Protein-disulphide Reductase Activity in Yeast

A STRAIN of *Saccharomyces cerevisiae* which in batch culture demonstrated little tendency to elongate was shown to do so in continuous culture when the supply of nitrogen was limited[1]. Because fewer cells elongated when sulphydryl compounds or sodium selenate were added to the medium, the sulphydryl–disulphide balance in the cells seemed to be implicated. Later investigations with the same yeast[2] revealed that the change in cell shape was not closely paralleled by changes in total and individual carbohydrates, proteins, nucleic acids and lipids of the cells. Attention was therefore directed to enzymes thought to be important in cell extension. Nickerson *et al.* placed special emphasis on a protein-disulphide reductase system controlling the formation of disulphide bridges in mannan-protein of the wall. They demonstrated in bakers' yeast[3] and *Candida albicans*[4] that after oxidation with potassium ferricyanide of isolated cell wall protein, the disulphide linkages were reduced by isolated mitochondria. The protein-disulphide-reductase activity was, however, very low in a mutant strain of *C. albicans* with elongated cells[4]. It was of interest, therefore, to compare activities of mitochondria isolated from elongated and ovoid cells of our strain of bakers' yeast.

In preliminary experiments, both the oxidized mannan-protein representing the cell wall substrate and the mitochondria were prepared by methods resembling those of Nickerson and Falcone[3,4]. Batch-grown ovoid cells were the source of the mannan-protein. Mitochondria were isolated from both ovoid and elongated cells of the same strain which had been continuously cultured at 25° C in glucose-limiting and nitrogen-limiting media respectively[2]. In all cases the dilution rate was 0·1 h^{-1}. Protein-disulphide-reductase activity of the mitochondria was measured after incubation for 1 h at 25° C with the substrate. The method of Boyer[5] was used for the assay of sulphydryl groupings. Mitochondria from ovoid cells showed much greater activity than an equal mass from elongated cells. The incubation mixtures of Nickerson and Falcone contained a "liver coenzyme concentrate", but a similar addition was shown to be unnecessary in the present experiments. Activity was not promoted by adding dihydronicotinamide adenine dinucleotide phosphate (NADPH), while less than 10 per cent increase in reduction was stimulated by 0·5 μmole of dihydronicotinamide adenine dinucleotide (NADH).

Electrophoretically pure ovalbumin (Sigma grade) furnished an alternative and convenient substrate in place of mannan-protein. Ovalbumin and mitochondria derived as before from ovoid and elongated cells were incubated for 30 min, but in further work the incubation period was reduced to 10 min (Table 1). The results were supplemented by using mitochondria of ovoid cells continuously cultured in phosphate-limited medium. There was no substantial increase in activity when NADH was added to the various incubation mixtures.

Table 1

Culture medium	Cell shape	Incubation period (min)	—SH production*
Nitrogen limiting	Elongated	30	183·1
Glucose limiting	Ovoid	30	649·3
Nitrogen limiting	Elongated	10	98·2 ± 15·2
Glucose limiting	Ovoid	10	248·1 ± 25·0
Phosphate limiting	Ovoid	10	208·1 ± 28·4

* Expressed as mμmoles cysteine/mg protein, means of four results after subtraction of control values. Protein-levels of mitochondria estimated routinely as total nitrogen × 6·25 but confirmed in selected cases by the Folin–Ciocalteau method (ref. 6). Mitochondria in phosphate buffer (*p*H 7·0) (0·5 ml.) and ovalbumin solution adjusted to $E_{1\,cm}^{280} = 1·0$ (0·25 ml.) and incubated in an evacuated desiccator at 25° C.

Table 2

Culture medium	Cell shape	—SH production* Ovalbumin	MP(*E*)	MP(*O*)
Nitrogen limiting	Elongated	101·7 ± 11·9	0·00	0·00
Glucose limiting	Ovoid	375·4 ± 25·5	111·4	123·2
Phosphate limiting	Ovoid	312·8 ± 17·5	—	—

* 0·1 ml. mitochondrial supernatant and 0·1 ml. ovalbumin solution or 0·5 ml. mannan-protein incubated for 1 min at 25° C in air with results expressed as mμmoles cysteine/mg protein, mean of three results after subtraction of control values. MP(*E*) and MP(*O*) represent mannan-protein of elongated ovoid cells, respectively.

The results suggested either a considerable difference in protein-disulphide-reductase activity of the mitochondria of ovoid and elongated cells or differences in permeability of the mitochondrial membranes. Accordingly the experiments with ovalbumin and mannan-protein were repeated using mitochondria preparations which had been disrupted sonically and the membranes removed by centrifugation at 20,000*g* for 20 min. The activities shown in Table 2 were not significantly increased when NADH was added. They demonstrated for the first time that the difference in activity between mitochondria of elongated and ovoid cells was not due to permeability effects, and provided evidence that there is little difference between the mannan-protein of elongated and ovoid cells as substrates.

Activity of the mitochondrial supernatant was lost when 40–60 per cent saturated ammonium sulphate was added to the preparation. The material precipitated by the addition was taken up in 5 mM phosphate or *tris* buffer at *p*H 7·0 and dialysed overnight against similar buffer. When presented with ovalbumin (0·25 mg) as substrate, the dialysed material (adjusted to the usual absorbance) displayed no disulphide-reductase activity after 1 min at 25° C. Addition of 0·25 μmole NADH to the incubation mixture, however, brought about the production of 1,360 mμmoles cysteine/mg protein. It is clear, therefore, that while protein-disulphide-reductase in yeast mitochondria is not dependent on the mitochondrial membrane, it is closely coupled with either NADH or an alternative coenzyme.

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Trypsin Inhibition by Phenylmethylsulphonylfluoride with Reduced Wool as Substrate

WE are trying to dissolve native proteins from wool by the method of limited proteolysis[1,2]. Unreduced wool is not attacked by trypsin and so, in our experiments, wool has been reduced under the mildest possible conditions which will allow trypsin digestion. We believe that these conditions do not cause denaturation of the wool[3]. Apparently, large protein fragments are dissolved from the reduced wool by trypsin, and in a synthetic boundary run[4] in the Spinco model *E* ultracentrifuge, the unfractionated solution gave a schlieren peak, sedimentation coefficient of 3·1. One obstacle to further characterization of the dissolved proteins is the enzyme in the solution, which may continue to digest the fragments it has released from the fibre. This communication describes experiments which show that the inhibitor phenylmethylsulphonylfluoride (PMSF)[5] will arrest the reaction of trypsin with reduced wool.

Accurately weighed samples of wool were reduced under controlled conditions, rinsed and treated with trypsin solutions. Fig. 1 shows the results of a series of experiments in which different amounts of inhibitor were added to trypsin solutions at different times before introducing the reduced wool sample. A comparison was made between the amount of protein dissolved by the inhibited