Isolation and Characterization of Twenty-three Ribosomal Proteins from Large Subunits of Yeast†

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ABSTRACT: The proteins of large ribosomal subunits from *Saccharomyces cerevisiae* were separated into 25 fractions by chromatography on columns of carboxymethylcellulose (CMC). Twenty-three proteins were then purified from the 12 CMC fractions by filtration through Sephadex G-75, Sephadex G-100, and Sephacryl S-200, and/or by phosphocellulose column chromatography. The isolated proteins are YP 1, YP 2, YP 9, YP 11, YP 13′, YP 16, YP 18, YP 26, YP 39, YP 41, YP 42, YP 42′, YP 44, YP 45, YP 47′, YP 52a, YP 53, YP 55, YP 59, YP 62, YP 68, YP A1, and YP A2. The molecular weight and amino acid composition of these proteins are presented.

In a previous paper (Higo & Otaka, 1979), we reported a large-scale isolation of small-subunit proteins from yeast ribosomes. Here, we have isolated 23 proteins in milligram quantity from large ribosomal subunits of the same organism. The detailed methods for isolation are described below.

Materials and Methods

Preparation of Ribosomes, Ribosomal Subunits, and Ribosomal Proteins. The methods described in Higo & Otaka (1979) were followed.

Fractionation of Large-Subunit Proteins. The proteins from large subunits were fractionated on columns of carboxymethylcellulose (CMC)[1] (2.5 × 55 cm) as described in a previous paper (Higo & Otaka, 1979) with the following modifications. After loading the sample, elution was done first with 900 mL of 0.05 M sodium acetate buffer (pH 5.6) containing 6 M urea and 0.5 mM dithiothreitol (DTT) and then with 6 L of a linearly increasing concentration of sodium acetate buffer (pH 5.6) from 0.05 to 0.65 M at a flow rate of 15 mL/(20 min tube). At the completion of the gradient, further elution was done with 300 mL of 0.85 M sodium acetate buffer (pH 5.6). The elution was monitored by assaying protein concentration (Lowry et al., 1951).

The systems for refractionations of the CMC fractions with Sephadex G-75, Sephacryl S-200 (Pharmacia, Uppsala, Sweden), phosphocellulose (P-cellulose; high capacity; Schwarz/Mann, New York), and/or diethylaminoethylcellulose (DEAE-cellulose; Bio-Rad, Richmond, CA) are listed in Table I.

Electrophoresis. Methods for all electrophoretic systems described in a previous paper (Higo & Otaka, 1979) were used. Molecular weight estimations of YP 44, YP 52a, YP 55, YP 59, YP 62, YP 68, YP A1, and YP A2 (Table III) were done by using the following standard proteins: myoglobin (M_r 17200), lysozyme (M_r 14300), cytochrome *c* (M_r 11700), and bacitracin (M_r 1450) (Schwarz/Mann, New York).

For the examinations of acidic proteins, two-dimensional (2-D) electrophoresis by Kaltschmidt & Wittmann (1970) and disc electrophoresis (pH 8.6; the same system with the first dimension of 2-D) were used.

Amino Acid Analysis. Amino acid analyses were done as described in a previous paper (Higo & Otaka, 1979).

Results and Discussion

Fractionation of Ribosomal Proteins by Chromatography on Carboxymethylcellulose Columns. In Figure 1a is shown a CMC column (0.8 × 40 cm) chromatographic profile of 50 mg of large-subunit proteins labeled with [^{3}H]lysine. The pattern was in essential agreement with that of a small-scale chromatography [about 3 mg of ^{3}H-labeled proteins; see Figure 2 of Higo & Otaka (1979)]. To analyze protein components in the respective CMC fractions, we subjected aliquots to 2-D electrophoresis by the method of Mets & Bogorad (1974) with some modifications. The YP numbers, our protein numbering system (Higo & Otaka, 1979), in Figure 1a indicate the protein species identified in the respective fractions, most of which contained more than one protein. We could not detect YP 15, YP 39, YP 67, and YP 69, which were previously found in large subunits (Otaka & Kobata, 1978). Since we did not analyze ill-defined peaks in this study, these proteins might have escaped detection. Alternatively, proteins, which could not be found in the standard 80S proteins, were detected in some fractions (not shown in Figure 1a). These may be aggregates or degradation products of certain proteins produced during chromatography.

To isolate an amount sufficient for sequencing studies, we fractionated the ribosomal proteins from about 60000 A_{260} units of large subunits by means of a preparative CMC column (2.5 × 55 cm) (Figure 1b). The separation was similar to that of Figure 1a, where 50 mg of proteins was chromatographed. Therefore, the fractions common to both figures were given the same designations, except for the fractions L-II through L-VII which might be aggregates or degradation products.

Isolation of Proteins. Purification of proteins from the respective chromatographic fractions (Figure 1b) was carried out as summarized in Table II (see Figures 2–10). Only the results requiring special comments are described below. Fraction L-I was chromatographed on a DEAE-cellulose column to obtain acidic proteins (Figure 2a). Fraction D3, obtained by washing the column with 0.5 M ammonium acetate (pH 5.0), was rechromatographed on the same column.

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[1] Abbreviations used: CMC, carboxymethylcellulose; DTT, dithiothreitol; P-cellulose, phosphocellulose; DEAE-cellulose, diethylaminoethylcellulose; 2-D electrophoresis, two-dimensional electrophoresis; $NaDodSO_4$, sodium dodecyl sulfate.

