groups of the inhibitors block Pi. A combination of both effects is also possible, especially in th case of UDPG. Thus, competitive effects with respect to the substrate (s) are likely.

It is highly probable that in vivo the enzyme is bound to glycogen particles, where the end-groups concentration is high. The intracellular Pi concentration in yeast has a value of around 0.02 M (16). Therefore, the enzyme in vivo appears to be present in an environment of high substrate saturation, and this counteracts the effects of competitive inhibitors.

The possibility that phosphoglucomutase is the rate-limiting step in glycogen degradation, thus masking the effects of phosphorylase inhibitors, can be eliminated by the observation, quoted before, that no increase in glycogen degradation occurs when the G-6-P concentration decreases sharply (14).

In order to account for anomalies in the levels of UDPG of glycogen deficient mutants of strain 4236, which we have also observed in mutants of strain 1338 (manuscript in preparation), it has been proposed that recycling of G-1-P, formed by phosphorylase, into UDPG and glycogen takes place (15). In the apparent absence of control of phosphorylase activity by intracellular metabolites, we feel that recycling of G-1-P may be a mechanism of major importance in controlling the overall rate of glycogen degradation in yeast cells. The degree of recycling may be determined by the intracellular concentration of G-1-P, and by the action of intracellular metabolites on the pyrophosphorylase and/or synthetase.

These observations, of course, do not eliminate the possibility of control of phosphorylase levels in yeasts, nor the possibility that the different forms of the enzyme may have different substrate and/or effector affinities and that their relative levels may be varied. Both of these, however, would be long-range mechanisms.

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pellets were suspended in 5.0 ml 0.15MKCl (adjusted to pH 7.4 with 0.02 M $KHCO_3$) and sonicated in a Biosonik (Bronwill, Rochester, N. Y.) at 70% maximal output for 30 sec. The supernatant collected after centrifugation at 800 x g for 10 min was divided into several aliquots and used for enzyme assays. For studies on the effect of nicotinamide and freezing-thawing, samples containing varying amount of nicotinamide were frozen in dry ice-acetone bath, stored in a freezer for 1 h, and thawed at 5°C in a cold room for 1 h.

All enzyme assays were carried out at 26°C in a Beckmann DB spectrophotometer by following the change in optical density at 340 mμ. Ingredients in assay mixtures were as follows. Pyruvate kinase: 50 mM Triethanolamine·HCl, pH 7.5, 0.23 mM ADP, 0.133 mM $NADH_2$, 0.77 mM phosphoenolpyruvate, 8 mM $MgSO_4$, 75 mM KCl, 25 μg/ml lactate dehydrogenase, and 0.05 ml cell extract. Hexokinase: 73 mM Triethanolamine·HCl, pH 7.5, 0.223 M glucose, 6.67 mM $MgCl_2$, 0.83 mM $NADP^+$, 0.54 mM ATP, 3.3 μg/ml glucose-6-phosphate dehydrogenase, and 0.1 ml cell extract. Glucose-6-phosphate dehydrogenase: 86 mM Triethanolamine·HCl, pH 7.5, 6.67 mM $MgCl_2$, 1.3 mM glucose-6-phosphate, 0.4 mM $NADP^+$ and 0.1 ml cell extract. 6-Phosphogluconate dehydrogenase: 41.6 mM Na-glycylglycinate, pH 9.0, 16.7 mM $MgCl_2$, 0.33 mM $NADP^+$, 0.83 mM 6-phosphogluconate and 0.2 ml cell extract. Lactate dehydrogenase: 0.2M Na-phosphate buffer, pH 7.4, 0.133 mM $NADH_2$, 0.133 mM Na-pyruvate and 0.01 ml cell extract. The final volume of all assay mixtures was 3.0 ml.

RESULTS AND DISCUSSION

Table 1 summarizes the effect of nicotinamide and freezing-thawing on the activity of pyruvate kinase (PK), hexokinase (HK), glucose-6-phosphate dehydrogenase (G6PDH), 6-phosphogluconate dehydrogenase (PGDH) and lactate dehydrogenase (LDH) in L1210 cell extracts. Extracts which had been frozen and thawed in the absence of nicotinamide retained full activity of these enzymes when it was added to freshly prepared extracts or to frozen ones just before thawing. Cell extracts which had been frozen and thawed in the

Table 1. Effects of Nicotinamide and of Freezing-thawing on Enzyme Activity in Extract of L1210 Cells

Extract / Addition / Enzyme	Fresh		Frozen and Thawed		
	No	50 mM Nicotinamide	No	50 mM Nicotinamide added before	
				Thawing	Freezing
Pyruvate kinase	1736	1804	1858	1877	1925
Hexokinase	105	75	79	68	32
Glucose-6-phosphate dehydrogenase	93	79	98	85	26
6-Phosphogluconate dehydrogenase	61	65	65	66	18
Lactate dehydrogenase	3838	3620	3463	3178	462

All values are results of two experiments. Enzyme activity is expressed as mμmole/min/ml supernatant. See "Material and Methods" for experimental details.

presence of 50 mM nicotinamide showed full activity of PK, but only 30% activity of HK, 28% of G6PDH, 30% of PGDH and 12% of LDH.

Fig. 1 shows % activity remaining in extracts which had been frozen and thawed in varying concentration of nicotinamide. The activity of PK remained constant with increasing amount of nicotinamide. Extracts frozen and thawed in the presence of 12.5 mM nicotinamide retained about 50% activity in HK, G6PDH and PGDH. LDH was most sensitive to nicotinamide, since 3.5 mM nicotinamide was enough to cause a 50% inhibition of its reconstitution.

The results of this communication suggest that nicotinamide prevents recombination of isoenzyme subunits of HK, G6PDH, PGDH and LDH. Thus it appears that in G6PDH, PGDH and LDH, the sites of attachment normally occupied by $NADP^+$ and $NADH_2$ recognize the pyridine moiety in these coenzyme molecules. This was also suggested by Shifrin, Kaplan and Ciotti (1959) in fluorescence studies on LDH. The fact that reconstitution of HK subunits

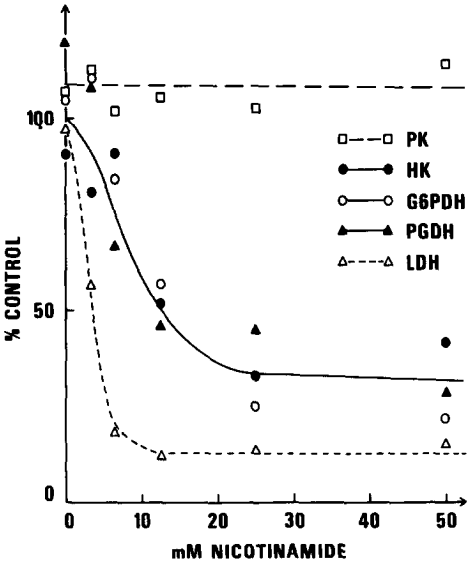

Fig. 1. The percentage of activity of pyruvate kinase (□), hexokinase (●), glucose-6-phosphate dehydrogenase (○), 6-phosphogluconate dehydrogenase (▲) and lactate dehydrogenase (△) recovered after freezing and thawing in increasing concentration of nicotinamide.

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is also inhibited by nicotinamide suggests that pyridine nucleotides may play a role in regulating the activity of HK. Finally, this report presents a convenient method for detecting enzymes with sites of attachment specific for pyridine nucleotides.

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