INHIBITION OF WHEAT GERM PORPHOBILINOGEN DEAMINASE ACTIVITY BY BUTANEDIONE

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1. Introduction

Porphobilinogen (PBG) deaminase or uroporphyrinogen I synthetase is the enzyme which catalyzes the tetramerization of porphobilinogen. The structural requirements for competitive inhibitors of the enzyme [1,2] suggest that there is at the substrate binding site at least one amino acid residue which binds carboxylate ion.

Arginyl residues can serve as recognition sites on a variety of enzymes when the substrates contain carboxylate or phosphate groups [3,4]. Butanedione in borate buffer inhibits such enzymes by binding to the guanidino groups of arginine residues in the binding site. This paper reports the results of studies of the effect of butanedione on PBG deaminase.

2. Experimental

PBG was purchased from Porphyrin Products (Logan, Utah) and Sigma (St. Louis, MO). Butanedione was purchased from Aldrich Chemical (Milwaukee, WI). All other chemicals were reagent grade or better. Wheat germ was obtained from Sigma. Spectrophotometric measurements were made with the Cary 15 and Zeiss M4QIII spectrophotometers. Wheat germ PBG deaminase was prepared as in [5] and carried through the heat-denaturation step. Protein was determined as in [6] with bovine serum albumin as standard. Butanedione stock solutions were made up in borate buffer and the pH adjusted to pH 8.2 with solid sodium hydroxide.

Deaminase activity was measured in final reaction mixture vol. 5.0 ml containing: 50 mM borate buffer, pH 8.2, 0.001 mM EDTA, 1 ml enzyme preparation (1.5 mg protein/ml assay mixture, spec. act. 2.97 nmol PBG consumed/h/mg protein) and PBG (60–70 nmol). After removing aliquots for zero time assays, the reaction mixture was evacuated and flushed with nitrogen repeatedly and then incubated for 4 h at 27°C. Aliquots were assayed for PBG with modified Ehrlich reagent [7]. Uroporphyrinogen was oxidized as in [8] and determined as uroporphyrin at 405 nm.

Modification of deaminase with butanedione was carried out at 25°C in final vol. 8.0 ml containing: 5 ml enzyme preparation (62 mg protein, spec. act. 2.87 nmol PBG consumed/h/mg protein), 50 mM borate buffer, pH 8.2 and concentrations of butanedione ranging from 0–100 mM. Aliquots (1.4 ml) were removed after incubation from 0–150 min, chilled to 4°C and dialyzed overnight at 4°C against 50 mM borate buffer, pH 8.2. Dialyzate, 1 ml, was assayed as described above. As a control, enzyme without butanedione was treated in the same way for the longest incubation time and then assayed.

When the ability of substrate to prevent inhibition was determined, PBG (80 μM) was added to the modification reaction mixture before the addition of butanedione (10 mM). PBG, uroporphyrinogen, uroporphyrin and butanedione were removed by extensive dialysis (24 h) at 4°C against 50 mM borate buffer before the enzyme was assayed.

The kinetic parameters were obtained using unmodified enzyme and enzyme which had been treated with 25 mM butanedione for 1 h at 25°C, dialyzed overnight at 4°C against 50 mM borate buffer, pH 8.2. PBG concentrations of 25 μM through

145 μM were used in separate incubation mixtures. Least squares analysis of data gave kinetic parameters with correlation coefficients greater than 0.97.

Reactivation of modified enzyme was attempted by treating deaminase at 25°C with 50 mM butanedione and dialyzing the mixture against either 50 mM borate buffer, pH 8.2 or against water for 23 h at 4°C.

3. Results and discussion

PBG deaminase was incubated with various concentrations of butanedione in borate buffer for a series of time intervals. In some experiments, PBG, the substrate, was added before the inhibitor. The enzyme aliquots were dialyzed individually against borate buffer before they were assayed for activity. The activity of deaminase, measured as PBG consumption and as porphyrin formation, was severely and rapidly reduced by butanedione in borate (fig.1). Borate alone had no effect on deaminase activity. Inhibition was not reversed by dialysis against borate or against water. The rate of inhibition was a function of butanedione concentration and the level of inhibition increased with time of incubation. For 100 mM butanedione, PBG uptake and porphyrin formation were almost completely inhibited before 60 min.

When deaminase was modified for 1 h in 25 mM butanedione, then dialyzed against borate and assayed with varying concentrations of PBG for 1 h and 2 h incubation times, the kinetic parameters in table 1 and fig.2 were obtained. K_m for porphyrin formation was slightly higher or the same for modified enzyme and V_{max} was lower by about a factor of 2. The results suggest that the modified enzyme mixture consists of totally inactivated enzyme and some native enzyme.

