Cyclic nucleotide-dependent inactivation of yeast fructose 1,6-bisphosphatase by ATP

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1. INTRODUCTION

When resting baker's yeast is added to a glucose solution the intracellular cAMP concentration increases several-fold in a few minutes and returns to normal in < 1 h [1]. This increase in cAMP concentration is believed to lead to the activation of trehalase by a cAMP-dependent protein kinase [1,2]. Lenz and Holzer [3] have shown that the irreversible inactivation of yeast fructose 1,6-bisphosphatase (FBPase) that occurs [4] in 1 h after addition of glucose to acetate-grown yeast is preceded by a rapid (3 min) reversible disappearance of $\sim 1/2$ the activity measured at 10 mM $MgCl_2$, with little change in the activity at 2 mM $MnCl_2$. Furthermore, stoichiometric amounts of phosphate are rapidly (< 6 min) incorporated into FBPase when glucose is added to derepressed yeast suspended in [^{32}P]orthophosphate [5].

These results suggested that yeast FBPase might be partially inactivated by a cAMP-dependent protein kinase that is stimulated by the transient increase in cAMP following addition of glucose to derepressed cells. This paper describes a cyclic nucleotide- and ATP-dependent reaction in vitro that decreases the activity of FBPase at 10 mM $MgCl_2$ by $\sim 60\%$ without changing its activity at 2 mM $MnCl_2$.

Abbreviations: cAMP, cCMP, cGMP, cIMP and cUMP, the 3′, 5′-cyclic phosphates of adenosine, cytosine, guanosine, inosine and uridine, respectively; FBPase, fructose 1,6-bisphosphatase (EC 3.1.3.11).

2. MATERIALS AND METHODS

Fructose 1,6-bisphosphate, nucleotides and accessory enzymes were from Boehringer. Commercial baker's yeast, grown with strong aeration on a mixture of molasses and ethanol, was obtained from our Rajamäki factory. It was suspended in an equal volume of 20 mM K-phosphate (pH 7.5) containing 1 mM $MgCl_2$ and 0.1 mM EDTA, and broken by shaking with glass beads in a Braun disintegrator. The disintegrates were centrifuged 20 min at 12 000 × *g* or 30 000 × *g* and then 60 min at 145 000 × *g*, and the supernatants dialysed for 3 h (or 20 h) against the same buffer (or with $MgCl_2$ omitted).

For activation/inactivation experiments, 250–500 μl portions of dialysed supernatant were adjusted to the required divalent metal ion concentration and preincubated for 4 min at 30°C before addition of nucleotides in 10–30 μl. Samples of 10 μl (FBPase) or 50 μl (trehalase) were transferred to 1.0 ml enzyme assay mixtures at various times after addition of the nucleotides. FBPase was assayed at 30°C as in [6] except that imidazole/HCl was replaced by 50 mM Pipes/KOH (pH 7.0) and [fructose 1,6-bisphosphate] was 0.3 mM instead of 0.1 mM. Where stated, 2 mM $MnCl_2$ was used instead of 10 mM $MgCl_2$. Trehalase was assayed at 30°C in 25 mM Pipes/KOH (pH 7.0) containing 10 mM trehalose and 1 mM $MgCl_2$, and the glucose produced determined enzymatically (cf. [1]).

3. RESULTS

The 12 000 × *g* supernatants of our commercial baker's yeast contained 13 mU FBPase/mg protein,

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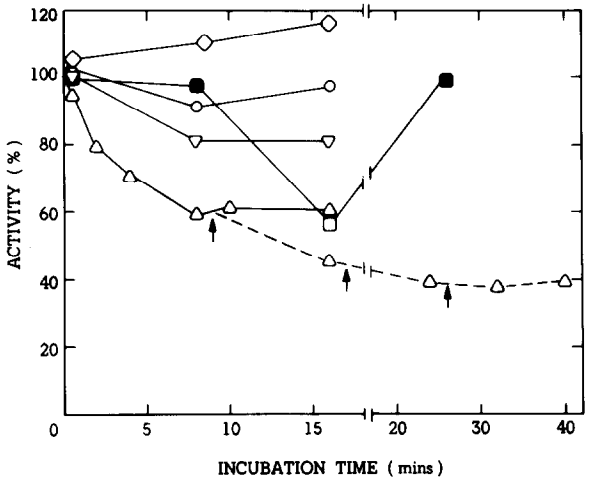

