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ACIDIC PROTEINS FROM Saccharomyces cerevisiae RIBOSOMES

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SUMMARY

Two dimensional gel electrophoresis of ribosomal proteins from Saccharomyces cerevisiae reveals the presence of three spots in the region corresponding to proteins of high acidic character. Washing the ribosomes with 0.4 M NH_4Cl and 50% ethanol, followed by chromatography of the extracted proteins on DEAE-cellulose, indicated the presence of two fractions of acidic proteins; (A and Ax), having very similar molecular weights (12.000-13.000), but phosphorylated to different extents. Fractions A and Ax are immunologically distinct and their immunologic properties differ from acidic proteins found in Escherichia coli, rat liver, and Artemia salina ribosomes.

Protein A can be resolved into two bands by electrofocusing, and two dimensional gel electrophoresis. The two components correspond to proteins L44 and L45 according to the standard nomenclature. Proteins Ax seems to correspond to the spot that moves above and to the left of L44 and L45 and is at least three times more phosphorylated than these two proteins.

INTRODUCTION

The presence of several proteins with strong acidic properties seems to be common to all types of ribosomes. Thus, ribosomal proteins having an isolectric point below pH 5.0 and molecular weights between 12.000 and 15.000 have been reported in bacterial and eukaryotic ribosomes (1-6). When these acidic proteins are present in the same ribosome in more than one copy, they differ only in simple chemical modifications such as acetylation (7) or phosphorylation (8,9) of one or two unmodified polypeptides. Although data are scarce some structural similarity seem to exist among the proteins from the few eukaryotic ribosomes studied (10), while the homology with the acidic proteins from Escherichia coli is rather poor (10). Perhaps the most striking characteristics of these

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proteins is the existance of alanine-rich regions in their molecules that might resemble some contractil proteins such as myosin (11).

The functional role of these proteins has been studied in great detail in E. coli ribosomes (12) and they seem to be required for ribosome activity in protein synthesis, although there are some contradictory reports (13,14). Specifically, they are implicated in the interaction of the ribosome with several supernatant factors. Reports suggest a similar role for the acidic proteins in eukaryotic ribosomes (3). Our results, however, indicate that Saccharomyces cerevisiae have a less stringent requirements for acidic proteins than ribosomes of bacterial cells (15).

In order to clarify the functional role of the acidic proteins, their identification and purification is required. We have analyzed the acidic proteins released by washing S. cerevisiae ribosomes with ammonium-ethanol. Results showing the presence of three main phosphorylated proteins are presented in this report.

MATERIALS AND METHODS

Cells and ribosome

S. cerevisiae Y166 was grown to late exponential phase in YEPD medium (15) or in low phosphate medium (16) when ^{32}P labeled cells were required. The cells were collected, broken and the ribosomes prepared as previously described (15).

Acidic proteins

The acidic proteins were extracted by washing the ribosomes with 0.4 M NH_4Cl in the presence of 50% ethanol at 0°C (according to Hamel et al. (17)) with the modifications reported (15). The proteins extracted ($SP_{0.4}$) were applied to DEAE-cellulose column and eluted essentially in the same conditions described for Artemia salina proteins (3) but using a steeper gradient (10 mM to 1.5 M) of sodium acetate. Proteins were labeled with ^{125}Iodine in the presence of chloramine T, when required (18).

Electrophoresis

Two dimensional gel electrophoresis was carried out according to the standard system of Kaltschmidt and Wittmann (19) with some modifications (20). When SDS was present in the second dimension, the conditions of Martini and Gould (21) were used. Electrophocusing was done using LKB ampholines between pH 3 and pH 5 in tubes of 10 cm x 0.5 cm following published conditions (9).

When required, spots were eluted from the gel plates with 67% acetic acid (22). The absorption at 605 nm and the radioactivity of the sample were estimated.

Immunological techniques

Antisera were rised in rabbit by intramuscular injection of the proteins in complete adjuvant followed by two subcutaneous booster injections in incomplete adjuvant every 15 days. Sera were processed as described (15). Immunodifusion tests were carried out on 1% agar plates, in 10 mM Tris-HCl, 150 mM NaCl pH 7.4.

