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Formylglycinamide ribonucleotide amidotransferase in a revertant of a Chinese hamster ovary purine auxotroph : implications for a mechanism of coordinate regulation of two enzymes in the de novo biosynthesis of purines

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FORMYLGLYCINAMIDE RIBONUCLEOTIDE AMIDOTRANSFERASE IN A REVERTANT OF A CHINESE HAMSTER OVARY PURINE AUXOTROPH

RICHARD LOUIS LEFF

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Implications for a mechanism of coordinate regulation of two enzymes in the de novo biosynthesis of purines

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ABSTRACT

FORMYLGLYCINAMIDE RIBONUCLEOTIDE AMIDOTRANSFERASE IN A REVERTANT OF A CHINESE HAMSTER OVARY PURINE AUXOTROPH:

Implications for a mechanism of coordinate regulation of two enzymes in the de novo biosynthesis of purines

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ABr, a revertant of the purine-requiring Chinese hamster ovary cell line Ade$^-P_{AB}$, is examined. A probably single mutational event is responsible for Ade$^-P_{AB}$, and for its reversion (ABr). Phosphoribosylpyrophosphate amidotransferase (PRPP ATase; EC 2.4.2.14) and formylglycinamide ribonucleotide amidotransferase (FGAR ATase; EC 6.3.5.3) have altered enzyme activities in Ade$^-P_{AB}$ and ABr. Ade$^-P_{AB}$ complements neither Ade$^-$A (deficient in PRPP ATase) nor Ade$^-$B (deficient in FGAR ATase). Mixing studies results in no alterations of PRPP ATase and FGAR Atase activities. Structural changes in Ade$^-P_{AB}$ and ABr have been described which alter the apparent glutamine affinity of PRPP ATase. In this thesis, FGAR ATase in dialyzed enzyme extracts from ABr is found to possess increased sensitivity to thermal inactivation at 50°C in comparison to extracts from wild type (CHO). Extracts from ABr and CHO demonstrate equivalent FGAR ATase apparent affinity for glutamine, with apparent binding constants (K_m) of 0.54mM and 0.86mM, respectively. Glutaminase sensitivity to thermal inactivation and apparent affinity for glutamine is found to be equivalent in ABr and CHO.

Given the complementation pattern of Ade$^-P_{AB}$ and the results of mixing studies, the findings of structural alterations in both PRPP ATase and FGAR ATase are discussed. Various models within the confines of

established theory do not explain the properties of Ade$^-$P$_{AB}$ and ABr. Another possible model presented involves the separation of these two enzymes from a single precursor. However, the recent mapping of PRPP ATase and FGAR ATase to different human chromosomes makes this theory less likely.

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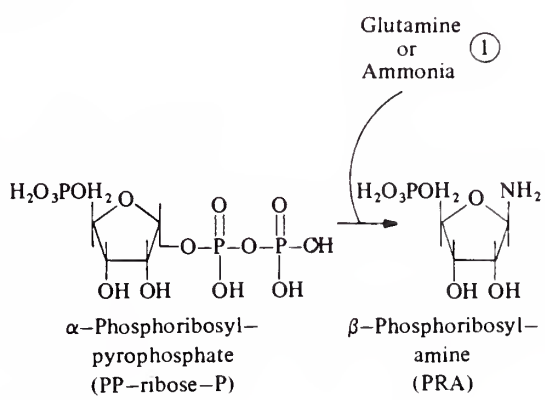
INTRODUCTION

The two enzymes of the purine de novo biosynthetic pathway classified as amidotransferases are phosphoribosylpyrophosphate amidotransferase (PRPP ATase, EC 2.4.2.14) and formylglycinamide ribonucleotide amidotransferase (FGAR ATase, EC 6.3.5.3). As amidotransferases, both enzymes catalyze the formation of a carbon-nitrogen bond utilizing glutamine as a nitrogen source. Additionally, both enzymes possess aminotransferase activity which substitutes ammonia for glutamine as the nitrogen donor. Chinese hamster ovary cell (CHO-K1) mutants have been isolated with altered PRPP ATase and FGAR ATase activities (1). A brief review of these two enzymes will be followed by a description of the mutant CHO cell lines studied in this paper.

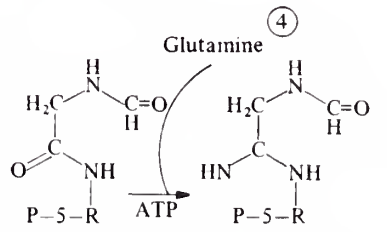
PRPP ATase catalyzes the formation of phosphoribosylamine (PRA) from phosphoribosylpyrophosphate (PRPP) and glutamine (or ammonia)(2). This



first committed step in the de novo synthesis of purines is a logical focus for the regulation of the enzymatic pathway. The complex regulation of de novo purine synthesis involves many factors including substrate availability, end product feedback inhibition, and enzyme stability (2-13).

The recent purification of PRPP ATase from a human source (14) has confirmed previous work on impure enzyme preparations. PRPP ATase is composed of 4-5 homogeneous subunits forming an active enzyme with a molecular weight of 133,000 daltons (14-17). The amidotransferase and aminotransferase activities copurify indicating that both activities are contained in one enzyme (14). The oxygen-sensitive and iron-sulfur components of PRPP ATase are necessary for mammalian enzymatic activity (18), but may not be directly involved with the active sites (19-21). PRPP ATase possesses separate binding sites for the substrates glutamine and PRPP, and for purine nucleotide feedback inhibitors (4,7,11,12,22).

FGAR ATase is the fourth enzyme in the de novo purine synthetic pathway. It catalyzes the formation of formylglycinamidine (FGAM) from formylglycinamide (FGAR) and glutamine (or ammonia) and the hydrolysis of



Phosphoribosyl–formylglycinamide (FGAR)

Phosphoribosyl–formylglycinamidine (FGAM)

ATP (2). FGAR ATase has been purified from avian liver as a single polypeptide with a molecular weight of 133,000 daltons (23). Separate sites for the binding of glutamine and an FGAR-Mg^{+2} complex have been characterized (24-28). FGAR ATase is not an iron-sulfur containing enzyme (23) as is PRPP ATase from avian and mammalian sources (18). In vitro studies reveal that FGAR ATase ammonia-dependent activity is much lower than its glutamine-dependent activity (24). This differs

from that found for PRPP ATase activity *in vitro* (1).

Several purine auxotrophic CHO cell lines, each deficient in an enzyme of the *de novo* purine synthetic pathway, have been characterized (29-33). Recently, Oates, Vannais and Patterson (1) described an interesting purine auxotroph. This cell line (Ade^-P_{AB}) was found to complement all the purine auxotrophic cell lines except those deficient in either PRPP ATase (Ade^-A) or FGAR ATase (Ade^-B). Further analysis revealed a loss of measurable FGAR ATase activity. Additionally, a virtual loss of measurable glutamine-utilizing PRPP ATase activity with an almost normal ammonia-utilizing PRPP ATase activity was identified. Mixing studies on cell-free extracts from Ade^-A, Ade^-P_{AB} and CHO-K1 produced no changes in the level of the separate PRPP ATase activities. Similarly, combinations of cell-free extracts from Ade^-B, Ade^-P_{AB} and CHO-K1 were not altered in the level of FGAR ATase activity (1).

