The Effect of Purine and Pyrimidine Nucleotides on Aspartate Transcarbamylase in *Dictyostelium discoideum*

by

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Submitted in Partial Fulfillment of the Requirements of the

University Undergraduate Fellows Program

1976 - 1977

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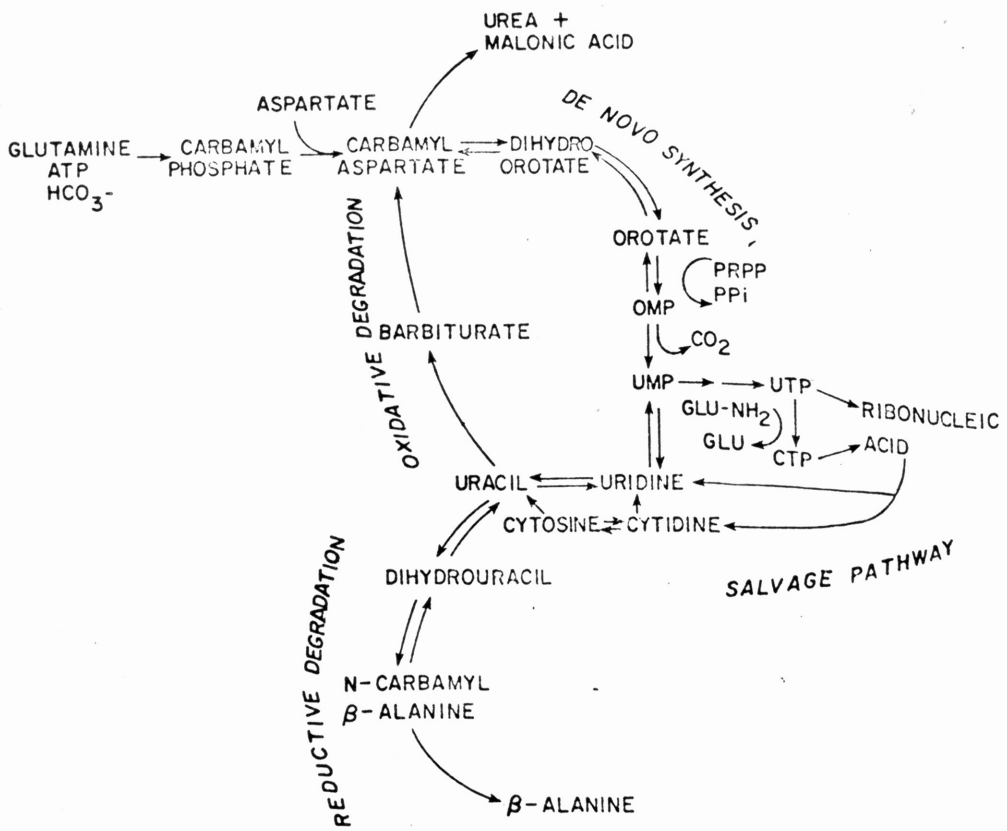
May 1977

Abstract

A key point in the regulation of pyrimidine biosynthesis by prokaryotic organisms is the enzyme Aspartate Transcarbamylase. This enzyme catalyzes the reaction of carbamyl phosphate plus aspartate to yield carbamyl aspartate, the first unique step in pyrmidine biosynthesis. While in prokaryotes ATCase appears as the main regulation factor, information on a control system for eukaryotic organisms is not well established. Studies done on Dictyostelium discoideum, a simple differentiating eukaryotic organism, have been carried out to discover what type of regulation it possesses over pyrimidine biosynthesis. ATCase enzyme assays performed using cytidine 5'-triphosphate and adenosine 5'-triphosphate resulted in no activation or inhibition of the enzymes' activity by these effectors. It appears that asparate transcarbamylase in D. discoideum is not allostericly regulated by pyrimidine or purine nucleotides.

Introduction

Pyrimidine biosynthesis of any cell, be it prokaryotic or eukaryotic must be regulated by some means to maintain the cell's metabolism at the proper level. Pyrimidine are formed through a series of intermediates from glutamine, ATP and bicarbonate as shown in the following diagram.



GENERALIZED SCHEME OF PYRIMIDINE BIOSYNTHESIS

Scheme for pyrimidine metabolism in eukaryotic cells.

