EFFECT OF CYCLIC AMP ON THE BIOGENESIS OF CYTOCHROME OXIDASE IN YEAST

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1. Introduction

Reversal of glucose or catabolite repression by cyclic AMP is well established, particularly in bacterial systems [1]. In yeast, cyclic AMP reverses the glucose repression of mitochondriogenesis [2]. Recent work in our laboratory (K.C., Ph. D. thesis) has suggested that the primary effect of glucose during mitochondriogenesis is probably on the synthesis of mitochondrial products. Evidence is presented here to show that glucose inhibits the synthesis of mitochondrially made subunits of cytochrome oxidase enzyme and that cAMP counteracts this inhibition.

2. Materials and methods

A diploid strain of *Saccharomyces cerevisiae* 3095 was employed in these studies. The growth condition for repression and derepression were as in [3]. Cytochrome oxidase was assayed by a standard method [4]. The enzyme was purified 20-fold as in [5] and antibody against it was raised as in [6].

The mitochondria were isolated after grinding the cells with celite-carborundum [7], solubilized and immunoprecipitated as in [8], and the immunoprecipitate was separated on SDS–polyacrylamide gels as in [9]. The gels were then sliced manually into 1.5 mm thick slices and digested with hydrogen peroxide and the radioactivity was counted in a scintillation counter (ECIL, India) with an efficiency of 70% for ^{14}C.

The experimental conditions were the same as in [3] and under these conditions, the cells are in a repressed state 2–2.5 h after inoculation into the repression medium and thereafter start derepressing as measured by the increase in several parameters like oxygen uptake and respiratory enzymes. If a fresh addition of glucose is made at this point, the repression is extended. This system is found convenient to compare the events during normal derepression and the effect of glucose on this.

3. Results

Preliminary experiments were carried out to check whether cAMP would counteract the repression by glucose. Even 20 mM cAMP showed only marginal (5%) positive response in restoring oxygen uptake and cytochrome oxidase activity.

This is likely due to the induction of lytic enzymes in yeast by glucose [3] and the consequent breakdown of cAMP.

We therefore resorted to radioactive labelling techniques. ^{14}C-labelled algal hydrolysate [5 μCi/ml medium] was added to the cells at the time of derepression and incubated for 60 min to label the cellular proteins. Any additions, which are mentioned below, were made at the same time as the radioactive compound. The mitochondria were isolated from the cells, and treated with antiserum to cytochrome oxidase. The immunoprecipitate was separated by electrophoresis and the radioactivity profile was determined.

Figure 1a shows the radioactivity profile of mitochondria obtained from normal derepressing cells. Six subunits of the enzyme are clearly identified and the molecular weights correspond to [10]. The corresponding radioactivity distribution data is given in table 1. When the cells are labelled in presence of chloramphenicol (CAP) (4 mg/ml medium) or cyclo-

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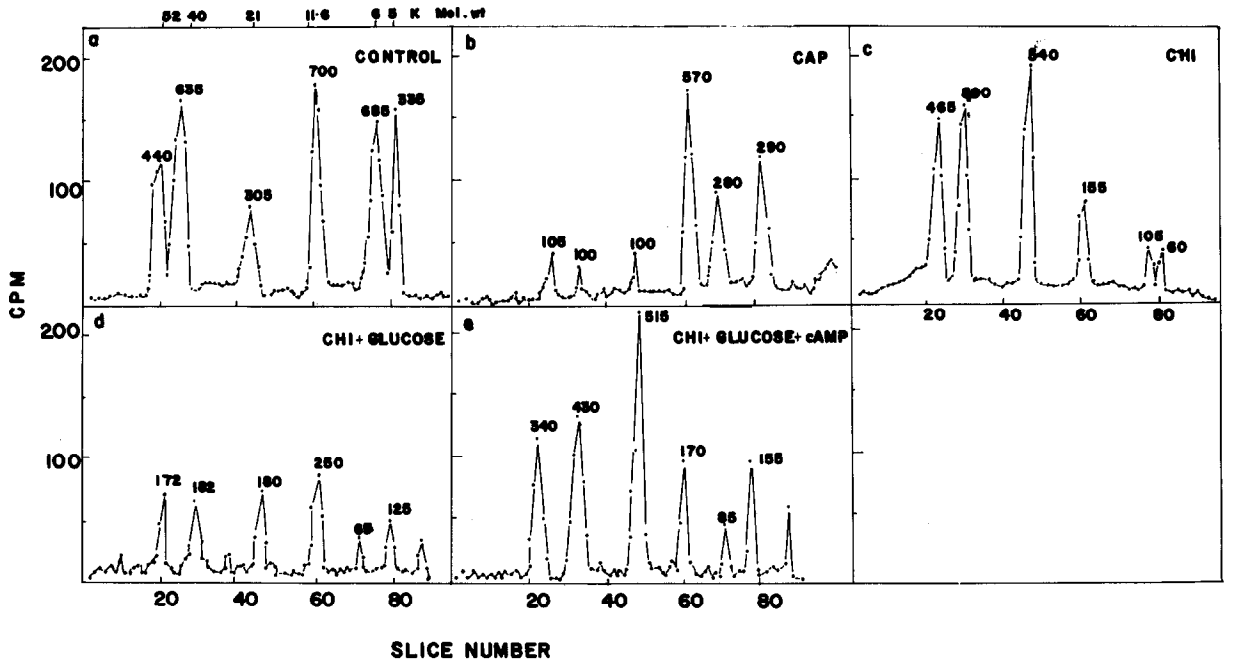