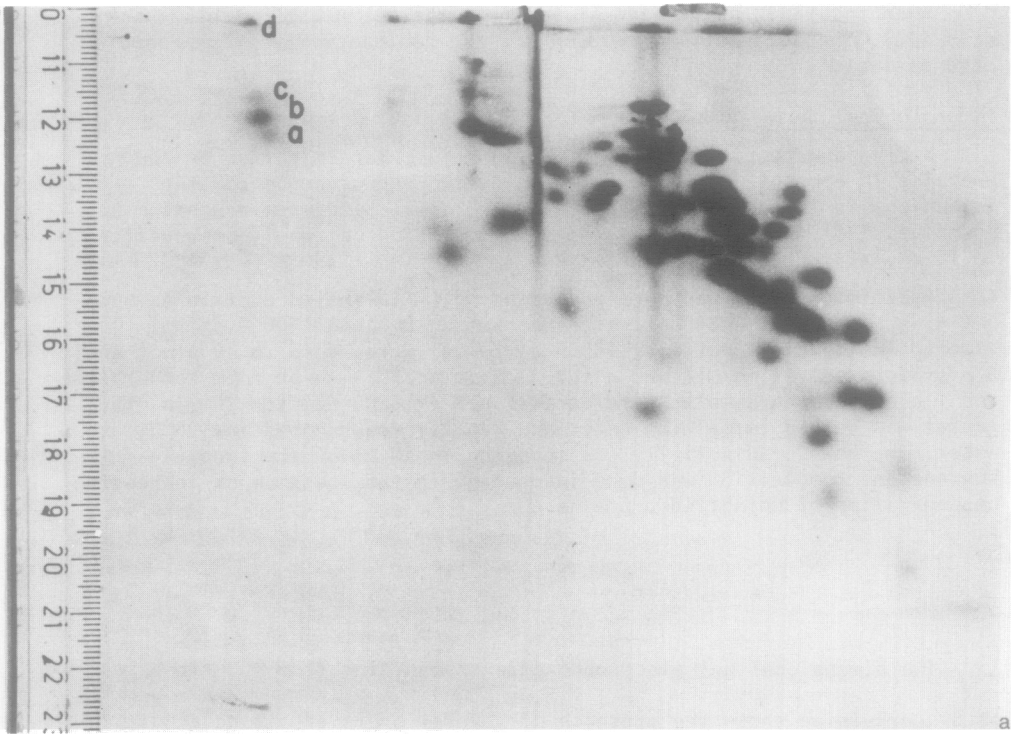
Radioimmunoassay was carried out using ^{125}I-labeled protein Ax and anti-$SP_{0.4}$ bound to CNBr activated Sepharose 4B. About 50000 cpm of labeled Ax (specific activity 18500 cpm/pmol) were added to 40 µl of anti-$SP_{0.4}$-Sepharose (1 mg/ml) and after incubation for 1 h at room temperature and 1 h at 0°C the samples were centrifuged at 5000 rpm for 10 min. The pellet was washed twice with water and finally resuspended in 100 µl of water. The bound radiactivity was measured in 50 µl of the final suspension. Competition was done incubating in the presence of increasing concentration of unlabeled proteins.

RESULTS

Two dimensional gel electrophoresis of proteins from 80S ribosomes of S. cerevisiae shows the presence of several spots of strong acidic character moving in the gels to a position similar to proteins L7 and L12 of E. coli ribosomes. Krujswijk and Planta (23) and Zinker and Warner (24) have reported the presence of two spots in that region that they named L44/45 and L35/36 in their respective nomenclature. However, we frecuently detect the presence of three spots in the region of L44/45 and a fourth that does not enter in the second dimension gel (Fig. 1A, spots a, b, c, and d). When the second dimension is carried out in the presence of SDS, the acidic proteins are resolved into two spots with apparently similar molecular weights (Fig. 1B).

In ribosomes obtained in the presence of $^{32}PO_4^{3-}$, spots a, b, c, and d are radioactive and the specific activity calculated from the ratio of radioactivity to the absorption at 605 nm in the eluted spots, is at least three times higher in spot c (Table 1).

The acidic proteins can be selectively removed from the ribosomes by washing them with 0.4 M NH_4Cl and 50% ethanol (15). When proteins thus


