RED PIGMENT OF ADENINE-DEFICIENT YEAST SACCHAROMYCES CEREVISIAE

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A number of adenine- and biotine-deficient mutants of yeast Saccharomyces cerevisiae are known to accumulate intracellularly red pigment when grown on medium containing suboptimal concentration of these components (Lindegren and Lindegren,I947; Ephrussi and Lederer,I948; Saito and Takahashi,I957; Chamberlain et al, I952; Srb,I958; Woodward and Rainbow,I96I; Itoch and Nosoh,I965; Smirnov et al,I966). The present report describes the isolation of red pigment and some study of its structure. The results suggest that red pigment is the mixture of poly(ribosylamino-imidazole) molecules varying in molecular weight and containing number of amino acids.

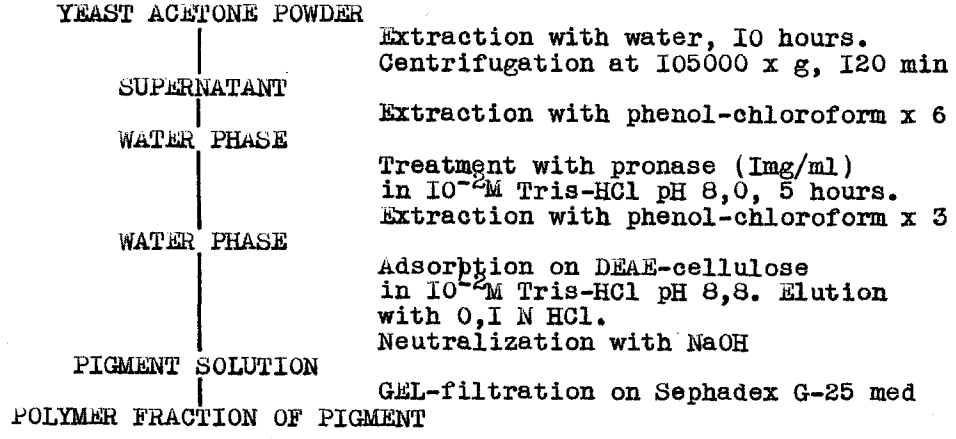

Fig.I. Scheme for isolation of red pigment

Adenine-deficient mutant 6-Π3 from Peterhoff genetic lines of Saccharomyces cerevisiae (Inge-Vechtomov,1963) was used in the experiments. Fig.I shows the procedure developed for isolation of red pigment.

In Fig.2 absorbtion spectra of pigment and its derivative obtained by diazotization according to Bratton and Marshall (1939) are shown. Native pigment has two absorption maxima at 490 and 540 mμ, its diazotized derivative exhibits maximum absorbtion at 515 mμ.

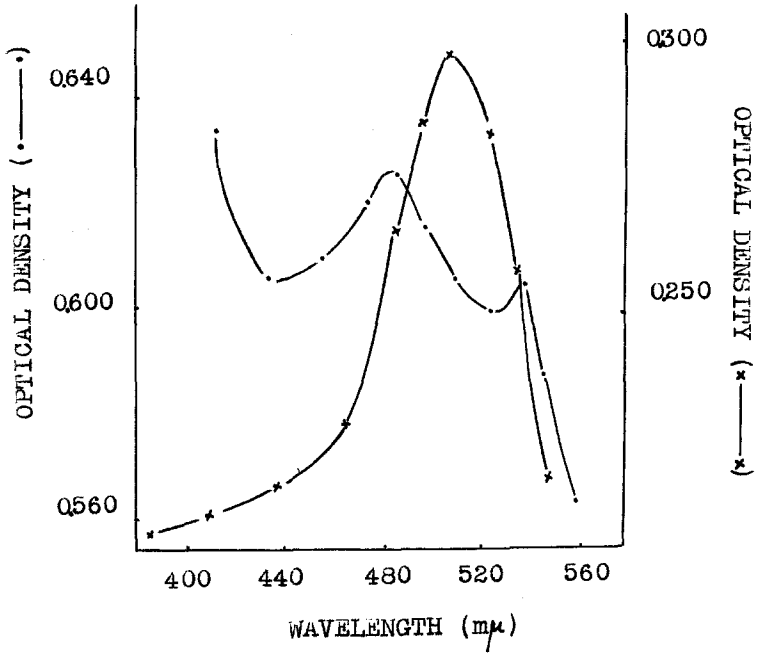

Fig.2. Absorption spectra of native (•——•) and diazotized (×——×) pigment.

The fractionation of pigment preparation on Sephadex G-25 is presented in Fig.3. It is evident that pigment is a polymer heterogeneous in molecular weight. Part of pigment is eluted immediately after V_o, other part undergoes fractionation. The

upper limit of molecular weight is less than 10000 as shown by fractionation of pigment on Sephadex G-50.