Work done with prokaryotic organisms has shown that regulation for most bacteria involves the allosteric enzyme aspartate transcarbamylase. Formation of N-carbamoyl-aspartic acid from carbamoyl phosphoric acid and aspartic acid is catalyzed by ATCase. In most cases the endproduct, cytidine 5'-triphosphate, exhibits feedback control on the enzyme with adenosine 5'-

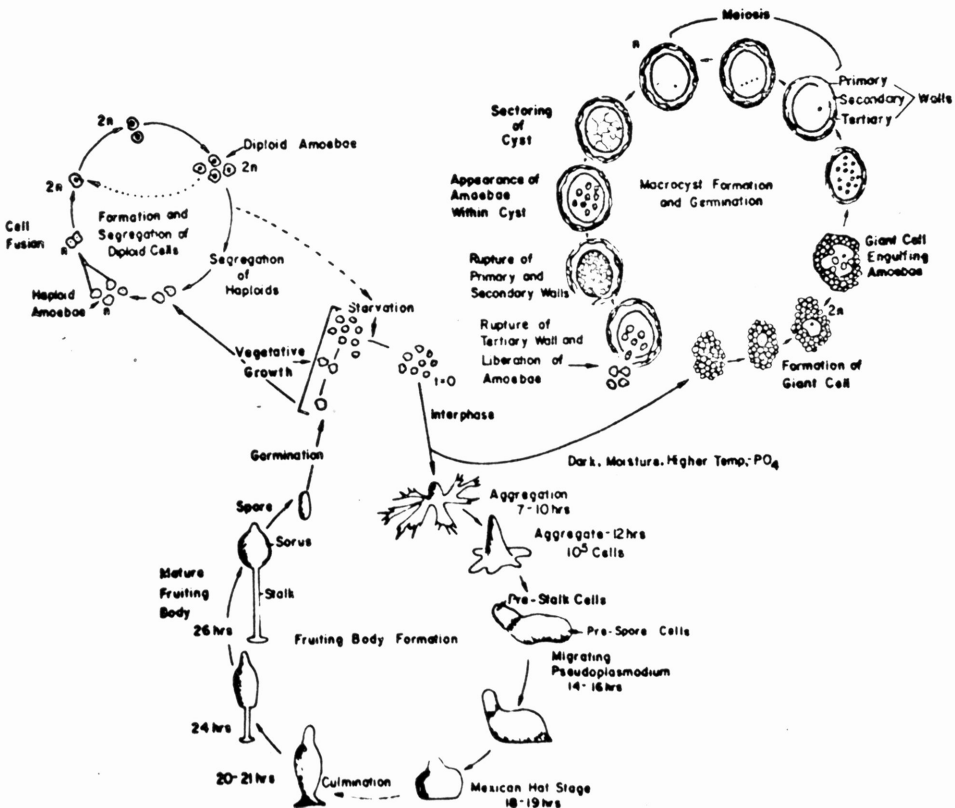
triphosphate showing activation. Three classes of bacteria have been set up on the basis of the activity, molecular weight, and allosteric effectors of aspartate transcarbamylase.

Class A, the first of these divisions, is represented by virtually all gram positive bacteria and exhibits no feedback inhibition. A protein of 100,000 daltons is present and consists of three catalytic subunits with no regulatory subunits evident. The Class B bacteria are further divided into Types I, II, III and IV, all of which show some form of allosteric regulation of ATCase. A regulatory protein of 300,000 daltons is found to be made up of six catalytic subunits and six regulatory subunits (7). The catalytic unit is composed of two trimers each weighing 100,000 and the regulatory unit consists of three dimers of 34,000 daltons each (7). In Type I, as expressed by E. coli, activity of the enzyme is increased by addition of adenosine 5'-triphosphate while inhibition of activity is seen with the addition of cytidine 5'-triphosphate. However in Type II (represented by Serratia and Proteus), CTP as well as ATP causes activation of aspartate transcarbamylase. Inhibition is seen within this group by the co-operative effect of ATP/CTP. Aeromonase and Arizona are typical of Types III and IV where no effect is seen on ATP/CTP. The last class of bacteria, Class C, contains a larger protein of 380,000 to 600,000 daltons which has not yet been separated into catalytic and regulatory subunits (1). Bacteria possessing this protein, such as Pseudomonas, show ATP activation and CTP inhibition.