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Table I: Elution System for Refractionation of Proteins

system[a] no.	material	column size	elution	flow rate	detection
1	Sephacryl S-200	2.5 × 140	5% acetic acid, 6 M urea	3 mL/(15 min tube)	A_{280}
2	Sephacryl S-200	2.2 × 140	5% acetic acid, 6 M urea	3 mL/(15 min tube)	A_{280}
3	Sephacryl S-200	2.5 × 140	0.05 M NaH_2PO_4, 0.3 M KCl, 6 M urea, pH 6.5 by methylamine (K buffer)	3 mL/(15 min tube)	A_{230}
4	Sephadex G-100	2.5 × 140	K buffer	3 mL/(15 min tube)	A_{230}
5	Sephadex G-100 or G-75	2.5 × 200	0.05 M pyridine formate, pH 4.5, 6 M urea	5 mL/(25 min tube)	A_{750}/folin
6	P-cellulose	1.5 × 40	0.1–0.7 M NaCl per 1.8-L gradient in 0.05 M NaH_2PO_4, 6 M urea, pH 6.5 by methylamine (P buffer)	5 mL/(15 min tube)	A_{750}/folin
7	P-cellulose	1.2 × 23	0.15–0.55 M NaCl per 0.5-L gradient in P buffer	1.5 mL/(20 min tube)	A_{280}
8	P-cellulose	1.2 × 23	0.2–0.6 M NaCl per 0.5-L gradient in P buffer	1.5 mL/(20 min tube)	A_{280}
9	P-cellulose	1.6 × 25	0.2–0.7 M NaCl per 0.5-L gradient in P buffer	2 mL/(22 min tube)	A_{280}
10	P-cellulose	1.2 × 30	0.2–0.4 M NaCl per 0.5-L gradient in P buffer	1.5 mL/(20 min tube)	A_{280}
11	DEAE-cellulose	1.5 × 30	0.01 (pH 5.7)–0.25 M (pH 5.0) ammonium acetate per 1.2-L gradient in 6 M urea, 0.5 mM DTT, and 0.5 M ammonium acetate (pH 5.0), 0.2 L	4 mL/(10 min tube)	A_{750}/folin
12	DEAE-cellulose	0.8 × 40	0.01 (pH 5.7)–0.5 M (pH 5.0) ammonium acetate per 0.4-L gradient in 6 M urea, 0.5 mM DTT	1.5 mL/(15 min tube)	A_{750}/folin

[a] The system numbers were indicated in the figures.