Ade^-P_{AB} cell lines were isolated from CHO-K1 using an ethyl methanesulphonate and BUdR-visible light procedure which usually produces point mutations (34). The alteration in both PRPP ATase and FGAR ATase in Ade^-P_{AB} can be easily explained by theorizing two mutational events; however, several properties of Ade^-P_{AB} argue against this possibility. First, the frequency of isolation of Ade^-P_{AB} is approximately that for either Ade^-A or Ade^-B. If Ade^-P_{AB} was the result of two independent events, then one would expect it to occur at a much lower rate than either Ade^-A or Ade^-B. Second, revertants of Ade^-P_{AB} (ABr) are not infrequently isolated. The final argument of Oates, Vannais and Patterson is the lack of a viable complement between Ade^-A (with normal FGAR ATase) and Ade^-P_{AB} (with near normal ammonia-dependent PRPP ATase), which should resemble ABr in PRPP ATase and FGAR ATase activities

(described below). These results are not easily explained in the context of the enzyme levels of PRPP ATase and FGAR ATase for ABr. Oates, Vannais and Patterson (1) therefore conclude that this implies a more complicated basis for Ade$^-P_{AB}$ than two distinct mutational events.

ABr, isolated in more than 30 independent reversions of Ade$^-P_{AB}$ (1), regains normal FGAR ATase activity, and retains near normal ammonia-dependent PRPP ATase, while possessing only minimal glutamine-dependent PRPP ATase activity. Further investigation by Holmes (personal communication) demonstrates the apparent decrease in (glutamine-dependent) PRPP ATase activity in ABr as being the result of a decrease in apparent affinity for glutamine. Additionally, the virtual loss of glutamine-dependent PRPP ATase activity in Ade$^-P_{AB}$ is due to a marked decrease in apparent affinity for glutamine of several orders of magnitude. Ade$^-P_{AB}$ does not appear to have altered apparent binding constant for PRPP or molecular weight as judged by sucrose density gradient (personal communications from Holmes). Since ABr does indeed possess some glutamine-dependent PRPP ATase activity whereas Ade$^-P_{AB}$ has virtually none, these findings clarify the previously mentioned lack of complementation between Ade$^-$A and Ade$^-P_{AB}$. Additionally, PRPP ATase in Ade$^-P_{AB}$ and in ABr is found to have greatly increased sensitivity to thermal inactivation (personal communication from Holmes). Therefore, these data suggest a structural mutation in PRPP ATase which causes a severe defect in Ade$^-P_{AB}$ and only a moderate change in ABr.

Studies on the growth rate of ABr in various media are consistent with the altered affinity for glutamine by PRPP ATase. In purine-free media, ABr has a slower rate of growth than CHO, with a doubling time of 20.6 hrs versus 12.5 hrs, respectively. However, in media supplemented

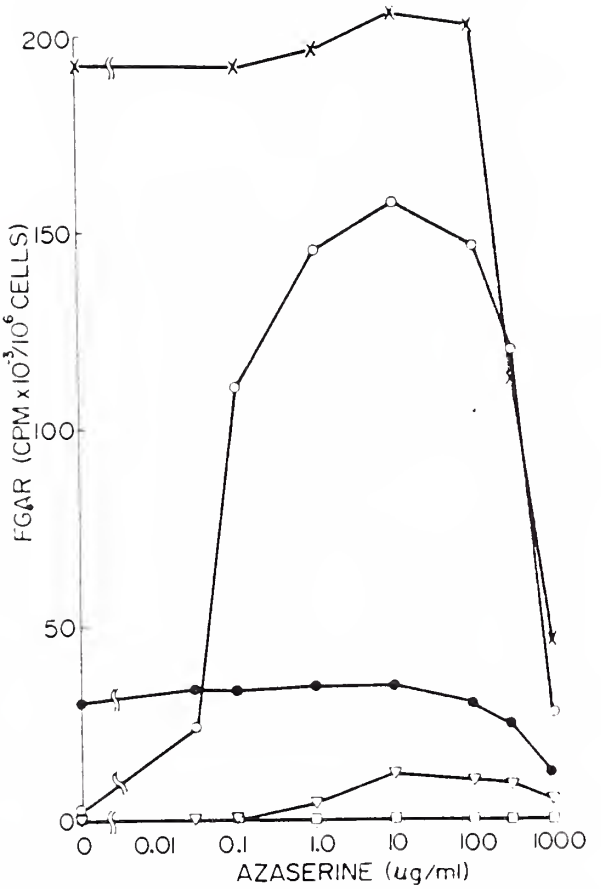
with hypoxanthine, ABr approaches wild-type growth rates (1). Additionally, all media contain some added glutamine since CHO-K1 cell lines are auxotrophic for glutamine. Supplementation of purine-free media with 10 times the normal level of glutamine increases the growth rate of ABr to near normal levels (personal communication from Holmes). These results imply that the decreased rate of de novo synthesis of purines in ABr can be accelerated by increasing the concentration of glutamine. These growth rate and enzyme activity studies of ABr indirectly demonstrate the importance of glutamine-dependent PRPP ATase activity in the rate of de novo purine synthesis.

Accumulation of FGAR and formation of completed purines by wild-type, purine auxotrophic mutants, and revertant cell lines are direct methods employed to examine the rate of de novo purine biosynthesis (1). Oates, et al incubated cells with ^{14}C-formate in purine-free media supplemented with either glutamine or ammonia to measure FGAR and completed purine synthesis. The results of Oates, et al (1) on formation of completed purines expressed as cpm/10^6 cells $\pm$ 100μg/ml azaserine (Aza) are:

	CHO-K1	Ade$^-$A	Ade$^-$B	Ade$^-$P$_{AB}$	ABr
Glutamine (1mM)	74,113	<100	<100	1,414	10,582
Gln (1mM) + Aza	1,056	<100	<100	<100	156
NH_4Cl (10mM)	32,947	<100	<100	826	17,873
NH_4Cl (10mM) + Aza	3,502	<100	<100	100	1,240

The addition of azaserine, a glutamine analog, inhibited glutamine-dependent FGAR ATase activity at much lower concentrations than it affected glutamine-dependent PRPP ATase activity, as seen in the figures from Oates, et al presented below (1).

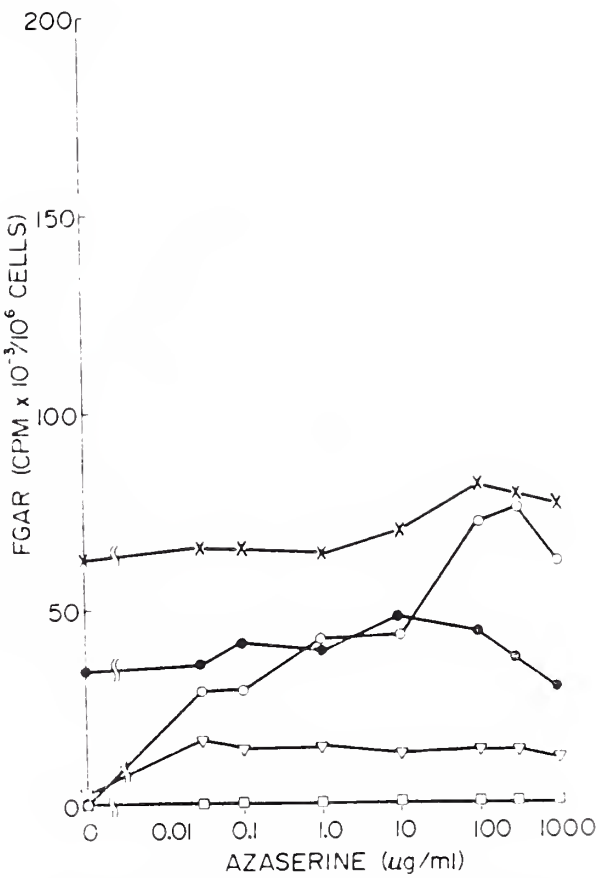
Glutamine



The Effect of Azaserine Concentration on Accumulation of Phosphoribosylformylglycinamide (FGAR) in Whole Cells Incubated with 1 mM Glutamine

(⊙—⊙) CHO-K1; (□—□) Ade A; (× ··· ×) Ade B; (● —●) Ade P_{AB}; (▽—▽) ABr34

Ammonia



The Effect of Azaserine Concentration on Accumulation of FGAR in Whole Cells Incubated with 10 mM NH_4Cl

(⊙—⊙) CHO-K1; (□—□) Ade A; (× ··· ×) Ade B; (● —●) Ade P_{AB}; (▽ ··· ▽) ABr34.

