In Figure 4 and Figure 6 the isolated proteins in a pure form in these studies are indicated with a YP number near the fraction and with filled circles on the schematic 2-D pattern, respectively.

Amino Acid Composition and Molecular Weight. Amino acid compositions of the isolated proteins are shown in Table I. A close examination of the table suggests that all the proteins listed are unique at least with respect to their amino acid compositions, except for YP 14′ and YP 14″. As mentioned before, these proteins revealed similar compositions and were at the same position on the 2-D pattern, suggesting that they are structurally quite similar, if not identical, proteins. However, unusually high aspartic acid content and the rather acidic nature of these proteins suggest the possibility that they may not be genuine ribosomal proteins.

The molecular weight of the purified proteins was estimated by electrophoresis in polyacrylamide gels containing $NaDodSO_4$ (Weber & Osborn, 1969). Approximate molecular weights of individual ribosomal proteins were estimated by running total 80S proteins together with the standard proteins on a 2-D slab (Otaka & Kobata, 1978). The molecular weights given here (Table I) are in good agreement with our previous data, with about 15% deviation at the most, except for YP 70. The deviation for YP 70 was larger than

15%. The molecular weight estimation obtained from 2-D slab electrophoresis might be subject to rather large errors for the low molecular weight protein species.

Isolation of small-subunit proteins from rat liver has been done by several workers [for a review, see Bielka & Stahl (1978)]. In particular, Wool and his co-workers reported the purification of almost all the small-subunit proteins (Collatz et al., 1976, 1977). However, there has been no report so far on the isolation of ribosomal proteins from lower eucaryotes such as yeast. The yeast proteins purified here do not represent a complete collection and consist of less than half of about 30 species in the small subunit. The remaining proteins could not be purified by the two-step chromatography described in this paper, and we are in the course of isolating them by other means.

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Quantitative Assessment of the Noncovalent Inhibition of Sickle Hemoglobin Gelation by Phenyl Derivatives and Other Known Agents†

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ABSTRACT: The ability of a variety of phenyl derivatives to inhibit sickle cell hemoglobin gelation was placed on a quantitative scale by parallel equilibrium and kinetic assays. Modifications of the phenyl ring studied include polar, nonpolar, and charged substituents, added aromatic rings, and loss of aromaticity. Other noncovalent inhibitors previously reported to have high potency were measured and placed on the same quantitative scale. Some phenyl derivatives were found to be as effective as any other known noncovalent antigelling agent. The phenyl compounds penetrate easily into red cells, and their potency is tolerant to chemical modification, which holds out the possibility of designing low-toxicity derivatives. On the negative side, the level of potency obtainable appears to be inadequate for clinical use. The best phenyl inhibitors display a functionally defined inhibitory constant (K_I) of 75 mM, and it can be estimated that inhibitor concentrations over 20 mM would be necessary to obtain minimal clinically significant benefit. Furthermore, with the variety of modifications tested here, no impressive increase in activity could be achieved over that found in the simplest phenyl compounds.

When sickle cell hemoglobin (HbS) is deoxygenated, it aggregates to form extended fibrillar structures (Murayama, 1966; Bertles et al., 1970). In the red cell these structures distort the membrane into bizarre shapes and rigidify the cell. The aggregation phenomenon can also be studied in concentrated solutions of HbS in which the result of self-association is the formation of semisolid gel. With other investigators, we reasoned that amino acids or small peptides might competitively inhibit specific contacts involved in the formation of HbS fibers. The literature contains reports of the inhibition of gelation by several amino acids and oligopeptides [see Dean & Schechter (1978)]. We tested the amino acids and found, in agreement with Noguchi & Schechter (1977, 1978), that only phenylalanine and tryptophan inhibit gelation at reasonable concentrations. Experiments with derivatives of these

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