Fig.1. Radioactive profiles after SDS–page of the immunoprecipitates of cytochrome oxidase. Experimental conditions as described in text. Where indicated, the final concentration of added cAMP was 1.5 mM. The figures near the peaks represent the total counts in that peak.

heximide (CHI) (50 μg/ml medium), the profiles are altered as shown in fig.1b and 1c, respectively. In conformity with [10,11] labelling of peaks I, II and III is sensitive to CAP and that of peaks IV, V and VI is CHI sensitive. It can also be seen from table 1, that under conditions of our experiments, 80% inhibition is achieved by the action of the antibiotics.

In the next series of experiments, the labelling was carried out in presence of CHI and glucose (10 mg/ml) and the corresponding gel pattern is given in fig.1d. General incorporation has been decreased almost 3-fold, but significantly the labelling of peaks I, II and III were abolished substantiating our contention that mitochondrial protein synthesis is sensitive to glucose repression. The profile obtained when 1.5 mM cAMP was added along with CHI and glucose during labelling is shown in fig.1e and it is clear that the peaks I, II and III are predominantly labelled, demonstrating the reversal by cAMP of the glucose effect. Data presented in table 1 also shows that almost 75% of total counts incorporated are in mitochondrially made products.

4. Discussion

Considering the many similarities between prokaryotes and mitochondria, it is not surprising that cAMP should be involved in the regulation of mitochondrial protein synthesis. In bacteria, it is well established that cAMP interacts at the level of transcription [12]. Preliminary studies on ^{32}P labelling of mitochondrial RNA has shown that glucose reduces the extent of labelling and cAMP reverses this. This suggests a similar mechanism for cAMP involvement as in bacteria. It is interesting to note the isolation [13] of a group of mitochondrial mutants called *box* mutants which lack both cytochrome *b* and cytochrome oxidase. The mutants map differently from the genes of both cyt *b* and oxidase and are extremely sensitive to glucose repression [13]. It would be worth exploring this mutant for its cAMP content or for the presence of cAMP receptor protein.

Glucose reduces the intracellular cAMP levels in yeast [14]. Perhaps this is the basic mechanism of action of glucose repression. Experiments are now in

Table 1
Relative distribution of radioactivity in cytochrome oxidase subunits

	Control	CHI	CAP	CHI + glucose	CHI + Glucose + cAMP
Total radio-activity loaded in gels (cpm)	3570	2320	1915	1085	1855
Recovered cpm in peaks	3100	1915	1441	974	1695
% CHI-sensitive products	55.39	16.71	79.10	45.16	24.17
% CAP-sensitive products	44.50	83.27	21.14	54.81	75.79
Relative labelling of subunits in comparison to control taken as 1.0					
Subunits					
I	1	1.056	0.238	0.390	0.772
II	1	0.929	0.157	0.286	0.677
III	1	1.770	0.327	0.590	1.688
IV	1	0.221	0.814	0.357	0.242
V	1	0.153	0.408	0.094	0.124
VI	1	0.179	0.865	0.373	0.462

In all cases the same amount of protein (100 μg) was loaded
Experimental conditions as described in section 2

progress to see whether such effects of cAMP are shown in the case of other mitochondrially made proteins.

Acknowledgements

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