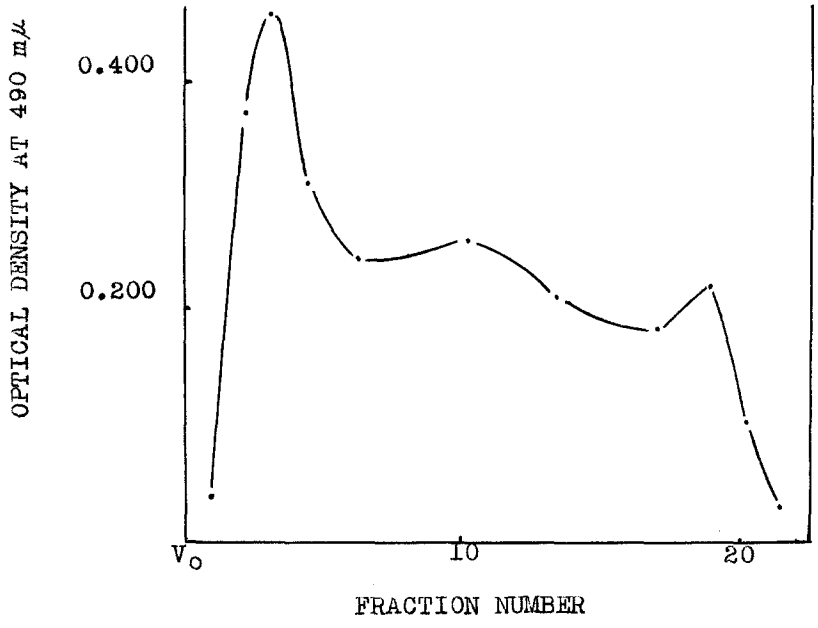

Fig.3. Fractionation of non-purified pigment on Sephadex G-25 med. Column size 2 x 40 cm, V_o 35 ml. Sample volume I ml, elution with H_2O, fraction volume 2 ml.

Fractionation of pigment on Sephadex G-25 med have also demonstrated that pigment is heterogeneous in spectral properties : the increase of molecular weight of pigment is accompanied by red shift of absorbance.

Preparations of pigment were found to contain amino acids. The set of amino acids characteristic for pigment fraction adsorbed on DEAE-cellulose at pH 8,8 is presented in Table I.

Table I

The amino acid content of red pigment

Amino acid	$\mu M \times 10^{-1}$/10 μM ribose	Amino acid	$\mu M \times 10^{-1}$/10 μM ribose
Lys	3,26	Gly	48,70
His	2,04	Ala	7,00
Arg	traces	Cys	15,30
Asp	34,60	Val	6,50
Glu	75,50	Met	traces
Thr	5,40	Ileu	3,16
Ser	6,50	Leu	2,65
Pro	8,67	Tyr	2,04
		Phe	1,22

Quantitative determination of amino acids in pigment was made by standard procedure on Hitachi automatic amino acid analyser after 24-hours hydrolysis of pigment samples with 6 N HCl at 110°C.

Pigment contains large amount of dicarboxylic acids which might determine its acidic properties. It is noteworthy that prolonged incubation of non-purified pigment with pronase does not lead to any significant drop in molecular weight.

Data on chemical composition of purified pigment are given in Table 2.

Table 2

Analysis of red pigment

	μM / 1 ml of pigment solution	Molar ratio (ribose=1)
Ribose[1]	9,8	1
Total nitrogen[2]	55,1	-
Amino acid nitrogen[3]	22,6	-
Total N - amino acid N	32,5	3,3
Acid labile nitrogen[4]	19,7	2,0
Total phosphate[5]	traces	

[1] Determination by orcinol method (Antoni et al, 1962).
[2] Determination by micromodification of Kjeldal procedure (Ma and Zuazaga, 1942).
[3] Calculated from figures of amino acid content (Table I).
[4] Determination with Nessler reagent after 90 min hydrolysis of sample with 2 N H_2SO_4 at 150°C.
[5] Determination according to Kondrashova et al (1965).

Discussion. Several facts suggest that part of red pigment is ribosylaminoimidazole.

I. Strain 6-Л3 Saccharomyces cerevisiae is adenine-deficient auxotroph analogous to AdI mutant from Dr.R.Mortimer collection unable to carry out the carboxylation of 5-amino-4-imidazole ribotide (Smirnov et al,I966).

2. The ratio ribose:acid labile nitrogen:total nitrogen (minus amino acid nitrogen) equal to I:2:3 is characteristic for ribosyl-5-amino-4-imidazole, the intermediate in purine biosynthesis (Levenberg and Buchanan,I957).

3. Absorbtion spectrum of diazotized pigment (Fig.2) resembles those of aminoimidazole derivatives (Levenberg and Buchanan, I957).

Discussing possible structure of pigment molecule one should emphasize the following:

I. The fact that pigment formation occures only in the presence of oxygen and is blocked by antioxidants e.g. mercaptoethanol (Smirnov et al, in press) suggests that most probable mechanism of pigment formation is oxidative polycondensation of ribosylaminoimidazole through imidazole ring leading to linear polymer with characteristic system of conjugated bonds. This type of mechanism is supported by spectral properties of pigment: the position of absorption maxima in red region of spectrum and red shift of absorption with the increase of molecular weight.

2. Chemical analysis of pigment demonstrates that the formation of polymer is not accompanied by deamination of aromatic NH_2-groups. At the same time relatively low ability of pigment to diazotization (comparing to corresponding amount of diazotizable monomer units) indicates that most of aromatic NH_2-groups are substituted.

3. Inability of pronase to cause significant changes in molecu-

lar weight of pigment suggests that amino acids are not the part of polymer backbone but rather bound to poly(ribosylaminoimidazole) chain by amide bonds with NH_2-groups of imidazole rings.

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