The results of the studies on FGAR accumulation and completed purine formation for Ade⁻A, Ade⁻B and CHO were as expected for cell lines possessing deficient PRPP ATase activity, deficient FGAR ATase activity, and wild-type enzymatic activities, respectively. Ade⁻P_{AB} accumulated FGAR at a constant low rate in the presence of either glutamine or ammonia. This is consistent with the absence of FGAR ATase in Ade⁻P_{AB}. The rate of FGAR accumulation is reduced at higher concentrations of azaserine (>100 μg/ml), consistent with inhibition of PRPP ATase activity. Conversely, in ABr azaserine concentrations that lead to the accumulation

of FGAR are consistent with the inhibition of FGAR ATase. The rate of production of FGAR is decreased in ABr due to decreased PRPP ATase activity (1). Therefore, only when FGAR ATase activity is sufficiently inhibited will FGAR accumulate in ABr.

These *in vivo* studies represent attempts to determine the significance of the mutations in Ade$^-P_{AB}$ (and in ABr) for *de novo* purine biosynthesis. The combined results on enzymatic activity, PRPP ATase thermal inactivation, apparent affinity for glutamine by PRPP ATase, accumulation of FGAR, and formation of completed purines (1, and personal communication from Holmes) are suggestive of a single mutational event affecting two distinct glutamine-utilizing enzymes. Increased sensitivity to thermal inactivation is indirect evidence in Ade$^-P_{AB}$ for a structural alteration in PRPP ATase. Data on FGAR ATase show a loss of measurable activity in Ade$^-P_{AB}$, with a virtually complete recovery of activity in ABr.

The following is a brief summary in tabular form of the findings on Ade$^-P_{AB}$ and ABr for PRPP ATase and FGAR ATase activities (1, and personal communication from Holmes).

Enzyme	Property	Ade$^-P_{AB}$	ABr
PRPP ATase	ammonia-dependent activity	+	+
	glutamine-dependent activity	++	+
	apparent glutamine affinity	++	+
	thermal stability	++	+
FGAR ATase	ammonia-dependent activity	o	+
	glutamine-dependent activity	o	+
	apparent glutamine affinity	?	?
	thermal stability	?	?

+ = near wild type level
↓ = decreased from wild type level
↓↓ = markedly decreased from wild type level
o = no measurable activity
? = unknown

The research described in this thesis entails the evaluation of FGAR ATase in ABr for alterations in structure and enzymatic properties. Specifically, FGAR ATase is examined for sensitivity to thermal inactivation and for apparent glutamine affinity. Since Ade$^-P_{AB}$ contains no measurable FGAR ATase enzymatic activity, these studies are performed on ABr. Observations by Holmes (personal communication from Holmes) on the apparent glutamine Km and the thermal inactivation of PRPP ATase in Ade$^-P_{AB}$ indicate that FGAR ATase in ABr might exhibit some alterations not examined by Oates, et al (1). In conjunction with past observations, the results on FGAR ATase can provide some insight for the genetic basis responsible for the altered enzymatic profilesof Ade$^-P_{AB}$ and ABr.

EXPERIMENTAL PROCEDURES

Materials

^{14}C-Glutamine (50mCi/mmole) and ^{14}C-glycine (1.4mCi/mmole) were obtained from New England Nuclear. One preparation of chicken liver acetone powder was obtained from Sigma. Another preparation was from chicken liver obtained from Gold Kist, Durham, N.C. and extracted with acetone as described in Methods of Enzymology (35). PRPP, ATP and ribose-5-phosphate were obtained from Sigma. All other chemicals were of the highest grade commercially available.

Cell Lines

Wild type CHO-K1 (CHO), Ade-P_{AB} revertant (ABr20), and azaserine-resistant (ASr) cell lines were generously supplied by Dr. David Patterson. Characterization and isolation of ABr20 beyond that described in the introduction can be found in the paper of Oates, Vannais and Patterson (1).

Cell lines were maintained under sterile conditions with Ham's F-12 medium plus dialyzed fetal calf serum (5%) supplemented with glutamine and nonessential amino acids. Fetal calf serum was dialyzed against 1000x volume of normal saline for 24 hours three times. Cultures were maintained at 37°C under 5% CO_2 and added moisture. Dialyzed culture medium, pH 7.4, was changed daily. Cultures were checked daily for infection and confluence. Cells were split 1:3 - 1:4 at 2-4 day intervals when confluent monolayers were observed. Cultures were kept in Petri dishes or roller bottles for increased production. Cells were split 1-2 days prior to harvesting and were harvested prior to confluence to insure rapid cell growth rates. This ensured maximal yield of the enzymes of the purine *de novo* biosynthetic pathway.

Enzyme Extraction

Cell-free extracts were prepared as follows. Cells were first dissociated with trypsin (1 mg/ml). Cells were then concentrated by centrifugation to $2x10^8$ cells/ml of phosphate buffered saline (containing the following in mmol/liter; NaCl 137, KCl 2.7, Na_2HPO_4 8.1, KH_2PO_4 0.62, dextrose 5.56, and chloroform 3.35). Cell disruption was by freeze-thawing three times with a dry ice-acetone bath. Centrifugation to remove cellular debris produced a supernatant of an "enzyme extract" which was then stored in small aliquots at -70°C. On the day of use enzyme extract was dialyzed against 1000 volumes of Tris buffer (50mM pH=7.4) for 2 hours at 4°C.

Protein concentration for enzyme extracts was determined by the method of Lowry, et al (36) modified to correct for the Tris buffer. Bovine serum albumin was used as a standard.

Preparation of FGAR

Commercial preparations of FGAR are not available. FGAR was synthesized using the enzymes of the purine de novo pathway from chicken liver acetone powder (37). FGAR ATase activity was blocked with azaserine as described in Methods of Enzymology (37). Serial dilutions of FGAR were used to determine that sufficient FGAR was available to maintain optimal enzyme activity for 45 minutes in the FGAR ATase activity assay described below (data not shown).

FGAR ATase Activity Assay

FGAR ATase activity was determined by measuring the FGAR-dependent conversion of radioactive glutamine to radioactive glutamate. For each experimental sample with FGAR present, a control was done with Tris buffer replacing FGAR. All assays were performed in duplicate. Assay mixtures of 50 microliters contained: Tris 50mM pH=7.4, $MgCl_2$ 10mM, KCl

100mM, ATP 10mM, ^{14}C-glutamine 4mM (specific activity 0.42mCi/mmole), FGAR solution (5 microliters), and dialyzed enzyme extract (25 microliters). Although FGAR concentration was not known, preliminary experiments demonstrated that this substrate was not limiting in this assay. All solutions were kept at 4°C until incubation at 37°C for 45 minutes unless otherwise specified. The reaction was stopped by immersion in an ice-water bath at 4°C.