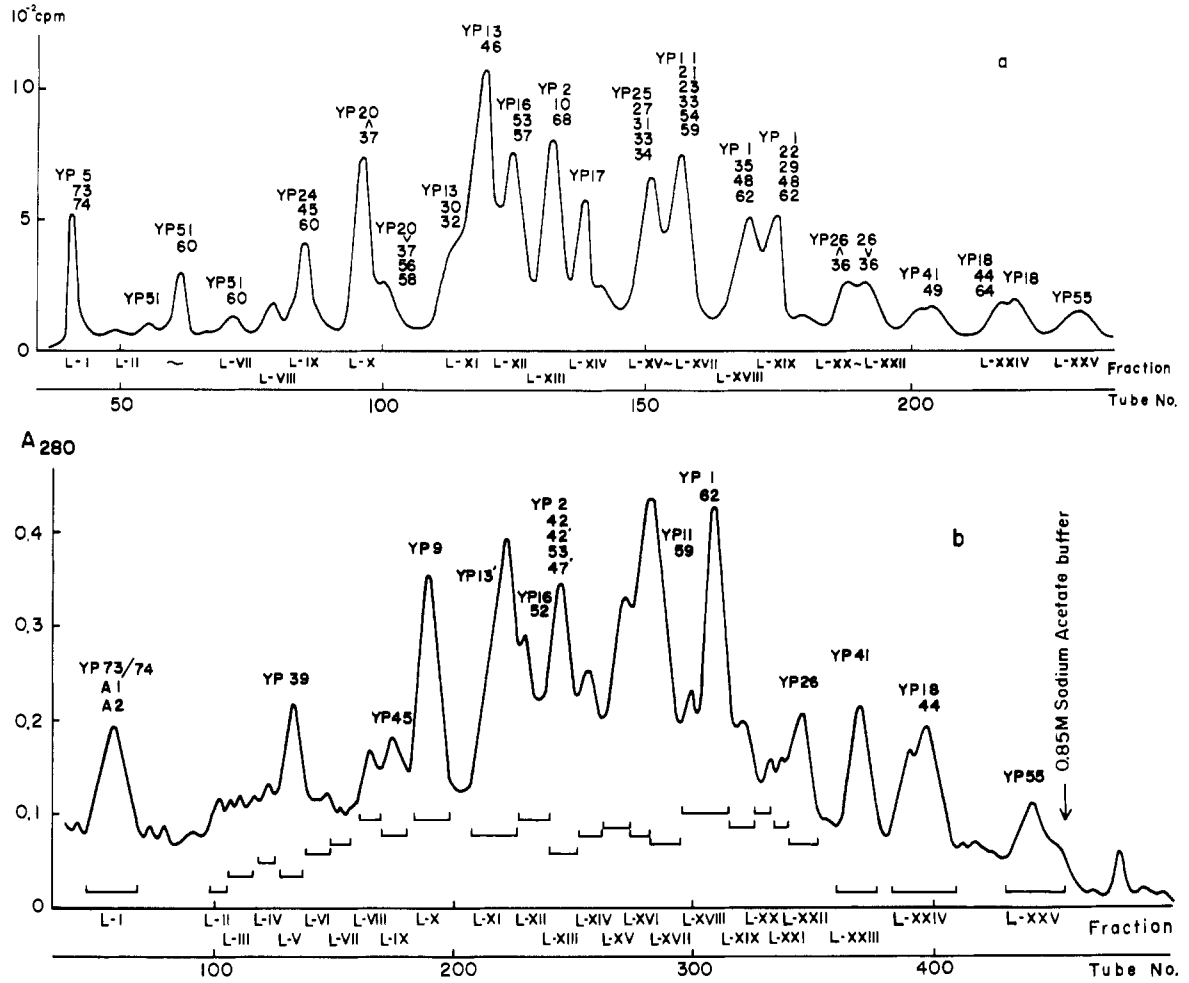

FIGURE 1: Carboxymethylcellulose column chromatography of yeast large-subunit proteins. (a) [^{3}H]Lysine-labeled large-subunit proteins with about 50 mg of carrier proteins. The inserted protein numbers indicate the protein species detected in the fractions. (b) Preparative chromatography. The inserted protein numbers indicate the protein species isolated from the fractions.

Three main peaks (indicated with brackets in Figure 2b) were observed. Each of all these three fractions produced virtually a single band on pH 4.5 disc electrophoresis. On a 2-D electrophoretogram, the first fraction yielded YP 74 as the major and YP 73 as a slightly fainter spot. The second fraction produced a major spot to the right of YP 74 and a third one situated still further to the right. Since they did not correspond to any standard 80S proteins, they were designated as YP A1 and YP A2, respectively (see Figure 12). As will be mentioned later, their amino-terminal amino acid sequences were already reported by Amons et al. (1978) (referred to as proteins A1 and A2 in that paper). YP 73/YP 74, as well as YP A1 and YP A2, was more acidic than L7/L12 from *Escherichia coli* 50S subunits as examined by pH 8.6 disc electrophoresis or by 2-D electrophoresis (Kaltschmidt & Wittmann, 1970). YP A1 and YP A2 could also be isolated in the same way from

Table II: Summary of Protein Isolation Procedure and Yield of Isolated Proteins

first (CMC) fraction	second fraction-ation	second fraction	third fraction-ation	isolated protein	yield (mg)	figure
L-I	DEAE-cellulose	D3	DEAE-cellulose	YP A1	1.8	2b
				YP A2	0.6	2b
L-V	Sephadex G-100			YP 39	5.5	3a
L-IX	Sephadex G-100			YP 45	1.0	3b
L-X	Sephacryl S-200			YP 9	1.1	3c
L-XI	P-cellulose	P1	P-cellulose	YP 13′	1.5	4b
L-XII	Sephadex G-100	S1	P-cellulose	YP 16	3.3	5b
		S3	P-cellulose	YP 52a	12.0	5c
		S4	P-cellulose	YP 52a		5d
L-XIII	Sephadex G-100	S1		YP 68	9.0	6a
				YP 2	13.7	6a
		S2	Sephacryl S-200	YP 2		6b
		S3	P-cellulose	YP 53	2.0	6c
				YP 42′	5.2	6c
				YP 42	10.3	6c
		S4	P-cellulose	YP 53		6d
				YP 42′		6d
				YP 47′	0.6	6d
L-XVII	Sephadex G-100	S1 + S2	Sephacryl S-200	YP 11	16.0	7b
		S5	P-cellulose	YP 59	24.0	
		S6	P-cellulose	YP 59		7c
L-XVIII	Sephadex G-100	S5		YP 62	27.5	8a
		S1	Sephacryl S-200	YP 1	27.4	8b
		S4	Sephadex G-75	YP 62		8c
L-XXII	Sephacryl S-200	A1		YP 26	5.0	9a
L-XXIII	P-cellulose	P3		YP 41	14.8	9b
L-XXIV	Sephadex G-100	S2		YP 44	20.5	10a
		S1	Sephacryl S-200	YP 18	27.3	10b
L-XXV	Sephadex G-100			YP 55	17.6	9c

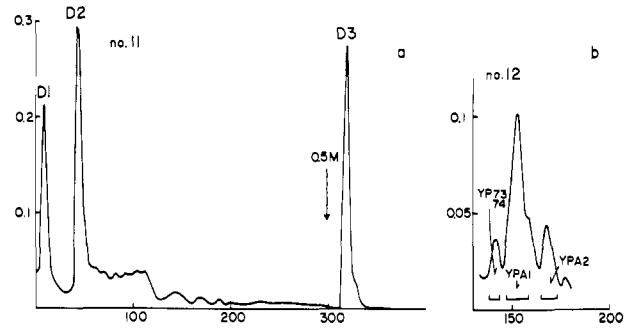

FIGURE 2: Isolation of proteins from L-I. (a) L-I; (b) D3 of (a). Throughout the figures for protein isolations, the inserted YP number indicates the protein species isolated from the fraction. Ordinate and abscissa represent protein concentration by absorption at 280 nm and fraction (tube number), respectively.

80S ribosomes with a slightly higher yield.

Fraction L-V was subjected to rechromatography on a Sephadex G-100 column (Figure 3a). The subfraction indicated with brackets in Figure 3a was homogeneous on pH 4.5 disc electrophoresis and was identified as YP 39 by 2-D electrophoresis.