d
c
b
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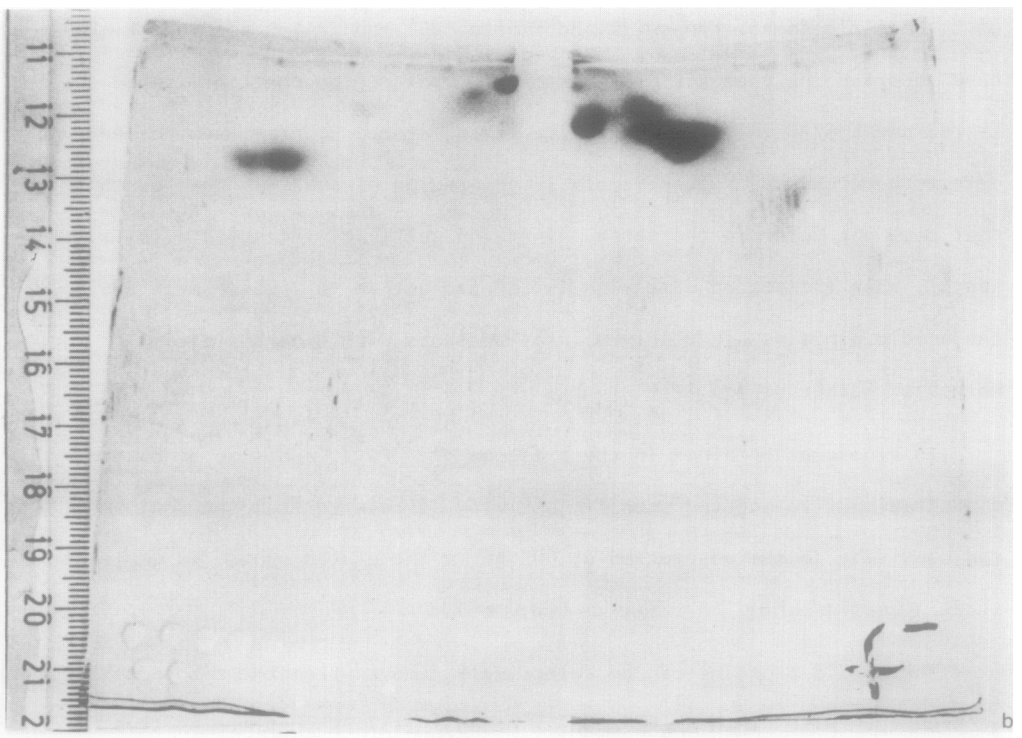

b

Table 1. Distribution of radioactivity among acidic proteins in two dimensional gel electrophoresis

Spot	^{32}P labeled proteins			Mixture of ^{125}I-labeled Ax and unlabeled A
	CPM	A_{605}	CPM/A_{605}	cpm
a	496	0.024	20.6×10^3	291
b	663	0.028	23.6×10^3	866
c	1092	0.018	60.6×10^3	2666
d	581	0.024	24.2×10^3	939

extracted from ribosomes labeled in vivo with ^{32}P were chromatographed in DEAE-cellulose as described in Materials and Methods, two peaks of phosphorylated proteins, fractions A and Ax, were obtained (Fig. 2). The ratio ^{32}P/protein in the fractions clearly indicated that Ax is more heavily labeled than A.

By polyacrylamide gel electrophoresis of a mixture of A and Ax in the presence of SDS, a single spot was detected in a position close to a cytochrome C marker (not shown). This suggests that both are made of one or several components of identical or very similar molecular weight (about 13.000 daltons). However two dimensional gel electrophoresis indicated that fraction Ax is made of only one polypeptide corresponding to spot c, whereas fraction A is made of two spots moving to the position of a and b and is slightly contaminated (21%) with spot c. Both fractions, A and Ax, seem to contribute to the formation of spot d, thus part of these proteins remain in the origin of the second dimension in variable amounts in different electrophoresis.

Figure 1. Two dimensional gel electrophoresis of 80S ribosomal proteins from S. cerevisiae. A) Second dimension in standard conditions. B) Second dimension in the presence of sodium dodecylfulphate.

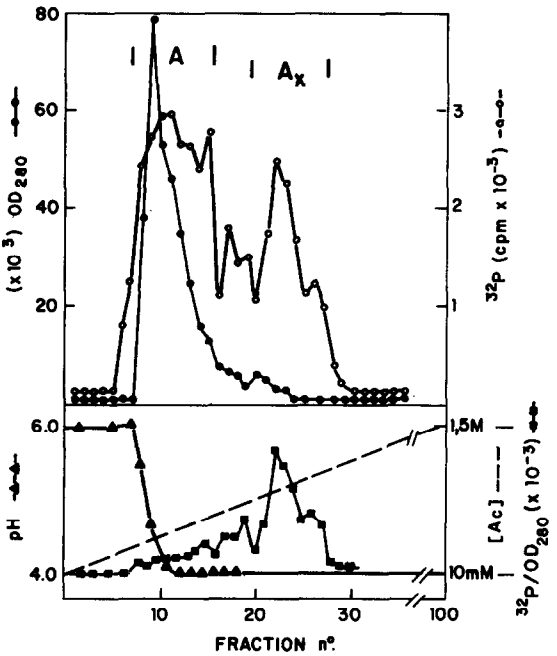


Figure 2. Chromatography on DEAE-cellulose of $SP_{0.4}$ fraction from 80S ribosomes. Proteins from the ammonium-ethanol treatment ($SP_{0.4}$) were applied to a column of 2.5 cm x 20 cm and eluted as indicated in Methods. Radioactivity and protein concentrations were tested in the fractions.