Electrophoresis on Whatman 3MM paper was promptly carried out. Ten microliters of a carrier solution of glutamine (4 mg/ml) and glutamate (4 mg/ml) was applied and dried prior to applying 15 microliters of assay mixture. Electrophoresis in borate buffer (50mM) with 2000 volts at 250 mAmps for 35 minutes was sufficient to separate glutamine and glutamate. The glutamate spot was identified with ninhydrin spray and cut out. Radioactivity was counted in a solution of PPO/POPOP (15.12 gm 2,5-Diphenyloxazole/378 mg P-bis[2-(5-Phenyloxazoyl)]-benzene/8 pints toluene) for 10 minutes with 60% efficiency.

The assay for FGAR ATase is linear with time (to 60 minutes) and protein concentration (data not shown).

Determination of Apparent Glutamine Affinity of FGAR ATase and Glutaminase

Glutamine concentrations were varied from 0.167mM to 4mM while all other assays conditions were held constant. Measurements without FGAR in the reaction mixtures were subtracted from each result. This difference determined the FGAR ATase activity. Glutaminase activity is measured by the conversion of glutamine to glutamate without FGAR present and is linear with time and protein concentration.

Thermal Inactivation of FGAR ATase and Glutamine

Following dialysis as described in Enzyme Extraction, the enzyme

extract was heated to 50°C in a water bath for variable lengths of time. The enzyme extract for each time point was heated in a single test tube and then aliquoted for duplicate assays. Enzyme extract was constantly kept at 4°C on an ice-water bath while being handled. Maximal enzyme activity was measured by maintaining a sample of enzyme extract at 4°C during the period of inactivation. Glutaminase activity was determined in controls without FGAR present.

Sucrose Density Gradient Study

PRPP ATase is known to exist in two forms (15). In the presence of PRPP a 133,000 dalton form exists, while in the presence of AMP a 270,000 dalton form predominates. In an attempt to determine an association between PRPP ATase and FGAR ATase, enzyme extracts with either PRPP or AMP present were applied to sucrose gradients and fractions assayed for FGAR ATase activity.

Enzyme extract from the ABr cell line was prepared as described in Enzyme Extraction and used on the same day. Separate aliquots of the dialyzed extract were incubated at 37° for 15 minutes with either 5.0mM AMP or 0.5mM PRPP. Each aliquot (200λ) was then placed on 11.6 ml of a sucrose density gradient of 10% to 28.2% with potassium phosphate buffer (50mM KPi pH=7.4, 15mM β-mercaptoethanol, and 5mM $MgCl_2$) and with either 5.0mM AMP or 0.5mM PRPP. Centrifugation at 100,000 x g was carried out for 16 hours in a 1.1 cm diameter tube. Fractions of 0.2 ml were collected and enzyme activity measured over a 60 minute incubation period at 37°C as previously described, except for the use of 32.5λ of extract and 17.5λ of reaction mixture. All reaction concentrations were otherwise unchanged from that previously described in the FGAR ATase activity assay.

RESULTS

Properties of the Enzyme Extract

Table 1 contains data comparing enzyme extracts from CHO and from ABr. These data are compiled from multiple experiments, including some not presented for the thermal inactivation and glutamine affinity studies. Overall, the enzyme extracts of ABr minimally differ by 1 to 1.5 standard deviations from the extracts of CHO in average specific activity and protein concentration. Protein concentration does vary significantly ($p < 0.05$); however, the 3 mg/ml (20%) difference between ABr and CHO is not large. Therefore, the actual enzyme extraction procedure produces equivalent preparations for ABr and CHO.

Thermal Inactivation Studies

Structural changes in enzymes can sometimes be indirectly demonstrated by an increased sensitivity to thermal degradation (1). PRPP ATase from ABr is more sensitive to inactivation at 37°C than that from CHO (personal communication from Holmes). FGAR ATase is relatively stable at 37°C (data not shown), but following dialysis it is rapidly inactivated at 50°C. The $T_{1/2}$ of FGAR ATase activity at 50°C in dialyzed extracts of ABr is half that of CHO, with decay constants of 0.9 minutes and 1.7 minutes, respectively (Figure 1). The data shown in Fig. 1 represent the combined results of two thermal inactivation experiments.

For the thermal inactivation studies, when compared to CHO enzyme extracts, those from ABr have a slightly greater FGAR ATase specific activity (13.8 nmol/mg protein/hr versus 10.1 nmol/mg protein/hr) and a slightly greater protein concentration (18.4 mg/ml versus 15.6 mg/ml). The differences between these parameters for the enzyme preparations of ABr and CHO are small in comparison to the nearly two-fold alteration in

the rate of inactivation of FGAR ATase at 50°C.

Additionally, glutaminase from both ABr and CHO decays to 50% of the original activity in approximately 3 minutes (Figure 2). Therefore, the rate of inactivation of glutaminase is essentially the same for ABr and CHO. These results on glutaminase demonstrate that under the conditions of this study ABr possesses an enzymatic activity which is virtually unaltered in its sensitivity to thermal inactivation. This is especially important to note when compared to the results demonstrating a two-fold difference between ABr and CHO in the thermal inactivation of FGAR ATase (Figures 1 and 2).

Apparent Affinity for Glutamine

The apparent affinity for glutamine of PRPP ATase is severely decreased in Ade$^-$ P_{AB} and only moderately affected in ABr (personal communication from Holmes). Figure 3 demonstrates the apparent affinity for glutamine of FGAR ATase from ABr as being similar to that from CHO. The apparent K_m for glutamine of ABr and CHO are 0.54mM and 0.86mM, respectively, with the least-squares plots having correlation coefficients of at least 0.86. These results are comparable to that previously found for the apparent glutamine K_m of FGAR ATase from avian liver, 0.2mM to 0.62mM (27,28) and from Ehrlich ascites tumor cells, 0.11mM (24). Least-squares analysis of a Michaelis-Menten plot in Cartesian coordinates for the data in Figure 3 yield large standard errors for the apparent glutamine K_m of FGAR ATase (mean standard error, T_S^2 = 0.41). Therefore, least-squares analysis of the data in the reciprocal plot (as in Figure 3) is the method employed throughout this paper. As demonstrated by the overlap on Figure 3, little difference is seen in the extrapolated values for the apparent glutamine K_m of FGAR

ATase from ABr and CHO.

The enzyme extracts from ABr and CHO for the study of apparent glutamine affinity of FGAR ATase possess approximately equal activities, under assay conditions, 18.1 nmol/mg protein/hr versus 18.6 nmol/mg protein/hr, respectively. Extrapolation of the data in Figure 3 yields FGAR ATase V_{max} for ABr and CHO of 22.2 nmol/mg protein/hr and 30.0 nmol/mg protein/hr, respectively. The slightly lower V_{max} of FGAR ATase from ABr, 74% of the wild-type level, is a small change given the complete absence of FGAR ATase activity in Ade$^-P_{AB}$. ABr and CHO enzyme extracts are also approximately equal in protein concentrations, 11.5 mg/ml and 13.4 mg/ml, respectively. Therefore, the enzyme extracts do not greatly differ for these parameters between ABr and CHO in this study.

As a further control the apparent glutamine affinity of glutaminase activity in ABr and CHO are virtually equal, 0.25mM and 0.30mM, respectively (Figure 4). These least-squares plots also demonstrate a slightly lower V_{max} for ABr in comparison to CHO, 15.8 nmol/mg protein/hr versus 21.8 nmol/mg protein/hr, respectively. The high values of the correlation coefficients for these results (at least 0.95) are due to the calculation of glutaminase activity from the measurement of one radioactive sample. Whereas FGAR ATase activity is the difference between two samples (one with and one without FGAR), glutaminase activity is only the result of one sample (without FGAR present). Therefore, much less variation is present in the plots for glutaminase (Figure 4) than in the plots for FGAR ATase (Figure 3).