YP 45 and YP 9 were isolated from L-IX and L-X, respectively. We localized these proteins in the small subunit (Otaka & Kobata, 1978). In fact, they could be purified from the S-VI and S-VIII fractions of small subunit proteins (Higo & Otaka, 1979). A rather low yield of these proteins from

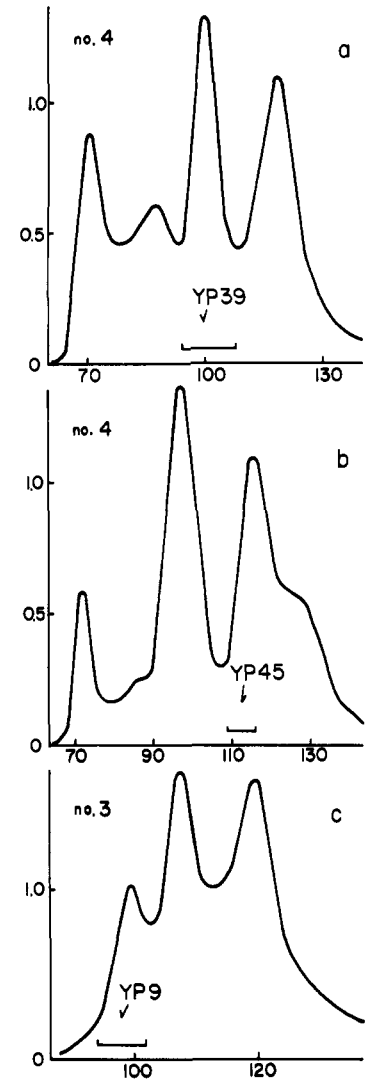

FIGURE 3: Isolation of proteins from L-V, L-IX, and L-X. (a) L-V; (b) L-IX; (c) L-X.

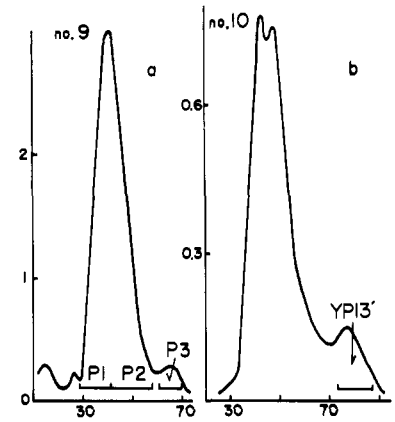

FIGURE 4: Isolation of proteins from L-XI. (a) L-XI; (b) P1 of (a).

large subunits may suggest that they are genuine components of the small subunits, and some of them became attached to the large subunit under certain conditions, such as during dissociation of 80S ribosomes.

The protein YP 13′ from L-XI (Figure 4b) appeared slightly below the YP 13 spot on the 2-D gel. Since this spot could not be detected in the standard 80S proteins (Otaka & Kobata, 1978) and the yield was very low, this may not be a genuine ribosomal protein.

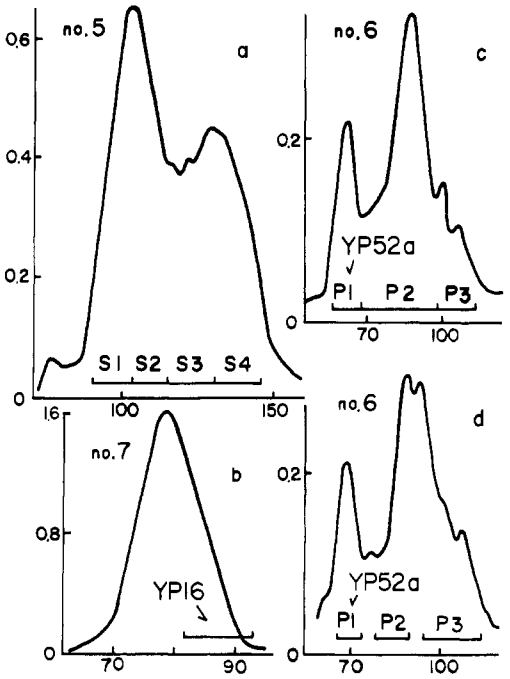

FIGURE 5: Isolation of proteins from L-XII. (a) L-XII; (b) S1 of (a); (c) S3 of (a); (d) S4 of (a).

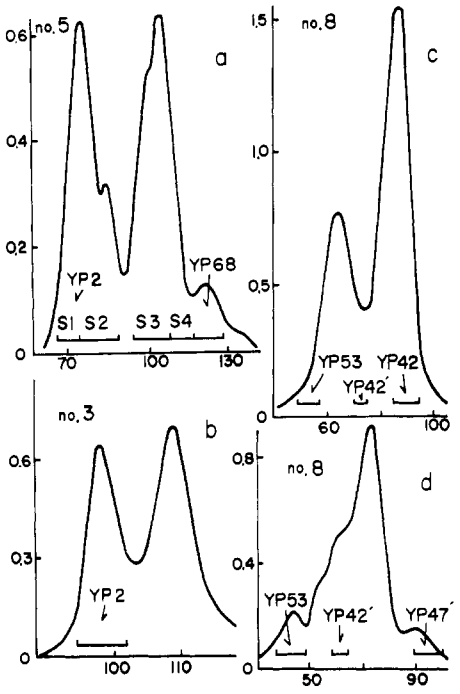

FIGURE 6: Isolation of proteins from L-XIII. (a) L-XIII; (b) S2 of (a); (c) S3 of (a); (d) S4 of (a).