Although much is known about the regulation of pyrimidines taking place in a prokaryote, the action occurring in a eukaryotic cell is not understood very well. It is doubtful that aspartate transcarbamylase is the site of regulation. Experiments with ATCase in rat cells show some degree

of inhibition by pyrimidine derivatives but only at very high levels of the nucleotides (2). Recent studies seem to point to Carbamyl Phosphate Synthetase, an enzyme found earlier in the synthesis, as the major site of feedback inhibition in eukaryotes (6). Inhibition has been seen with addition of uridine 5'-triphosphate (4) and activation of the enzyme occurs with 5-phosphoribosylpyrophosphate (5).

One problem related to eukarotic cells is that their metabolite levels charge as the cell undergoes differentiation. This makes it difficult to determine what concentrations of pyrimidines should be found at different stages in the cell's life. Dictyostelium discoideum offers a useful system for study as it undergoes a rather complex life cycle as outlined in Figure 2. During its life cycel, D. discoideum undergoes various stages in which its pyrimidine biosynthesis also must be charging. By studying pyrimidines at various periods in its life cycle one can hopefully begin to understand the workings of a differentiating eukaryotic cell. The major thrust of this experiment deals with the amoeboid stage of D. discoideum and what regulation is taking place, if any, on the activity of ATCase.



Materials and Methods

Salmonella typhimurium was run as a control along with strain AX2 of D. discoideum in performing ATCase enzyme assays. Cells of D. discoideum were grown up in 500 mls of HL-5 media described by Cocucci and Sussman (1970) (3). The cells were then harvested by centriguging at 200 rpm for five minutes and resuspending the pellet in phosphate buffer (50 mM, pH 7.5) with ZnAc and β-mercapthoethanol. Breakage was performed by sonicating the cells for thirty seconds. Breakage of cells was tried by homogenizing them with no success.

The cell free extract, after being purified, was placed into tubes containing water, postassium phosphate buffer ($0.05 \cdot 10^{-3}$ M, pH 7.0) potassium aspartate buffer ($10 \cdot 10^{-3}$ M, pH 7.0) and the appropriate effector, either ATP or CTP. The reaction was then started with the addition of carbamyl phosphate. Samples were taken at 10', 20', and 30' intervals and added to water and a color solution of antipyrine:oxime. These tubes were then incubated in a 60° water bath for two hours and finally read at 466_{nm} on a Coleman Spectrophotometer. The data from these readings was then used in determining the activity of aspartate transcarbamylase with various effectors.

Results

With the first extracts of D. discoideum purification was tried by dialysis in a mixture of 0.05 M potassium phosphate buffer, 0.2 mM $ZnSO_4 \cdot 6H_2O$ and $2 \cdot 10^{-3}$ M β-mercapthoethanol for times of up to sixteen hours. Upon

Upon completion of the assay no activity was seen. A shorter time sequence did result in activity appearing. Assuming ATCase in D. discoideum was an unstable enzyme, a column of G-25 sephedex was prepared and used for separation yielding good results. It was also found out that a higher concentration of aspartate was required to achieve activity then was allotted for in the assay procedure as set forth. A final amount twice as concentrated as called for was used in order to determine activity. The specific activity of aspartate transcarbamylase in both S. typhimurium and D. discoideum under various conditions was then determined and the results are seen Table 1.

