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PURIFICATION AND PROPERTIES OF GUANYLATE KINASE FROM BAKER'S YEAST

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Guanylate kinase (ATP:(d)GMP phosphotransferase, EC 2.7.4.8) was purified about 200-fold with 4% yield from baker's yeast. The enzyme preparation showed a single band on polyacrylamide gel electrophoresis and the molecular weight of the enzyme was calculated to be 25 000 by gel filtration. With ATP as a phosphate donor, the kinase used only GMP as a phosphate acceptor. K_m values for ATP and GMP were 0.5 and 0.048 mM, respectively. The enzyme reacted optimally at pH 7.5. The enzyme was labile during storage at 4°C and inactivation was prevented by 20% glycerol.

The phosphorylation of nucleoside monophosphates is catalyzed by nucleoside monophosphate kinases. In mammalian tissues, it was demonstrated that there are at least four distinct nucleoside monophosphate kinases, adenylate kinase, guanylate kinase, pyrimidine nucleoside monophosphate kinase and thymidylate kinase. The purification of guanylate kinase from mammalian tissue [1–4] and *Escherichia coli* [5] has been reported. In yeast, however, no investigation has been carried out with purified enzyme with the exception of adenylate kinase. In this report, we describe a procedure for the purification of guanylate kinase to homogeneity from baker's yeast and some of its properties.

Baker's yeast was air-dried and then completely dried over P_2O_5 in vacuo. Guanylate kinase activity was measured spectrophotometrically by the method of Miech and Parks [1]. One unit of guanylate kinase is defined as the amount of enzyme which catalyzes the phosphorylation of 1 μmol GMP/min at 25°C. All operations for purification were carried out at 0–4°C.

50 g dried cells of baker's yeast were ground with an equal weight of alumina for 2 h. The crude enzyme was extracted with 300 ml 0.01 M potassium phosphate buffer (pH 7.5) followed by centrifugation at 10000 × *g*. To the crude extract was added 1 ml of 1% protamine sulfate (pH 7.5)/100 mg protein with stirring. After 30 min, the mixture was centrifuged. The precipitate forming at 40–80% saturation $(NH_4)_2SO_4$ was dissolved in 0.01 M potassium phosphate buffer (pH 7.5) and dialyzed against same buffer. The insoluble material formed during dialysis was removed by centrifugation. The crude enzyme preparation was applied to a DEAE-cellulose column (3.8 × 20 cm) previously equilibrated with 0.01 M potassium phosphate buffer (pH 7.5). Active enzyme was not adsorbed to the column. These fractions were pooled and precipitated at 80% saturation $(NH_4)_2SO_4$. The precipitate was dissolved in a small volume of 0.001 M potassium phosphate buffer (pH 7.5) and dialyzed against the same buffer. The enzyme solution was applied to a hydroxyapatite column (2.4 × 11 cm) previously equilibrated with this buffer. After the column was washed with the same buffer, the enzyme was eluted with 0.005 M potassium phosphate buffer (pH 7.5). The active fractions were pooled and concentrated with an Amicon ultrafiltration apparatus. This fraction contained a high

TABLE I

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Step	Total protein (mg)	Total units	Specific activity (units/mg)	Yield (%)
1. Crude extract	9010	441	0.049	100
2. Protamine sulfate	4040	307	0.076	70
3. $(NH_4)_2SO_4$	3040	252	0.083	57
4. DEAE-cellulose	792	221	0.278	50
5. Hydroxyapatite	54.5	184	3.38	42
6. Sephadex G-75	1.7	17.9	10.6	4

level of adenylate kinase activity. In recent years, it has been demonstrated that adenylate kinase, like some kinases and dehydrogenases, bound to a Blue Dextran affinity column in the presence of low ionic strength solvents [6]. Therefore, gel filtration with Blue Dextran at low ionic strength was carried out. Concentrated enzyme (54.5 mg protein) was run on a Sephadex G-75 column (2.4 X 98 cm) with 1 ml 2% Blue Dextran 2000, then eluted with 0.005 M potassium phosphate buffer (pH 7.5). After adenylate kinase had eluted together with the Blue Dextran in the void volume, guanylate kinase was eluted.

A summary of the purification procedure is presented in Table I. The enzyme was purified approx. 200-fold. The purified enzyme was shown to be homogeneous by polyacrylamide gel electrophoresis. The molecular weight of guanylate kinase from *E. coli* was reported to be 88 000. In contrast to that, the molecular weight of yeast guanylate kinase was calculated to be 25 000 by Sephadex G-75 gel filtration, this is similar to that of the mammalian enzymes. The optimal pH for guanylate kinase was 7.5 (Fig. 1) and the enzyme was most stable at this pH. At pH values below 4 and above 9, no activity remained after 48 h storage at 4°C. Thermal stability was investigated by incubating the enzyme at various temperatures for 10 min. Guanylate kinase was rather heat labile and 50% activity was lost at 45°C. At 60°C, 90% of the original activity was lost. The enzyme was quite labile during storage at 4°C and the activity was completely lost after 2 weeks. Some compounds were tested to stabilize the enzyme, and 20% glycerol (w/v) was found to be most effective. With 20% glycerol, 90% of the original activity still remained, even after 1 month.

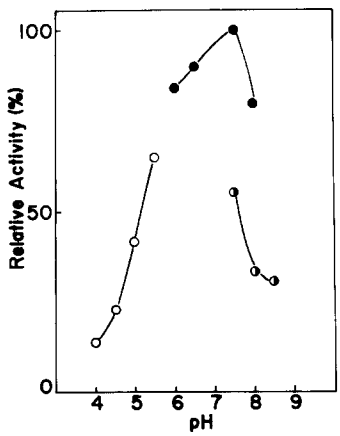

Fig. 1. Effect of pH on the enzyme activity. Solutions containing 25 μg/ml enzyme were incubated for 10 min. ○———○, acetate buffer; ●———●, potassium phosphate buffer; ◑———◑, Tris-HCl buffer.

The enzyme was highly specific for ATP as a phosphate donor. Among the nucleoside monophosphates investigated (GMP, AMP, CMP, UMP, IMP and XMP) only GMP reacted with ATP. Initial velocity studies using either GMP or ATP as the changing fixed substrate indicate that the reaction proceeded by a sequential (either 'random' or 'ordered') mechanism rather than by a 'ping-pong' mechanism. K_m values for ATP and GMP were 0.5 and 0.048 mM, respectively. The enzyme required divalent cations for reaction. Mg^{2+} and Mn^{2+} were most effective and Co^{2+} could partially fulfill the requirement.

The investigation of purified yeast guanylate kinase reveals that it is highly specific to GMP and its properties differ from that of bacterial enzyme but are similar to those of mammalian enzymes.

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