The 2-D position of YP 52a (Figure 5c,d) was indistinguishable from that of YP 52 from small subunits [see Higo & Otaka (1979)] and yet their amino acid compositions were different, suggesting that they are different proteins. The YP 42′ spot was located at the upper right hand of YP 42 on the 2-D gel, and YP 47′ was between YP 47 and YP 52 (Figure 6c,d); YP 42′ and YP 47′ did not correspond to any spot of the standard 80S proteins (Figure 12). The yield of YP 47′ was very low. As shown in parts a and c of Figure 7, YP 59 could be obtained from both subfractions S6 and S5 (elution profile not shown).

The subfraction S1 derived from L-XVIII yielded three peaks (Figure 8b) by Sephacryl S-200 filtration. All of them,

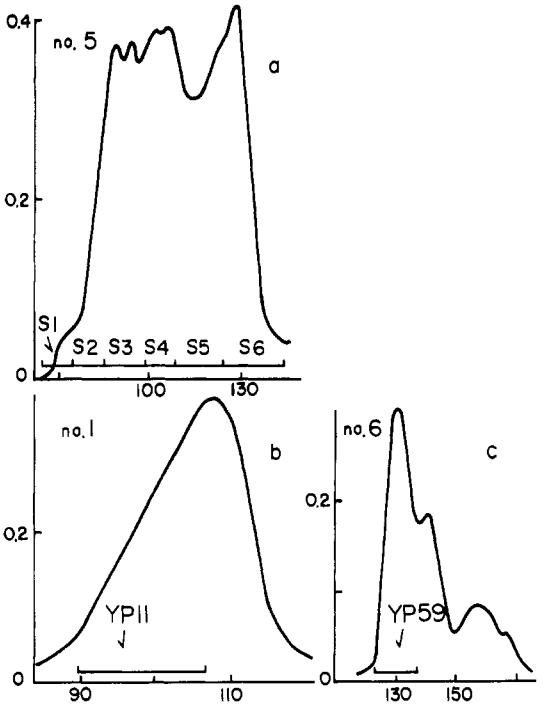

FIGURE 7: Isolation of proteins from L-XVII. (a) L-XVII; (b) S1 and S2 of (a); (c) S6 of (a).

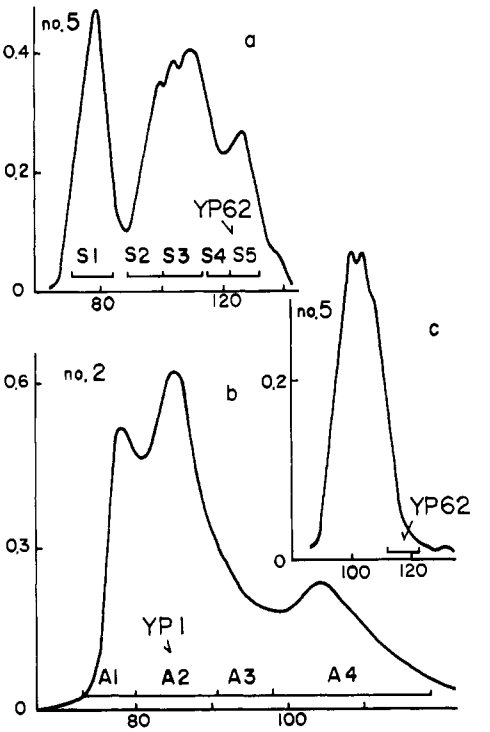

FIGURE 8: Isolation of proteins from L-XVIII. (a) L-XVIII; (b) S1 of (a); (c) S4 of (a).

A1–A4, produced a single band with the same mobility by pH 4.5 disc electrophoresis and also a single spot only at the position of YP 1 by 2-D electrophoresis. Their amino acid compositions were also quite close. These facts suggest that A1–A4 are similar proteins and their different chromatographic behaviors might be a reflection of different aggregation states of YP 1; under the electrophoretic conditions, dissociation occurred.

Filtration of L-XXIV gave YP 44 in a pure state (subfraction S2 in Figure 10a). The complete amino acid sequence of this protein has already been determined (Itoh & Witt-

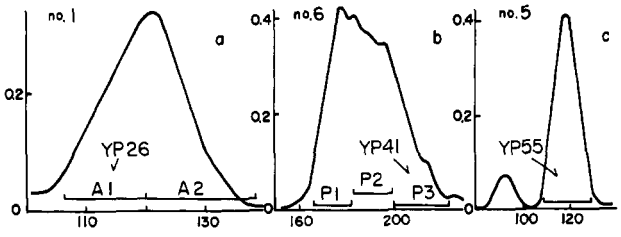

FIGURE 9: Isolation of proteins from L-XXII, L-XXIII, and L-XXV. (a) L-XXII; (b) L-XXIII; (c) L-XXV.

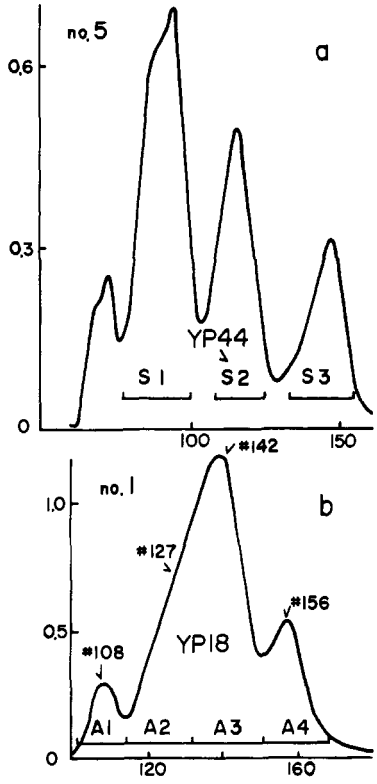

FIGURE 10: Isolation of proteins from L-XXIV. (a) L-XXIV; (b) S1 of (a).