In order to determine whether the substrate can

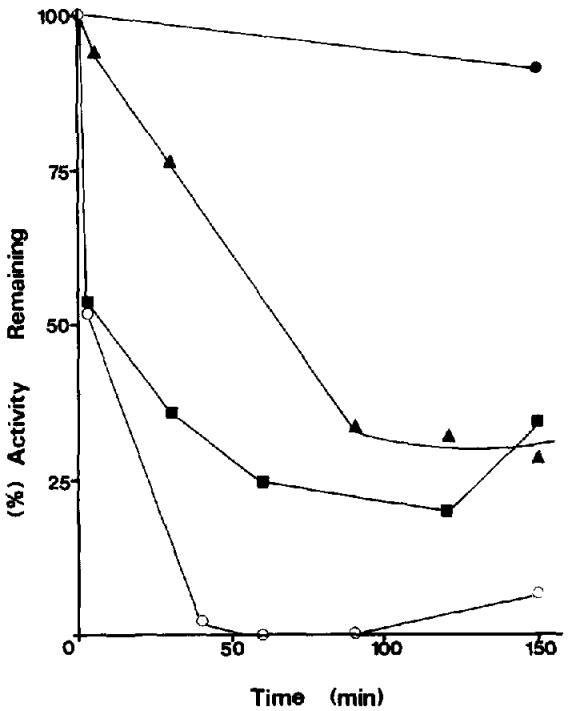

Fig.1. Inactivation of PBG deaminase by butanedione in borate buffer. The enzyme (7.8 mg/ml modification mixture; spec. act. 2.87 nmol PBG consumed/h/mg protein) was incubated with various concentrations of butanedione. Aliquots (1.4 ml) were removed at indicated times and dialyzed against 50 mM borate buffer, pH 8.2 at 4°C and 1 ml was assayed for PBG-consuming activity. The incubations included butanedione in the following concentrations: (•) 0 mM; (▲) 10 mM; (■) 50 mM; (○) 100 mM. Uroporphyrinogen formation gave similar curves.

Table 1
Kinetic parameters for wheat germ PBG deaminase before and after inhibition by 25 mM butanedione measured after one hour and two hour incubation times

Incubation time	K_m (μM)	V_{max} (nmol/ml/h)	K_m (μM)	V_{max} (nmol/ml/h)
Unmodified	26	7.5	22	7.3
Modified run 1	36	2.9	42	3.4
Modified run 2	24	3.3	27	3.1
Modified run 3	30	2.6	24	2.2

Activity was measured as porphyrin formation

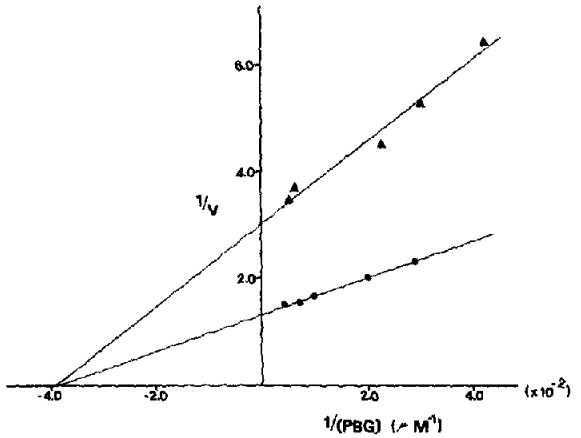

Fig.2. Double reciprocal plot for uroporphyrinogen formation by unmodified (•) and 2,3-butanedione modified PBG deaminase (▲). PBG deaminase was incubated with 25 mM 2,3-butanedione for 60 min at 25°C. Velocity is expressed as nmol uroporphyrinogen formed/ml/h.

protect the enzyme from inactivation by butanedione, deaminase was mixed with PBG (80 μM) before addition of butanedione (10 mM) and borate buffer. Aliquots were removed at various time intervals and dialyzed against borate buffer at 4°C before assay.