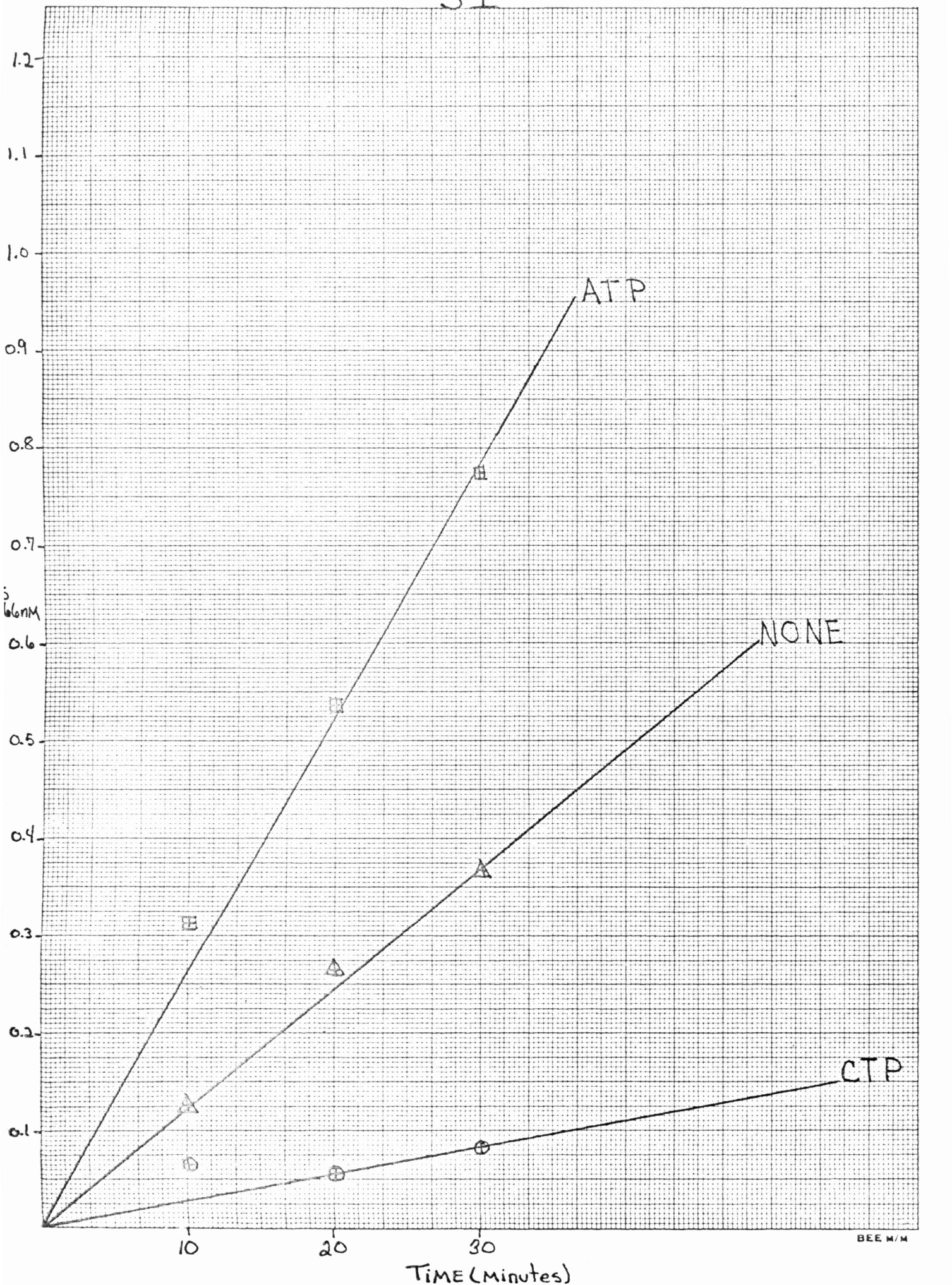
Table 1

	S1	AX2, dialyzed	AX2, sephadex
Effectors	sp. act.	sp. act.	sp. act.
None	5	9	6.25
CTP	1.06	7.05	6.75
ATP	13	8.73	6.8