mann-Liebold, 1978). Subfraction S1 was subjected to Sephacryl S-200 column chromatography, yielding three peaks (Figure 10b). All of them, however, gave a single band with almost the same mobility by pH 4.5 disc electrophoresis as in the case of YP 1. When aliquots of the fractions in tube numbers 108, 127, 142, and 156 (Figure 10b) were analyzed by 2-D electrophoresis, only fraction 142 produced a single spot at the position of YP 18 (Figure 11). Both fraction 127 and fraction 156 gave, in addition to YP 18 as the major spot, three additional spots in a rectangular distribution with YP 18 at the lower right corner. Fraction 108 gave primarily one spot at the upper left corner of the rectangle. The amino acid compositions of the proteins in these four fractions are the same within experimental error. The peculiar chromatographic and electrophoretic behavior may reflect various states of aggregates of YP 18; each chromatographic fraction represents different states of aggregation with varying stability against 8 M urea (in the first-dimension run) and/or against 0.1% $NaDodSO_4$ (in the second-dimension run). A1–A4, indicated with brackets in Figure 10b, were separately pooled as the YP 18 preparation.

A similar electrophoretic behavior was also observed for YP 1, YP 2, YP 13′, and YP 26; these proteins yielded a rectangular distribution with one major spot at the lower right corner and three very faint spots at the other three corners.

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

protein:	YP 1	YP 2	YP 9	YP 11	YP 13′	YP 16	YP 18	YP 26	YP 39	YP 41	YP 42	YP 42′	YP 44	YP 45	YP 47′	YP 52a	YP 53	YP 55	YP 59	YP 62	YP 68	YP A1	YP A2
M_r ($\times 10^{-3}$):	59.0	45.0	27.0	31.0	27.6	26.7	32.0	27.0	20.0	17.5	17.0	17.0	10.8[b]	14.5	13.5	16.0[b]	14.0	10.8[c]	12.5[b]	10.1[b]	8.0[b]	13.0[b]	13.0[b]
Asp	6.7	6.8	7.8	8.1	9.4	7.8	9.2	8.4	9.6	5.9	6.9	9.6	3.4	9.9	6.4	4.9	7.4	5.6	6.9	5.3	8.3	9.3	10.8
Thr	6.5	7.2	6.8	4.0	5.5	3.7	4.9	3.1	3.4	3.6	3.3	6.3	8.6	2.0	5.3	5.1	2.5	9.3	4.8	5.8	7.4	2.1	2.6
Ser	4.8	6.5	4.6	5.5	4.1	4.0	2.8	4.4	5.0	3.7	7.7	3.6	3.0	6.5	4.2	4.7	7.9	10.3	7.4	6.5	5.1	7.9	7.9
Glu	8.0	8.9	10.8	7.1	9.7	11.9	9.8	10.9	12.1	7.0	7.6	6.3	11.8	12.3	9.4	9.8	9.2	3.3	7.1	7.3	8.8	16.6	15.5
Pro	3.4	5.1	6.1	4.0	4.7	4.5	3.3	1.1	6.9	4.9	4.5	4.0	1.7	4.1	4.7	3.8	3.7	3.8	3.4	2.8	4.2	2.2	3.1
Gly	8.8	9.0	10.8	14.3	5.6	7.0	7.3	5.5	8.2	12.0	6.2	4.4	10.1	10.1	7.8	6.9	6.1	12.2	8.3	8.7	5.2	12.5	11.1
Ala	8.9	13.3	8.5	10.7	12.9	10.4	7.3	14.3	10.4	9.5	10.0	3.5	6.6	9.2	9.6	14.0	8.7	8.7	7.9	11.7	9.0	20.3	18.8
Val	9.0	9.3	8.4	7.1	7.9	6.1	7.2	7.0	7.5	6.4	6.7	9.7	6.2	7.9	7.5	11.2	9.1	2.0	10.1	7.0	6.5	5.1	4.8
Met	1.3	0.2	0.4	0.5	0.7	0.4	0.0	0.0	0.6	1.5	1.2	1.3	0.2	1.3	3.4	0.5	0.1	0.6	0.8	1.2	0.1	1.5	1.6
Ile	4.7	3.9	6.3	5.8	4.3	7.6	2.6	3.3	8.7	5.6	8.4	4.9	0.9	6.5	4.5	5.6	4.6	0.5	5.8	6.7	5.6	3.8	4.5
Leu	6.0	8.4	8.6	6.6	8.9	8.6	6.9	8.8	7.1	6.4	9.9	8.1	7.3	6.4	7.9	8.6	11.3	3.0	6.1	5.9	9.8	8.9	9.5
Tyr	3.2	0.7	1.3	2.2	3.4	4.1	5.8	1.9	1.5	3.3	4.8	5.6	3.6	3.3	3.0	1.5	2.5	3.7	5.6	3.0	2.0	1.4	1.6
Phe	4.2	2.4	3.0	2.2	2.6	4.1	2.4	0.6	2.6	3.5	1.6	1.5	5.0	2.4	4.0	3.0	1.8	5.1	3.9	2.4	3.9	2.5	2.3
His	4.2	2.7	1.5	3.7	1.4	1.7	2.7	3.7	1.3	6.2	2.5	0.9	4.7	1.5	2.9	0.4	1.6	7.2	2.8	1.3	0.7	0.2	0.3
Lys	11.6	9.0	8.4	8.0	13.9	12.1	11.6	12.1	10.8	12.7	9.1	5.3	19.4	8.2	9.1	12.7	13.9	13.4	9.4	13.5	15.6	6.2	6.6
Arg	9.0	6.9	6.9	10.4	5.0	6.2	16.5	14.9	4.4	7.9	9.7	5.2	7.5	8.5	10.3	7.5	9.6	11.6	9.6	10.9	7.8	0.7	0.0

[a] The values are in mole percent. [b] See Materials and Methods.