Fig.1. Inactivation of FBPase by ATP plus cAMP. At time zero 1.2 mM ATP plus 180 μM cAMP (△, ▪ and □), 1.2 mM ATP + 18 μM cAMP (▽), 1.2 mM ATP alone (○) or 180 μM cAMP alone (◇) were added to dialysed 12 000 × *g* supernatant containing 40 mg protein/ml and 2.8 mM $MgCl_2$. The broken line shows the effect of repeated additions of 1.2 mM ATP plus 180 μM cAMP at the arrows. FBPase was assayed in 10 mM $MgCl_2$ (open symbols, including □) or 2 mM $MnCl_2$ (▪).

which is ~30% of that found in cells grown on ethanol alone [5]. Incubation with ATP and $MgCl_2$ in the presence of 180 or 18 μM cAMP, respectively, caused inactivations of 40% and 20% to develop within 8 min, but < 10% inactivation in the absence of cyclic nucleotide (fig.1). Repeated addition of ATP plus cAMP caused the inactivation to increase to a maximum of ~60%. With 180 μM cAMP alone a small activation occurred, which has not been investigated. With no added nucleotides, the activity of FBPase did not change during 1 h at 30°C (not shown). No inactivation of FBPase by ATP plus cAMP was observed when the activity was measured at 2 mM $MnCl_2$ instead of 10 mM $MgCl_2$ (fig.1).

The inactivation also occurred when the post-microsomal supernatant was incubated with ATP + cAMP (table 1(a)). Investigation of the nucleotide requirement (table 1(b)) showed that cAMP could be replaced by cGMP or cIMP but only by higher concentrations of cUMP. ATP could be replaced by ADP, but with less effect. Presumably some disproportionation of ADP to ATP plus 5′-AMP occurs. No cAMP-dependent inactivation occurred when ATP was replaced by 5′-AMP: instead an initial inhibition of 14% decreased with time, presumably representing inhibition by the 13 μM 5′-AMP introduced with the enzyme sample to the assay mixture and metabolic removal of 5′-AMP by the yeast extract (5′-AMP is a potent inhibitor of FBPase with a K_i ~ 80 μM [7]).

With yeast supernatant dialysed for 20 h against 20 mM K-phosphate (pH 7.5)/0.1 mM EDTA the inactivation was clearly dependent on added divalent metal ion (Mg^{2+} or Ca^{2+}, but not Co^{2+} or Mn^{2+}) (table 1(c)). The inactivation was smaller (25%) than usual in this experiment, possibly be-

Table 1

Metal- and nucleotide-dependence for inactivation of FBPase

Addition to:	Remaining activity (%) after		
	0.5 min	8 min	16 min
(a) 145 000 × *g* supernatant containing 2.8 mM $MgCl_2$			
1.2 mM ATP	94	91	94
+ 180 μM cAMP	86	64	67
(b) 12 000 × *g* supernatant containing 2.8 mM $MgCl_2$			
1.2 mM ATP	101	91	97
+ 180 μM cAMP	91	57	60
+ 180 μM cGMP	82	65	65
+ 180 μM cIMP	89	58	56
+ 180 μM cUMP	97	93	93
3.0 mM ATP + 600 μM cUMP	98	74	68
1.6 mM ADP	95	96	99
+ 180 μM cAMP	92	69	80
1.3 mM 5′AMP	86	97	ND
+ 180 μM cAMP	86	93	ND
(c) 30 000 × *g* supernatant containing 0.1 mM EDTA, 1.2 mM ATP, 180 μM cAMP			
No addition	95	90	ND
2.8 mM $MgCl_2$	97	75	ND
2.8 mM $CaCl_2$	86	75	ND
2.8 mM $Co(NO_3)_2$	102	92	ND
2.8 mM $MnCl_2$	94	93	ND

Experimental details in section 2. Protein concentrations (mg/ml) before the FBPase assays were: (a) 26, (b) 42 and (c) 31; ND, not determined