Additionally, in ABr the slightly greater apparent affinity for glutamine (lower apparent K_m) of glutaminase parallels that of FGAR

ATase. This tends to minimize the slight difference in apparent glutamine affinity of FGAR ATase, since a similar finding is demonstrated for glutaminase. Therefore, the result that enzyme extracts from ABr are equivalent to those from CHO in apparent glutamine affinity of FGAR ATase can be made with greater certainty.

Other Results

Preliminary studies were performed on a sucrose density gradient for ABr and on enzyme dilutions for an azaserine-resistant cell line (ASr). These results are briefly presented below without the actual data.

FGAR ATase retains activity in conditions of a sucrose density gradient. When applied to a sucrose density gradient, insufficient FGAR ATase activity is present to be accurately distinguished from background. Therefore, the determination of any multienzyme complex with PRPP ATase is not practical with the crude dialyzed enzyme extract in use.

Isolation of a cell line which overproduces FGAR ATase would have aided in the sucrose density gradient studies. Since azaserine is a potent inhibitor of FGAR ATase, ASr was thought to be a possible overproducer of FGAR ATase. ASr cells are resistant to growth inhibition at 1μg/ml concentration of azaserine was assayed for FGAR ATase enzyme activity. FGAR ATase is inhibited at azaserine concentrations of 0.1μg/ml or less. Serial dilutions (1:2, 1:5, 1:10) of enzyme extracts from ASr and CHO possess comparable activities. Therefore, ASr does not appear to be an overproducer of FGAR ATase.

DISCUSSION

Alterations of PRPP ATase and FGAR ATase in Ade$^-P_{AB}$ might be regulatory or structural in their nature. In Aerobacter aerogenes both PRPP ATase and FGAR ATase are coordinately repressed and derepressed (38). However, research has identified structural alterations in both enzymes for Ade$^-P_{AB}$. The structural change in PRPP ATase involves the utilization and binding of glutamine. Glutamine-dependent PRPP ATase in Ade$^-P_{AB}$ has markedly decreased affinity for glutamine while in ABr it is only moderately decreased (1, personal communication). Additionally, increased sensitivity to thermal inactivation of PRPP ATase in Ade$^-P_{AB}$ and ABr, indirectly demonstrating structural alteration, has been found (personal communication from Holmes).

FGAR ATase activity lost in Ade$^-P_{AB}$ is almost completely recovered in ABr (1). The results of experiments on FGAR ATase from ABr demonstrate an increased sensitivity to thermal inactivation (Figure 1). This is not unusual in that increased sensitivity to thermal inactivation is found to be common among revertant cell lines (1). The most likely explanation for this increased sensitivity is a structural change(s) in FGAR ATase from ABr.

Other possible causes of the increased sensitivity to thermal inactivation involve an altered intracellular environment which offers less protection to FGAR ATase in crude dialyzed enzyme extracts from ABr. Diffusible, low molecular weight compounds are removed by dialysis. This includes glutamine, potassium, FGAR, magnesium, and ATP which have been shown to increase the stability of FGAR ATase (23,39). A non-diffusible glutamine-enzyme complex for FGAR ATase with increased stability at 50°C has also been described (23,25,39). The presence of any glutamine-enzyme

complex cannot be totally eliminated from having a potential effect upon the measured rates of thermal inactivation. However, the studies on FGAR ATase affinity for glutamine (Figure 3) demonstrate only a minimal difference between ABr and CHO, with binding constants of 0.54mM and 0.86mM, respectively. Any protection from a greater amount of glutamine-enzyme complex in ABr (due to increased glutamine affinity) would be expected to increase the thermal stability of FGAR ATase. Since just the opposite is observed, it is unlikely that a glutamine-enzyme complex can be responsible for the increased sensitivity to thermal inactivation of FGAR ATase from ABr.

The decreased thermal stability in ABr (and Ade$^-P_{AB}$) might be due to altered transport of glutamine. CHO-K1 cells are auxotrophic for glutamine. Then, although the media for CHO and ABr contain the same concentration of glutamine, the intracellular concentration of glutamine could be lower in ABr than in CHO. Lowered intracellular glutamine concentration might then lead to less protection of FGAR ATase by decreasing the amount of glutamine-enzyme complex. This is felt to be unlikely in that structural changes in PRPP ATase from Ade$^-P_{AB}$ and ABr have been documented (1, and personal communication from Holmes). Additionally, loss of measurable FGAR ATase activity in Ade$^-P_{AB}$ is well-established (1). Therefore, decreased glutamine transport would involve yet a third alteration in CHO to produce Ade$^-P_{AB}$ from a probable single mutation.

As a further control, glutaminase was examined for thermal sensitivity and apparent glutamine affinity. The rate of inactivation of glutaminase at 50°C is the same for ABr and CHO (Figure 2). This finding serves to emphasize the two-fold difference found for the thermal inactivation of

FGAR ATase between ABr and CHO (Figure 1). Conversely, the slightly greater apparent glutamine affinity of glutaminase from ABr (Figure 4) minimizes the slightly greater apparent glutamine affinity of FGAR ATase from ABr (Figure 3). Therefore, the increased thermal inactivation of FGAR ATase from ABr is indirect proof of some structural alteration, and most likely is not due to decreased glutamine-enzyme complex from either decreased apparent glutamine affinity or decreased amounts of intracellular glutamine.

The evaluation of Ade$^-P_{AB}$ as a result of a single mutational event producing structural changes in two enzymes will be elaborated on later. It is of interest to first point out an important conclusion that may be drawn from the preceding investigations of Oates, et al (1) and E.W. Holmes (personal communication). As mentioned in the Introduction, Ade$^-P_{AB}$ retains only measurable ammonia-dependent PRPP ATase activity (1), while ABr also possesses some measurable glutamine-dependent PRPP ATase activity. Additionally, both Ade$^-P_{AB}$ and ABr have normal ribose-5-phosphate aminotransferase which converts ribose-5-phosphate and ammonia to phosphoribosylamine (PRA) (1). However, in complementation of Ade$^-P_{AB}$ and Ade$^-$A (with normal FGAR ATase activity), neither ammonia-dependent PRPP ATase nor ribose-5-phosphate aminotransferase form sufficient PRA *in vivo* to sustain cellular function. Therefore, in this mammalian system the *de novo* synthesis of purines requires glutamine-dependent PRPP ATase activity. This conclusion is further supported by finding that increased concentrations of glutamine raises the growth rate of ABr to wild-type levels (CHO-K1 is a glutamine auxotroph), thereby overcoming the decreased apparent affinity for glutamine by PRPP ATase in ABr (personal communication from Holmes).

It should also be mentioned that Ade$^-$P$_{AB}$ represents a separation of glutamine and ammonia-dependent PRPP ATase activities. Glutamine-dependent activity is virtually lost in Ade$^-$P$_{AB}$ due to a marked decrease in apparent affinity for glutamine (personal communication), while ammonia-dependent activity is almost normal (1). There are some reports of separating these enzymatic activities in partially purified preparations (40). However, single mutational events were clearly shown to form cell lines deficient in both glutamine and ammonia-dependent PRPP ATase (30,31,33). Additionally, purified human PRPP ATase has recently been found to maintain a constant ratio of glutamine-dependent to ammonia-dependent activities (14). Therefore, the enzyme alterations in Ade$^-$P$_{AB}$ which keep the ammonia-dependent PRPP ATase activity basically intact and markedly decreases the apparent glutamine affinity of (glutamine-dependent) PRPP ATase activity imply a more complicated explanation than if both activities were simultaneously altered.