These results were confirmed by resolving a mixture of ^{125}I-labeled Ax and unlabeled proteins from 80S ribosomes by two dimensional electrophoresis. As indicated in Table 1, radioactivity is associated mainly to spot c.

The purity of fraction Ax was confirmed by electrophocusing the sample in polyacrylamide gels between pH 5 and pH 3 (Fig. 3). Ax has an isolectric point close to pH 4.0 and corresponds to the most acidic of the three main bands detected when the total acidic proteins ($SP_{0.4}$) are electrophocused in the same conditions. In the $SP_{0.4}$ samples faint, bands whose intensities vary with the preparation of the ribosomes, are occasionally detected just below the larger ones, probably corresponding to their dephosphorylated products.

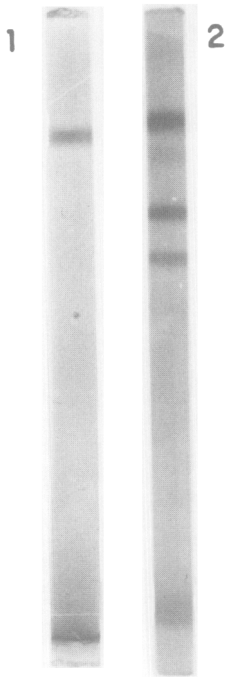


Figure 3. Electrophocusing in polyacrylamide gels. 1, Fraction Ax. 2, Fraction $SP_{0.4}$.

Using antisera rised against the total acidic proteins ($SP_{0.4}$) it was possible to show that fractions A and Ax give different precipitation bands when tested by immunoprecipitation (Fig. 4). The precipitation of band Ax is clearly distinguishable from the precipitation band of A and the latter in fact appears as a double band.

The same conclusion can be drawn from a radioimmunoassay using anti-$SP_{0.4}$ and ^{125}I-labeled Ax. As shown in Fig. 5 fraction Ax does not compete with fraction A.

We have previously shown that neither of the two fractions A and Ax cross reacts with antisera rised against proteins L7 and L12 from E. coli (15).

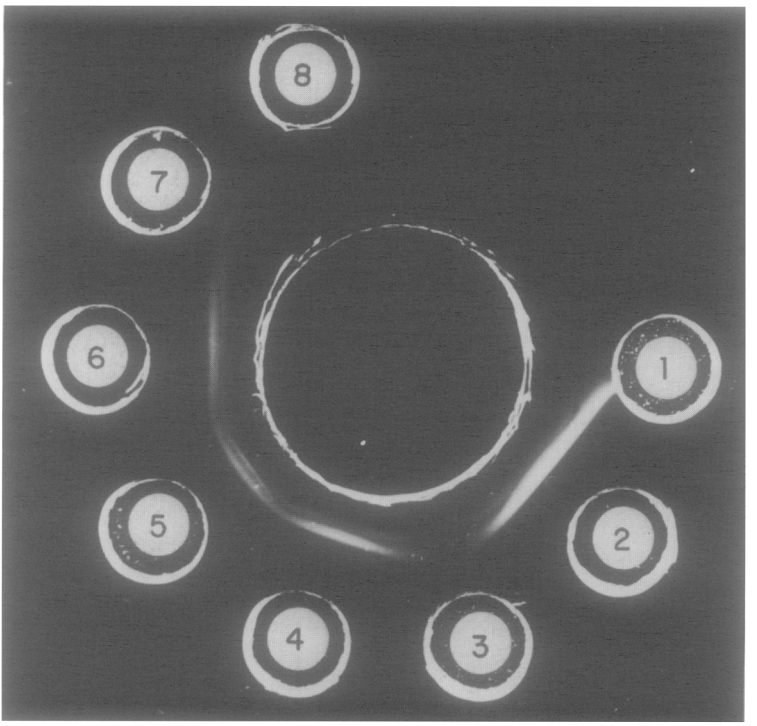
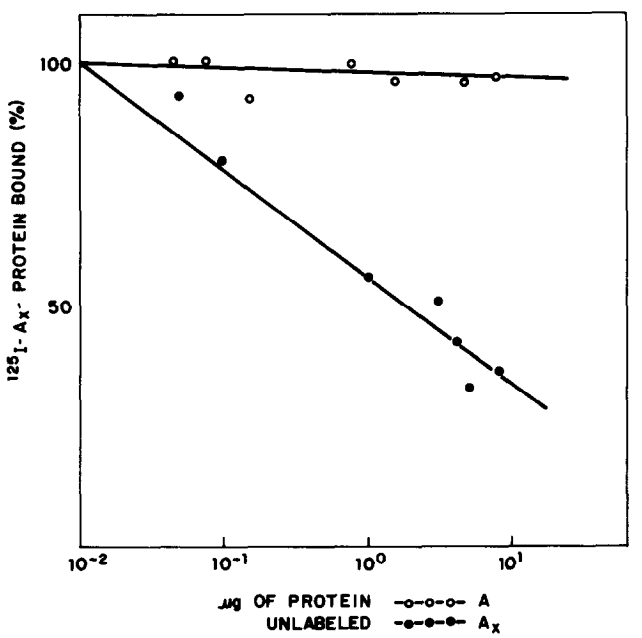