Table 2

Metal- and cyclic nucleotide-dependence for activation of trehalase

Addition to:	Activity (mU/mg protein) after	
	2 min	8 min
(a) 30 000 × *g* supernatant containing 4 mM $MgCl_2$		
None	ND	29.5
4 mM ATP	ND	30.4
+ 200 μM cAMP	ND	94
+ 200 μM cGMP	ND	94
+ 200 μM cIMP	ND	91
+ 200 μM cUMP	ND	66
(b) 145 000 × *g* supernatant containing 0.1 mM EDTA, 1.7 mM ATP, 200 μM cAMP		
None	31.2	28.6
3.0 mM $MgCl_2$	71	77
3.0 mM $MnCl_2$	48	68
3.0 mM $Co(NO_3)_2$	65	71
3.0 mM $CaCl_2$	42	43

Experimental details in section 2. Protein concentrations (mg/ml) before the trehalase assays were: (a) 27 and (b) 22; ND, not determined

cause of the long dialysis in the absence of $MgCl_2$. No inactivation occurred with ATP + Ca^{2+} in the absence of cyclic nucleotide (not shown).

These results were compared with the ATP + cAMP-dependent activation of trehalase reported in [1,2]. With our yeast, the activation of trehalase by ATP was completely dependent on cyclic nucleotides and was 3.3-fold with cAMP, cGMP and cIMP and somewhat less (2.2-fold) with cUMP (table 2(a)). The activation with cUMP was initially slower than that with cAMP and could not be increased to the level observed with cyclic purine nucleotides by prolonging the incubation time to 16 min (not shown). As reported [2], Mg^{2+}, Mn^{2+} and Co^{2+} were effective divalent metal ions. Ca^{2+} was found to be much less effective (table 2(b)).

4. DISCUSSION

These results show that yeast FBPase in the 12 000 × *g* or 145 000 × *g* supernatants of disintegrated yeast can be inactivated in vitro in a reaction dependent on ATP, cyclic nucleotide and divalent metal ion. Coupled with the knowledge [5] that the rapid and reversible phase of the inactivation of FBPase in vivo is accompanied by phosphorylation of a serine residue in the enzyme, this strongly suggests that yeast FBPase is inactivated by a soluble cyclic nucleotide-dependent protein kinase. Like the rapid (3 min) inactivation in vivo when glucose is added to derepressed yeast [3] the inactivation in vitro decreased the activity of FBPase measured at 10 mM $MgCl_2$ by a maximum of ~60% and did not change the activity measured at 2 mM $MnCl_2$ (fig.1). Kidney [8] and liver [9] FBPase are phosphorylatable by purified protein kinases, but in the former case the activity is not affected and in the latter case the activity apparently increases by ~40%.

Although cAMP concentrations in yeast are generally lower in glucose repressed cells than in derepressed cells (see [10] and references therein), addition of glucose to commercial baker's yeast causes a sudden transient increase in cAMP concentration [1], which is thought to be responsible for the activation of trehalase and degradation of trehalose that occur in the lag phase before growth on glucose. These results suggest that it is also responsible for the rapid phase of the inactivation of FBPase that occurs under similar conditions [3–5]. Both phenomena re-direct the flux in the Embden–Meyerhof pathway into the glycolytic direction.

There is little information about the cyclic nucleotide specificity of yeast cAMP-dependent protein kinases, although the mammalian enzymes are also activated by low concentrations of cIMP and higher concentrations of cGMP, cUMP and cCMP (reviewed in [11]). Under our conditions, cAMP, cGMP and cIMP were equally effective both for activation of trehalase and inactivation of FBPase, and cUMP was less effective in both cases. The effects of repetitive additions of ATP plus cAMP shown in fig.1, however, indicate that the kinetics of inactivation of FBPase in crude yeast supernatants reflect primarily the consumption of ATP and cAMP, especially the latter, since a second addition of cAMP alone, but not one of ATP alone, caused a further inactivation of ~15% (not shown). The apparent differences in divalent metal ion requirement are not sufficient reason to suppose that different protein kinases are involved in the activation

of trehalase and inactivation of FBPase, since the metal ion requirement of the free catalytic subunit of the kinase may vary with its substrate.

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