Before presenting various explanations to account for the enzymatic characteristics of Ade$^-$P$_{AB}$, I will briefly summarize some of the relevant information. Both PRPP ATase deficient cell lines (Ade$^-$A) and FGAR ATase deficient cell lines (Ade$^-$B) have been isolated (29-31,33,41,42). Both PRPP ATase and FGAR ATase have been purified as separate and distinct enzymes (14,22,23). Human PRPP ATase has been purified and is composed of 4-5 homogeneous subunits with molecular weights of 30,000 each (14). FGAR ATase from chicken liver is composed of a single polypeptide with a molecular weight of 133,000 (23). The existence of the Ade$^-$P$_{AB}$ cell line with structurally altered PRPP ATase and FGAR ATase from a probably single mutational event is an interesting occurrence. Evidence for a single mutation producing Ade$^-$P$_{AB}$ is based upon isolation technique,

isolation frequency, and reversion frequency. Evidence for the enzymatic profile of Ade$^-$P$_{AB}$ as the result of structural changes in both PRPP ATase and FGAR ATase is as follows. Ade$^-$P$_{AB}$ possesses almost normal ammonia-dependent PRPP ATase, markedly altered apparent glutamine affinity of (glutamine-dependent) PRPP ATase and loss of FGAR ATase. Similarly, ABr possesses almost normal ammonia-dependent PRPP ATase, moderately altered apparent glutamine affinity of (glutamine-dependent) PRPP ATase and virtually normal FGAR ATase (1, personal communication from Holmes). Also, increased sensitivities to thermal inactivation indirectly demonstrate structural alterations in PRPP ATase and FGAR ATase from Ade$^-$P$_{AB}$ and ABr. Experiments on the glutamine affinity of FGAR ATase from ABr demonstrate no difference between ABr and CHO. Therefore, I postulate that some structural alteration of FGAR ATase in Ade$^-$P$_{AB}$ results in an enzyme which is catalytically inactive and/or extremely labile, but this mutation need not affect glutamine binding.

Addition of the data presented in this thesis to the tabular summary present in the Introduction on the findings for Ade$^-$P$_{AB}$ and ABr yields:

Enzyme	Property	Ade$^-$P$_{AB}$	ABr
PRPP ATase	ammonia-dependent activity	+	+
	glutamine-dependent activity	↓↓	↓
	apparent glutamine affinity	↓↓	↓
	thermal stability	↓↓	↓
FGAR ATase	ammonia-dependent activity	o	+
	glutamine-dependent activity	o	+
	apparent glutamine affinity	(+)	+*
	thermal stability	(↓)	↓*

+ = near wild type level
↓ = decreased from wild type level
↓↓ = markedly decreased from wild type level
0 = no measurable activity
* = result presented in this thesis
( ) = postulated from results presented

Possible explanations for the existence of Ade$^-$P$_{AB}$ must also take into account that Ade$^-$P$_{AB}$ does not complement Ade$^-$A, Ade$^-$B nor itself, but does complement all other purine auxotrophs (Ade$^-$C, Ade$^-$D, Ade$^-$E...) (1). Additionally, coincubation of crude extracts of Ade$^-$P$_{AB}$, Ade$^-$A, Ade$^-$B, and CHO in mixing studies does not result in any alteration of PRPP ATase or FGAR ATase activities (1). Since normal enzymatic activities are possible in the presence of Ade$^-$P$_{AB}$ extract, any theory involving modification of PRPP ATase and FGAR ATase in the cytosol is unlikely. This precludes the presence of a diffusible inhibitory substance. In complementation studies, a normal post-translational modification would be expected to modify enzyme from both cell lines; however, an inability to complement Ade$^-$A and Ade$^-$B demonstrates that such is not the case for Ade$^-$P$_{AB}$ (1). Since FGAR ATase retains little enzymatic activity when blocked with the glutamine analog azaserine, a diffusible inhibitory substance present in Ade$^-$P$_{AB}$ that could affect both glutamine-dependent PRPP ATase and FGAR ATase might be a glutamine analog. However, mixing studies of extracts from Ade$^-$P$_{AB}$ fail to inhibit PRPP ATase and FGAR ATase from other cell lines (1). Therefore, like alterations in post-translational modification, the presence of a diffusible inhibitory substance is doubtful.

If the alterations in PRPP ATase and FGAR ATase in Ade$^-$P$_{AB}$ are not inherent in the cytosol, then the two enzymes may have effects upon each other in the cytosol. For example, the existence of multienzyme

complexes is a possibility. A multienzyme complex would seem favorable since many of the intermediates of the de novo purine biosynthetic pathway are unstable (43). Early reports on the characteristics of the enzymes involved revealed some partial co-purification (44). However, further research demonstrated that these enzymes are not present in a tight multienzyme complex (45).

If PRPP ATase and FGAR ATase do not affect each other in a multienzyme complex, then perhaps they share a common subunit which when altered could affect both enzymes. It has been postulated that some of the glutamine-utilizing amidotransferases are the union of separate glutaminase and ammonia-utilizing aminotransferase polypeptides (27). Glutaminase activity would reside on a separate polypeptide, with enzymatic specificity conferred by the individual aminotransferase polypeptide. Such a mechanism seems unlikely in that PRPP ATase is formed of 4-5 homogeneous subunits with a molecular weight of 30,000 (14) and FGAR ATase is a single polypeptide with a molecular weight of 133,000 (23). Although PRPP ATase and FGAR ATase are from different species (human and avian sources, respectively), subunits of PRPP ATase are also found in bacteria (22,46,50). Additionally, no distinct glutaminase activity has been separated from purified preparations of either PRPP ATase or FGAR ATase (14,22,23,46). Therefore, it seems doubtful that PRPP ATase and FGAR ATase share some common subunit, especially when one considers that PRPP ATase is separable into 4-5 homogeneous subunits and FGAR ATase is a single polypeptide.

Postulating the theoretical existence of a single multifunctional polypeptide could explain many of the mutational findings for Ade-P_{AB}. Such a polypeptide is not without precedent, in that a multifunctional

polypeptide has been identified which catalyzes the first three reactions of the de novo pyrimidine biosynthetic pathway (47-49). A multifunctional polypeptide is consistent with the production of Ade$^-P_{AB}$ by a single mutational event which then alters more than one enzyme activity. The mixing studies, the complementation pattern of Ade$^-P_{AB}$, and the not uncommon formation of revertants are in agreement with such a theoretical multifunctional polypeptide.