Figure 4. Double immunodifusion test. Central well, anti-$SP_{0.4}$. Wells 2, 4 and 6, fraction $SP_{0.4}$. Well 3, fraction Ax. Well 5 fraction A.



DISCUSSION

Our results show the presence in the 80S ribosome of S. cerevisiae of three main proteins of strong acidic properties. Two of them, spots b and a, must correspond respectively to proteins named previously as L44 and L45 and henceforth we will refer to them using this nomenclature. They form part of the 60S subunit and seem to be partially released upon dissociation of the ribosomes (25). In agreement with other reports (24), we find that both proteins are phosphorylated and from the ratio of ^{32}P incorporated to the intensity of the spots, the extension of phosphorylation must be similar in the two molecules. Hence it seems unlikely that they represent different degrees of modification of the same protein. However, data from Itoh and Osawa (reported in ref. 8) on the amino acid composition of two yeast proteins, A1 and A2, probably equivalent to L44/45, suggest that both proteins are chemical variants of a single polypeptide. It is therefore possible that, if phosphorylation is the modifying process, the site and not the extent of the modification is the differentiating factor.

This situation is different in A. salina, where two proteins, eL7 and eL12, with characteristics similar to L44 and L45 have been shown to be the phosphorylated and unphosphorylated forms of a single polypeptide (9).

The third protein, spot c or fraction Ax, has not been reported previously in yeast, although it might correspond to spot P_5^{II} detected by Zinker and Warner only by autoradiography (24).

Protein Ax has a molecular weight very close or identical to proteins L44/45 and therefore its high specific activity indicated that it is phosphorylated at least three times more than the other acidic proteins. However, Ax is clearly distinguishable from L44/45 in its antigenic

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Figure 5. Radioimmunoassay. ^{125}I-labeled fraction Ax and anti-$SP_{0.4}$, bound to Sepharose 4B, were allowed to react in the presence of increasing concentrations of unlabeled fraction A (○) or fraction Ax (●). Results obtained with fraction A were corrected for the 21% contamination of Ax.

determinants, and appears to be a different protein. In A. salina a protein with characteristics similar to Ax has been suggested to be related to the proteins equivalent to L44/45 (9).

The relative amounts of the three proteins L44, L45 and Ax change in different ribosomal preparations. Our preliminary results indicate that Ax and L44 predominate over L45 in ribosomes from cells in stationary growth phase. These results would be in agreement with the changing ratio of proteins L7 and L12 in E. coli (26).

The data available from four eukaryotic species studied in some detail indicate that two types of acidic proteins, having chemical or immunigenic differences exist in the cell, namely proteins P1 and P2 in rat liver (8), L40 and L41 in HeLa cells (4), L7/12 and "top band" in A. salina (9), and L44/45 and Ax in yeast (this report). All of them are susceptible to phosphorylation originating one or several derivatives, up to nine in rat liver (5). It is not yet known whether all these modified proteins are present in all the ribosomes or whether several types of ribosomes exist having different acidic proteins. In some ways eukaryotic cells seem to have duplicated to one polypeptide-two proteins system found in bacterial cells.

Nothing is known about the significance of such a diversity of ribosomal acidic proteins. In our experience eukaryotic ribosomes are less dependent on these acidic proteins than bacterial ribosomes for functioning (15). If this is so, these proteins might have a modulator effect on the ribosome function that is compatible with the existance of a larger number of acidic protein species in a more sophisticated cell machinery.

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