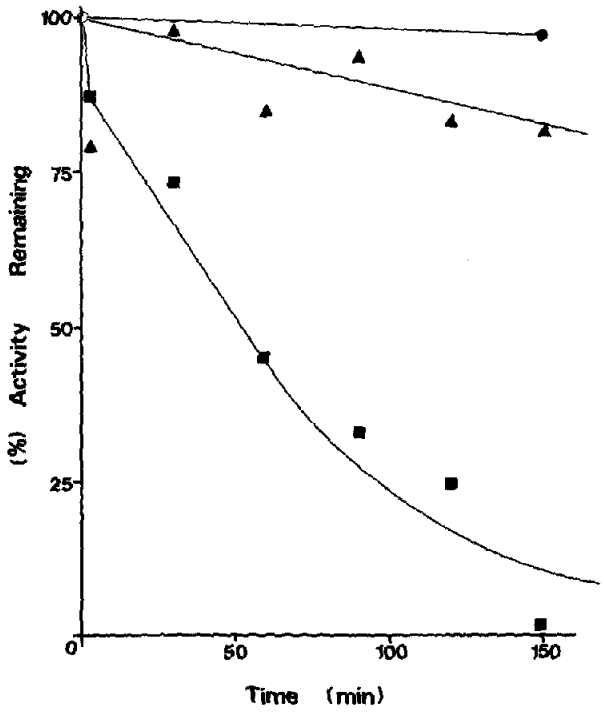

Fig.3. Protection by substrate of PBG deaminase against inactivation by butanedione. The enzyme (7.9 mg/ml modification mixture with spec. act. 1.7 nmol/h/mg protein) was incubated with various concentrations of butanedione with and without PBG. Aliquots (1.4 ml) were removed at indicated times and dialyzed overnight against 50 mM borate buffer, pH 8.2 at 4°C and 1 ml was assayed for PBG-consuming and uroporphyrinogen-forming activities. The incubation mixtures included butanedione and PBG in the following concentrations: PBG consumption: (•) 0 mM butanedione and 0 μM PBG; (▲) 10 mM butanedione and 80 μM PBG; (■) 10 mM butanedione and 0 μM PBG.

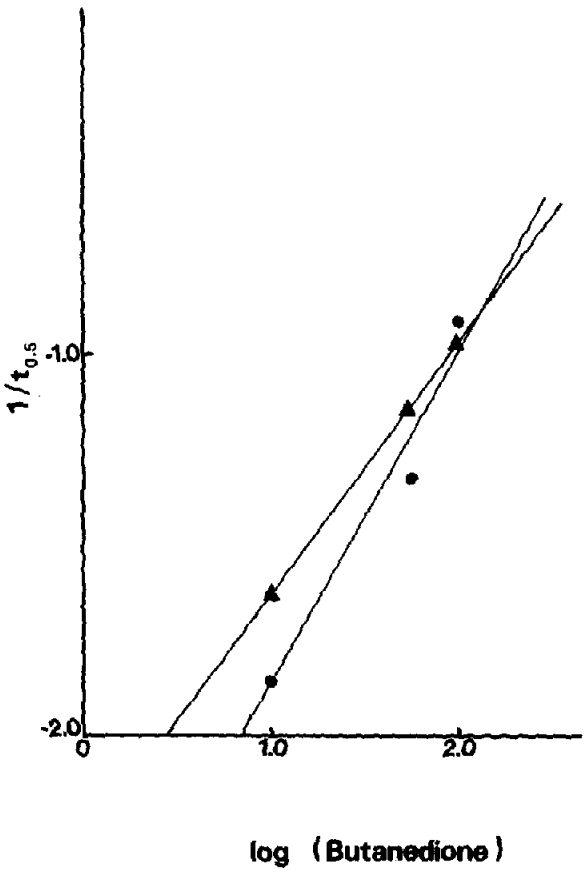

Fig.4. Semilogarithmic plot of PBG deaminase activity after treatment with various concentrations of butanedione. Half-times of inactivation ($t_{0.5}$) values were calculated from semilogarithmic plots of log PBG deaminase activity versus time (min) for initial reaction times up to 60 min. (•) PBG consumption; (▲) uroporphyrinogen formation.

The substrate showed a pronounced ability to protect deaminase activity from butanedione inhibition. Both PBG-uptake (fig.3) and porphyrinogen formation (results not shown) were appreciably protected by addition of PBG before inhibitor.

For data in the first hour of reaction when log $1/t_{0.5}$, where $t_{0.5}$ is the time required for 50% inhibition, was plotted against log butanedione concentration the slope was 0.89 for PBG and 0.65 for porphyrin formation (fig.4). These preliminary results suggest that one arginine is being inactivated and is required for deaminase activity [9].

Carboxypeptidase A [3], aspartate amino transferase [10], and isocitrate dehydrogenase [11] are enzymes which have substrates with carboxylate groups and which have been shown to require one or more arginine residues to bind these substrates. PBG deaminase may be another enzyme of this kind because butanedione inhibits its activity and the substrate lifts this inhibition. It is probable that PBG deaminase catalyzes at least two different types of reactions: condensation of PBG units and cyclization of a linear tetrapyrrole. How the PBG-binding site, the intermediate(s) binding site and the cyclization site relate to each other is not known.

Acknowledgement

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