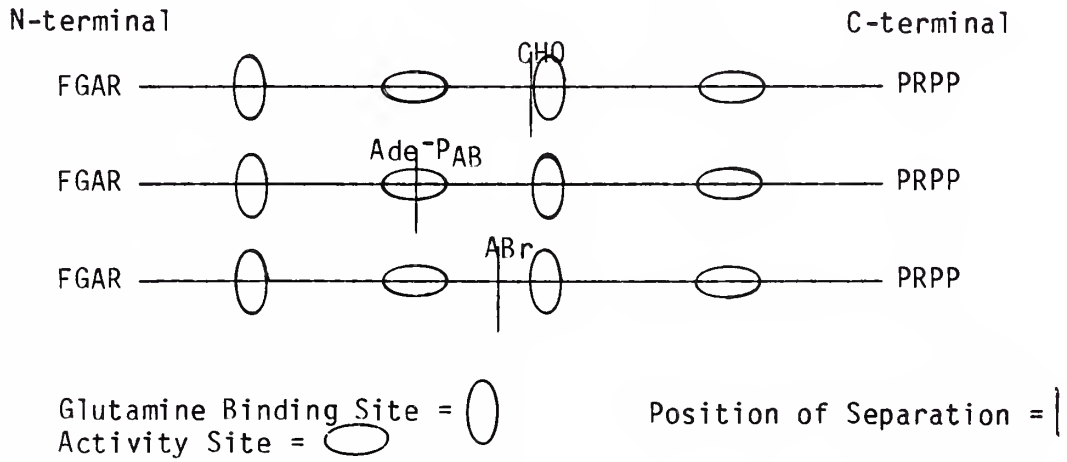
Additionally, coordinate control in the production of PRPP ATase and FGAR ATase has been demonstrated for bacteria (37). This type of regulation of the level of enzymes of a pathway has been described for the (previously mentioned) first three enzymes of the de novo pyrimidine biosynthetic pathway (47,48) and for two non-sequential enzymes of the de novo purine biosynthetic pathway (49). The identification of a multifunctional protein catalyzing the first three reactions of the de novo pyrimidine biosynthetic pathway explains the findings for these enzymatic activities. The two non-sequential enzymes of the de novo purine biosynthesis are the third and the sixth enzymes of the pathway, phosphoribosylglycinamide synthetase (GAR synthetase, EC 6.3.4.13) and phosphoribosylaminoimidazole synthetase (AIR synthetase, EC 6.3.3.1), respectively. Both GAR synthetase and AIR synthetase have been assigned to chromosome 21. Also, a purine auxotrophic cell line (Ade$^-P_{CG}$) and its revertants have been reported which first lost and then regained both GAR synthetase and AIR synthetase (49). Therefore, the coordinate regulation of GAR Synthetase and AIR Synthetase may be under the direction of a single regulatory unit which is mutated in Ade$^-P_{CG}$ and its revertants. However, for Ade$^-P_{AB}$ the structural changes in both PRPP ATase and FGAR ATase cannot be easily explained by mutation in a similar regulatory unit.

None of the possible explanations presented above satisfactorily deals with all of the enzymatic and genetic characteristics of Ade$^-P_{AB}$ (and ABr). A theoretical multifunctional polypeptide can explain the structural alteration of two enzymes from one mutational event. However, the purification of separate and distinct enzymes makes this theory unlikely. Additionally, the complementation pattern of Ade$^-$A and Ade$^-$B point to two separate genes for these enzymes. The separate complementation group Ade$^-P_{AB}$ could be explained by its affecting a multienzyme complex. Such a complex is demonstrated for the last two enzymes of the purine de novo biosynthetic pathway leading to formation of IMP (phosphoribosyl-aminoimidazolecarboxamide transformylase, EC 2.1.2.3 and inosine-5'-monophosphate cyclohydrolase, EC 3.5.4.10; reference 51). Additionally, in Salmonella typhimurium, identification of a third complementation group (distinct from the two groups for each enzyme) has been shown to affect the interaction between these two enzymes (52). This is demonstrated by the restoration of low transformylase activity from this third complementation group by the addition of normal cyclohydrolase. However, a similar mixing study for PRPP ATase and FGAR ATase from Ade$^-P_{AB}$ does not affect any enzyme activities (1). Therefore, PRPP ATase and FGAR ATase are probably not in a tight multienzyme complex similar to that of transformylase and cyclohydrolase.

Ade$^-P_{AB}$ is a third complementation group to Ade$^-$A and Ade$^-$B. Therefore, it appears to involve a third gene distinct from the two genes coding for PRPP ATase and FGAR ATase. A model for Ade$^-P_{AB}$ might involve the theoretical existence of a single precursor for PRPP ATase and FGAR ATase. Given the complexities of mammalian systems, such a precursor

could exist at the DNA, RNA or polypeptide level. Ade$^-P_{AB}$ would then be the result of a mutation in the separation of the PRPP ATase and FGAR ATase segments at one of these three levels.

The marked decrease in apparent glutamine affinity of (glutamine-dependent) PRPP ATase in Ade$^-P_{AB}$ with reversion to a moderate decrease in ABr could be explained by the following hypothesis. The γ-glutamyl intermediate formed during glutamine hydrolysis has recently been shown to bind to the N-terminal cysteine residue on PRPP ATase in E. coli (53). If one assumes that the FGAR ATase gene is located "proximal" or on the 5'-end of the PRPP ATase gene, a mutation could lead to an error in the splicing of DNA or RNA or in the separation of these two enzymes such that the glutamine site of PRPP ATase (at its N-terminal end) and a crucial enzymatic site of FGAR ATase (at its C-terminal end) would both be affected.



Since the active cysteine residue is normally a N-terminal residue, then the presence of several extra amino acids in Ade$^-P_{AB}$ might severely disrupt the glutamine-utilizing section of PRPP ATase and only minimally affect the ammonia-utilizing section of PRPP ATase. FGAR ATase activity would be simultaneously lost due to a mutation in the C-terminal

portion of this protein. For reversion to take place, the position for DNA or RNA splicing or for separation of two polypeptides would change to a position which added at least one (but not too many) amino acids to the N-terminus of PRPP ATase in ABr. The altered glutamine binding of PRPP ATase would then be less marked. Also, FGAR ATase could be almost fully active, and might only exhibit the loss of some non-functional amino acids with a decrease in thermal stability.

The presumed change in the position of the separation between PRPP ATase and FGAR ATase would of necessity be due to alterations in the genetic code. There is little information to speculate even further on the nature or mechanism of the putative defect in the splicing process. However, the model presented does fit all of the known properties of Ade$^-$P$_{AB}$ and ABr.

The complementation properties of Ade$^-$P$_{AB}$ and ABr are in accord with this model; however, the recent mapping of PRPP ATase and FGAR ATase to different human chromosomes does cast doubt on this model. PRPP ATase has been tentatively assigned to human chromosome 4 (54). FGAR ATase was initially localized to human chromosome 4 by linkage to esterase production (55), but has been reassigned to human chromosome 14 (56). Although the genome of the Chinese hamster cell may differ from human cells with regard to the chromosomal localization of PRPP ATase and FGAR ATase, the model proposed above for Ade$^-$P$_{AB}$ is not supported by the finding for human cells.

Short of isolating and sequencing PRPP ATase and FGAR ATase from Ade$^-$P$_{AB}$ and ABr, a simpler experimental method involves examination of the N-terminus of PRPP ATase. If PRPP ATase from Chinese hamster ovary cells possess the same N-terminal, glutamine binding cysteine residue as

found for E. Coli (53), then this could be identified by first irreversibly binding a radiolabelled glutamine analog such as azaserine or DON (6-diazo-5-oxo-L-norleucine). Next, a single step of Edman degradation could be performed to determine if a radioactive glutamine analog-cysteine unit was the N-terminal residue. If the alterations which formed Ade$^-$P$_{AB}$ and ABr produced extra N-terminal amino acids on PRPP ATase, then both cell lines would demonstrate an absence of the radioactive glutamine analog-cysteine residue. This finding would be consistent with the model presented above.