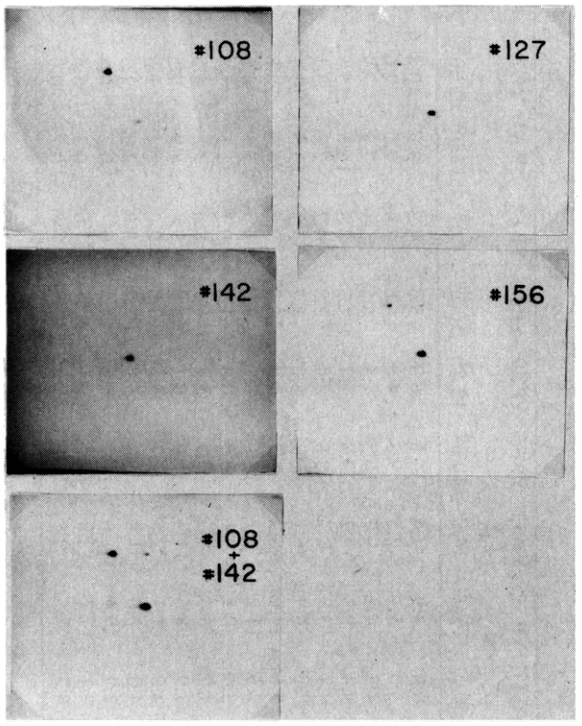

FIGURE 11: Two-dimensional electrophoresis of YP 18.

In all cases, the faint spots did not correspond to any standard ribosomal protein spots.

All the isolated proteins in a pure form in these studies are indicated with YP numbers in Figure 1b and with filled circles on the schematic 2-D pattern in Figure 12.

Amino Acid Composition and Molecular Weight. Amino acid compositions suggest that each of the 23 proteins purified here is a unique species (Table III). Protein YP 55 had a particularly low content of hydrophobic amino acids and was relatively rich in hydrophilic amino acids; the total of valine, isoleucine, and leucine was only 5.39%, while in all other proteins even valine content alone exceeded this value.

Two acidic proteins, YP A1 and YP A2, are similar in amino acid composition (with a possible difference in proline and arginine content). The amino-terminal amino acid sequences (Amons et al., 1978) as well as tryptic peptide maps of these two proteins (unpublished data) are also indistinguishable from each other. Moreover, their molecular weights and tryptic peptide maps (over 90%) are similar to those of YP 73/YP 74 (unpublished data), suggesting that YP A1, YP A2, YP 73, and YP 74 have similar, if not identical, primary amino acid sequences with slightly different electrophoretic charges possibly due to their different degrees of phosphorylation, etc.

Molecular weights of those proteins that were estimated by $NaDodSO_4$–polyacrylamide gel electrophoresis (Table III) are in general agreement with those deduced from 2-D electrophoresis (Otaka & Kobata, 1978). However, molecular weights of YP 44, YP 55, YP 62, and YP 68 deviated considerably from those reported earlier (Otaka & Kobata, 1978). The molecular weight estimations obtained from 2-D slab electrophoresis might be subject to rather large errors for the low molecular weight protein species.

Several workers reported the purification of rat liver large-subunit proteins [for a review, see Bielka & Stahl (1978)]. In addition, acidic ribosomal proteins from large subunits were purified from brine shrimp (*Artemia salina*) (van Agthoven et al., 1978). Wool and co-workers reported the purification of altogether 80 proteins from rat liver large and small subunits and presented their amino acid composition and molecular weight (Collatz et al., 1976, 1977; Tsurugi et al., 1976, 1977, 1978).

We isolated several proteins which were undetectable in the standard 2-D pattern of 80S ribosomal proteins. They were YP 13′, YP 42′, and YP 47′ in this paper and YP 14′ and YP 14″ in a previous paper (Higo & Otaka, 1979). A similar observation was made by Wool and co-workers. They were L35′ (Tsurugi et al., 1976), L7′, L23′, L27′, L36′, and L37′ (Tsurugi et al., 1977), Sa, Sb, S3a, S3b, S5′, S15′, and S27′