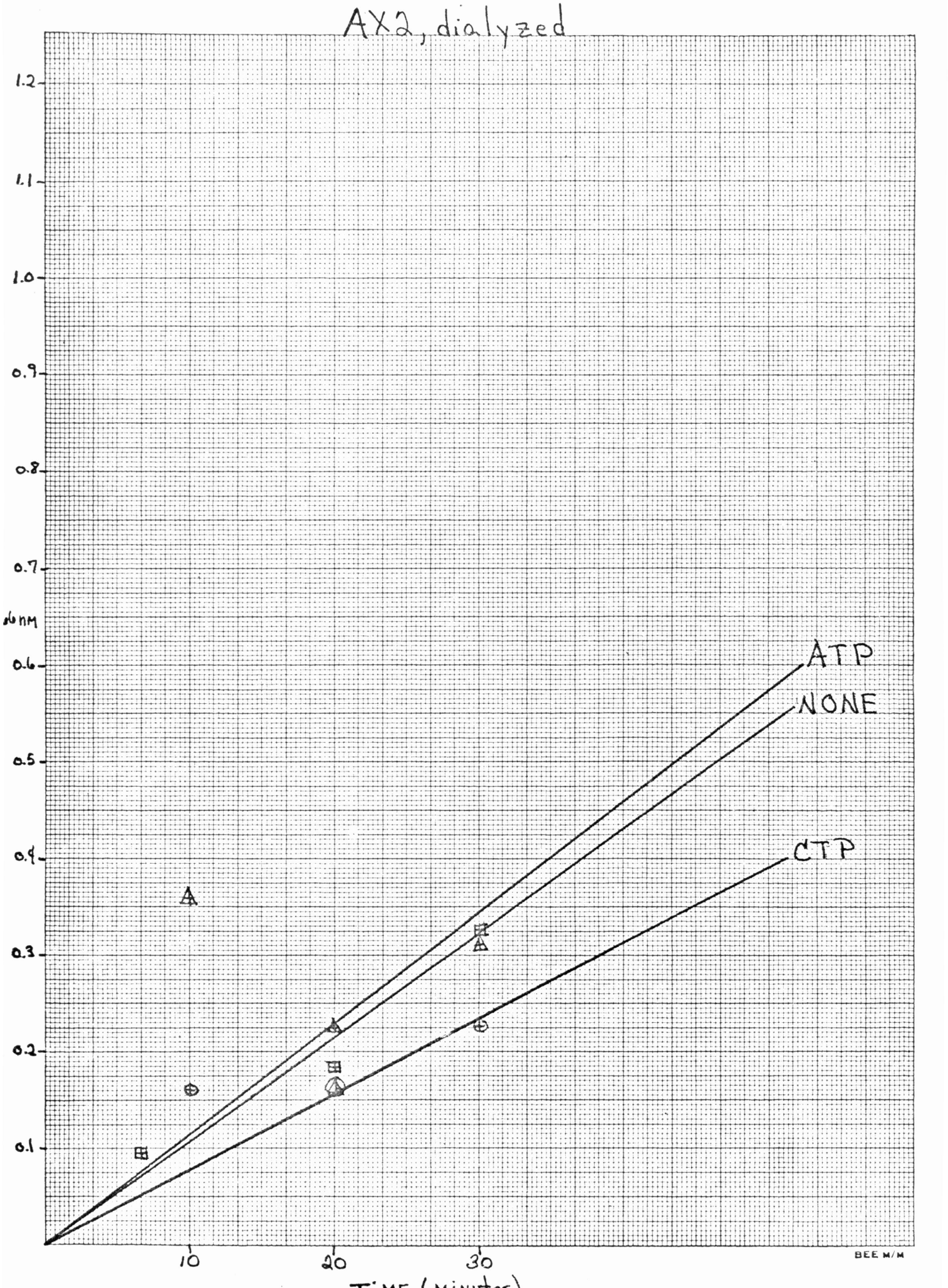
sp. act. = moles CAA/min/mg protein

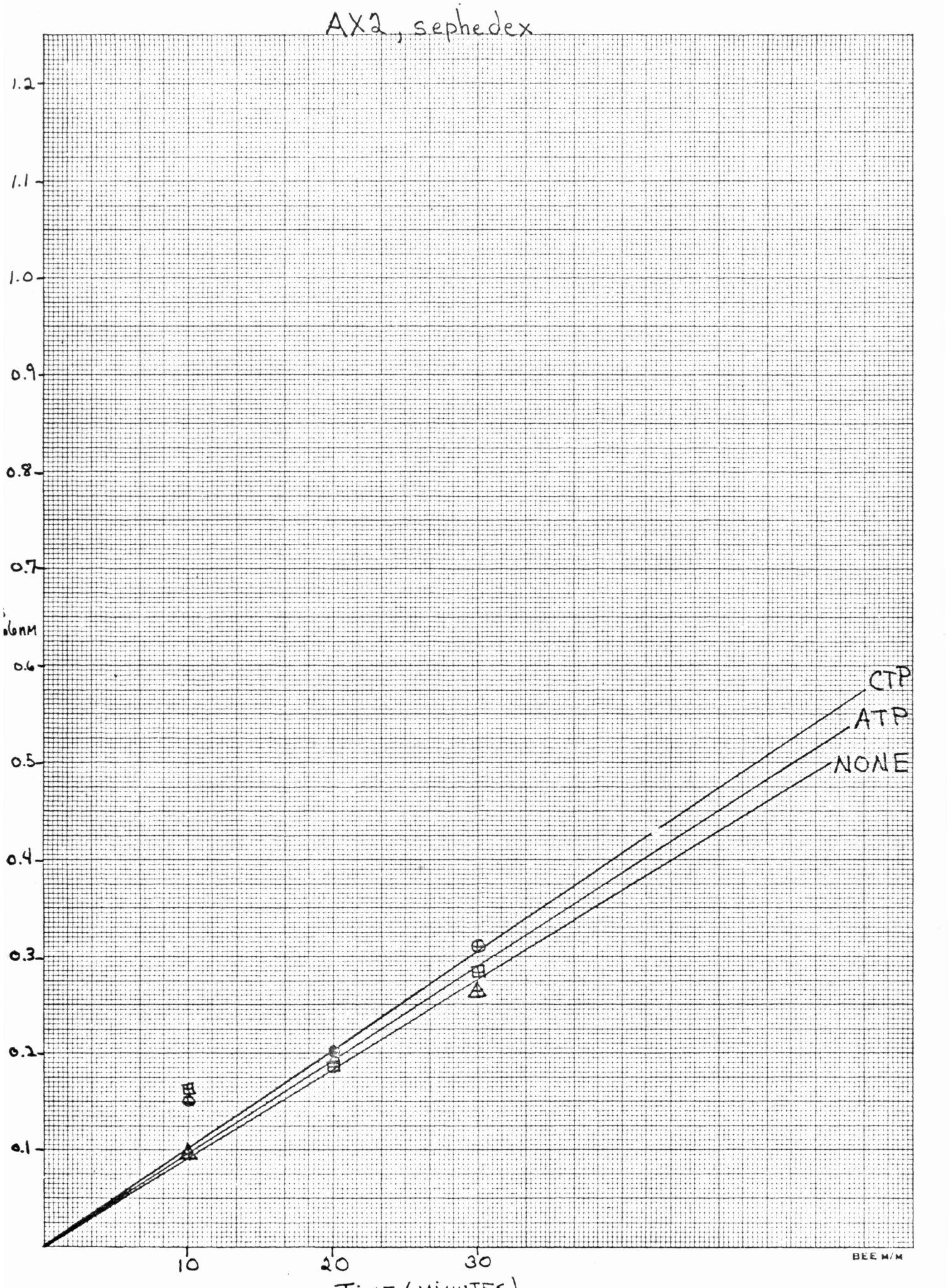
As expected S. typhimurium shows inhibition by CTP and activation by ATP. In D. discoideum neither effector seems to have any regulating effect upon the enzyme. This can best be seen by comparison of the activity graphs of S1 to those of AX2 (see following pages).

S1


1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
ATP
NONE
CTP
10
20
30
TIME (minutes)
BEE M/M

66nM


AX2, dialyzed
1.2
1.1
1.0
0.9
0.8
0.7
Δ nM
0.6
0.5
0.4
0.3
0.2
0.1
ATP
NONE
CTP
10
20
30
TIME (MINUTES)
BEE M/M


AX2, sephedex
1.2
1.1
1.0
0.9
0.8
0.7
0.6nM
0.6
0.5
0.4
0.3
0.2
0.1
CTP
ATP
NONE
10
20
30
TIME (MINUTES)
BEE M/M

Discussion

The results of this work give only a small clue as to the workings of D. discoideum's pyrimidine biosynthesis. Aspartate transcarbamylase appears to be an unstable enzyme not subject to allosteric regulation by purine or pyrimidine nucleotides. Experiments need to be done concerned with other life stages in order to see if they too follow in the same manner. It may be possible to see that at a specific stage synthesis is controlled by ATCase regulation. However, eukaryotes probably control their pyrimidine synthesis de novo by allosteri regulation of carbamyl phosphate synthetase (CPSase) and not through ATCase as in prokaryotes. Further work needs to be undertaken in which D. discoideum's CPSase enzyme activity can be determined. Once knowledge on CPSase activity and regulation at different periods in D. discoideum's life cycle is known, this information along with that of ATCase activity can be used to set up a working, functional model of pyrmidine biosynthesis in a differentiating cell.

References

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