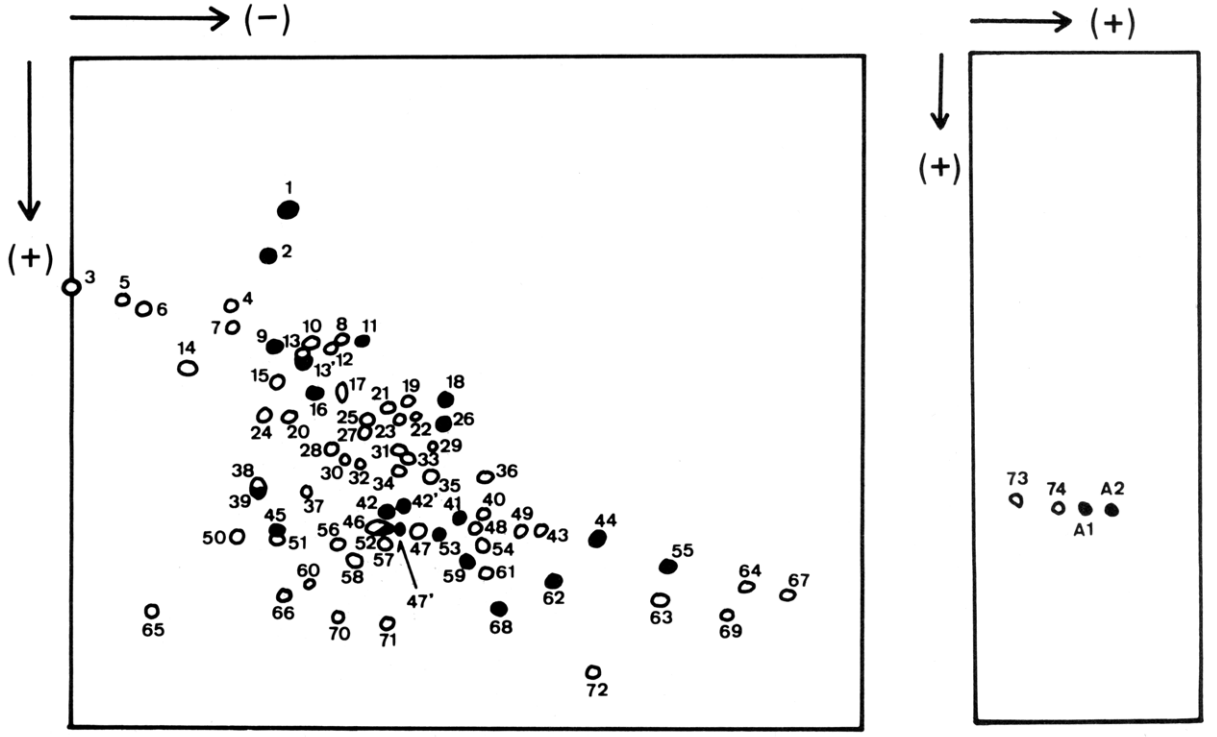

FIGURE 12: Schematic diagram of two-dimensional electrophoretogram of 80S ribosomal proteins. Isolated proteins are indicated by filled circles.

(Collatz et al., 1977), and L13′ and L18′ (Tsurugi et al., 1978). Some, if not all, of these proteins may have been produced from genuine ribosomal proteins as similar artifacts by aggregation or degradation.

Itoh and Wittmann-Liebold (unpublished data) recently determined the sequence of YP 55 almost completely and compared it with amino-terminal sequences of rat liver proteins. The highest homology (53% in the first 30 amino acid residues of the amino-terminal region) was obtained between YP 55 and rat liver L37.

Amons et al. (1977) reported the amino-terminal sequence of YP A1 and YP A2, which revealed a considerable sequence homology with an acidic ribosomal protein not only from *A. salina* but also from *Halobacterium cutirubrum*. This suggests the general occurrence of these proteins in eucaryotes and at least in one bacteria. Using our data, Tsurugi et al. (1978) compared the amino acid compositions of YP A1 and YP A2 with acidic protein(s) from other organisms, but as yet there is no conclusive evidence indicating homology between eucaryotic acidic proteins and *E. coli* L7/L12.

In the course of this study we have purified 23 proteins, which represent nearly half of some 40 protein species generally reported for the eucaryotic large subunit. Purification of the remaining proteins is now under way.

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The *Escherichia coli* Phosphoenolpyruvate-Dependent Phosphotransferase System: Observation of Heterogeneity in the Amino Acid Composition of HPr†

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ABSTRACT: Resonances of the aromatic protons of tyrosine have been observed in the proton nuclear magnetic resonance (^{1}H NMR) spectrum of purified HPr from *Escherichia coli*. Analysis of the NMR spectrum of native HPr suggests that the tyrosine is located in a single position in the secondary structure and that this position is on the interior of the molecule inaccessible to solvent. Previous reports suggested that *E. coli* HPr contained no tyrosine [Anderson, B., Weigel, N., Kundig, W., & Roseman, S. (1971) *J. Biol. Chem. 246*, 7023–7033]. In contrast, we find, by amino acid analysis and ultraviolet and NMR spectroscopy, that *E. coli* HPr does contain tyrosine but at a subintegral level of 0.5 ± 0.1 mol of tyrosine per mol of HPr.

HPr is a phosphoryl group carrying protein which functions as an intermediate in the transfer of a phosphoryl group during the energized transport of sugars according to eq 1.[1]

$$\begin{aligned} \text{PEP} + \text{E}_\text{I} &\xrightarrow{\text{Mg}^{2+}} \text{P–E}_\text{I} + \text{pyruvate} \\ \text{P–E}_\text{I} + \text{HPr} &\leftrightarrow \text{P–HPr} + \text{E}_\text{I} \\ \text{P–HPr} + \text{hexose}_{(\text{out})} &\xrightarrow[\text{Mg}^{2+}]{\text{E}_\text{II}} \text{HPr} + \text{hexose–P}_{(\text{in})} \end{aligned} \quad (1)$$

The phosphoryl group transfer and sugar transport processes are catalyzed by the enzymes E_I and E_{II}, respectively (Kundig & Roseman, 1971). *Escherichia coli* HPr was first isolated and characterized by Roseman and co-workers (Roseman,

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[1] Abbreviation used: PEP, phosphoenolpyruvate.

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