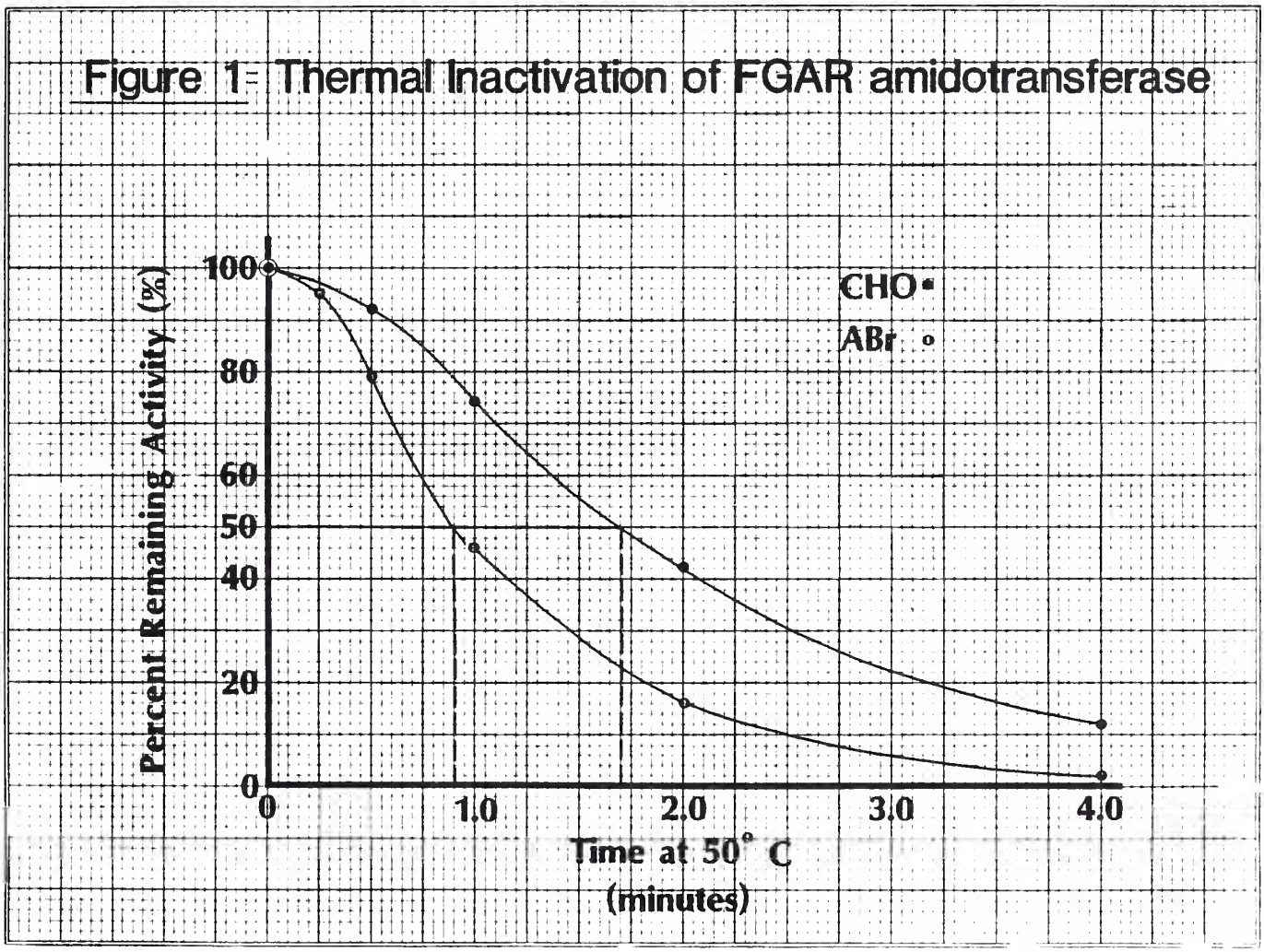
TABLE 1

FGAR ATase Specific Activity and Protein Concentration

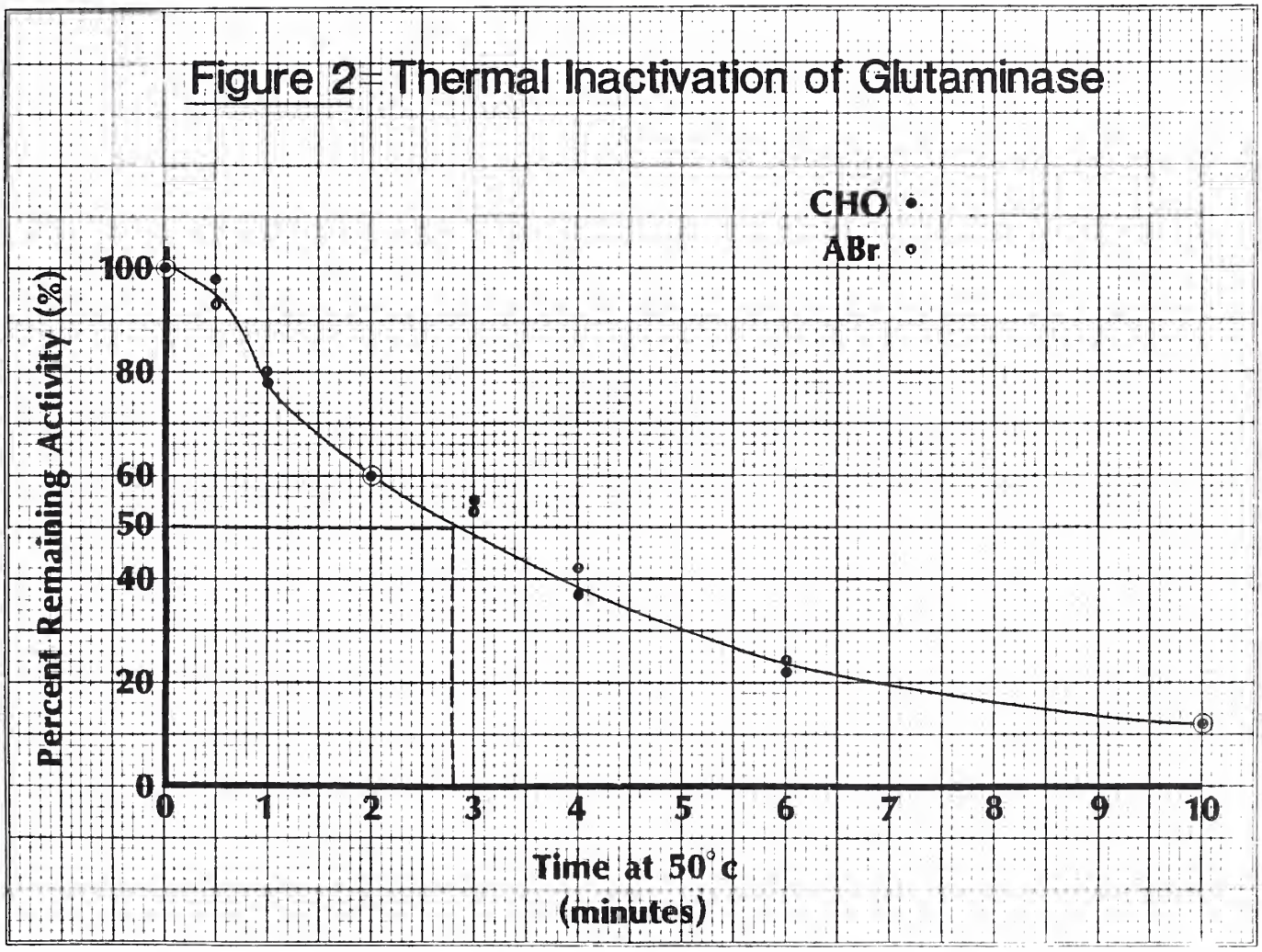
Means and standard deviations are compilations of multiple experiments with ABr and CHO enzyme extracts. Values are expressed as mean ± standard deviation (sample size).

	ABr	CHO
FGAR ATase Specific Activity* (nmol/mg protein/hr)	13.8 ± 3.55 (10)	14.5 ± 3.80 (11)
Protein Concentration (mg/ml)	12.5 ± 3.35 (11)	15.3 ± 1.86 (12)

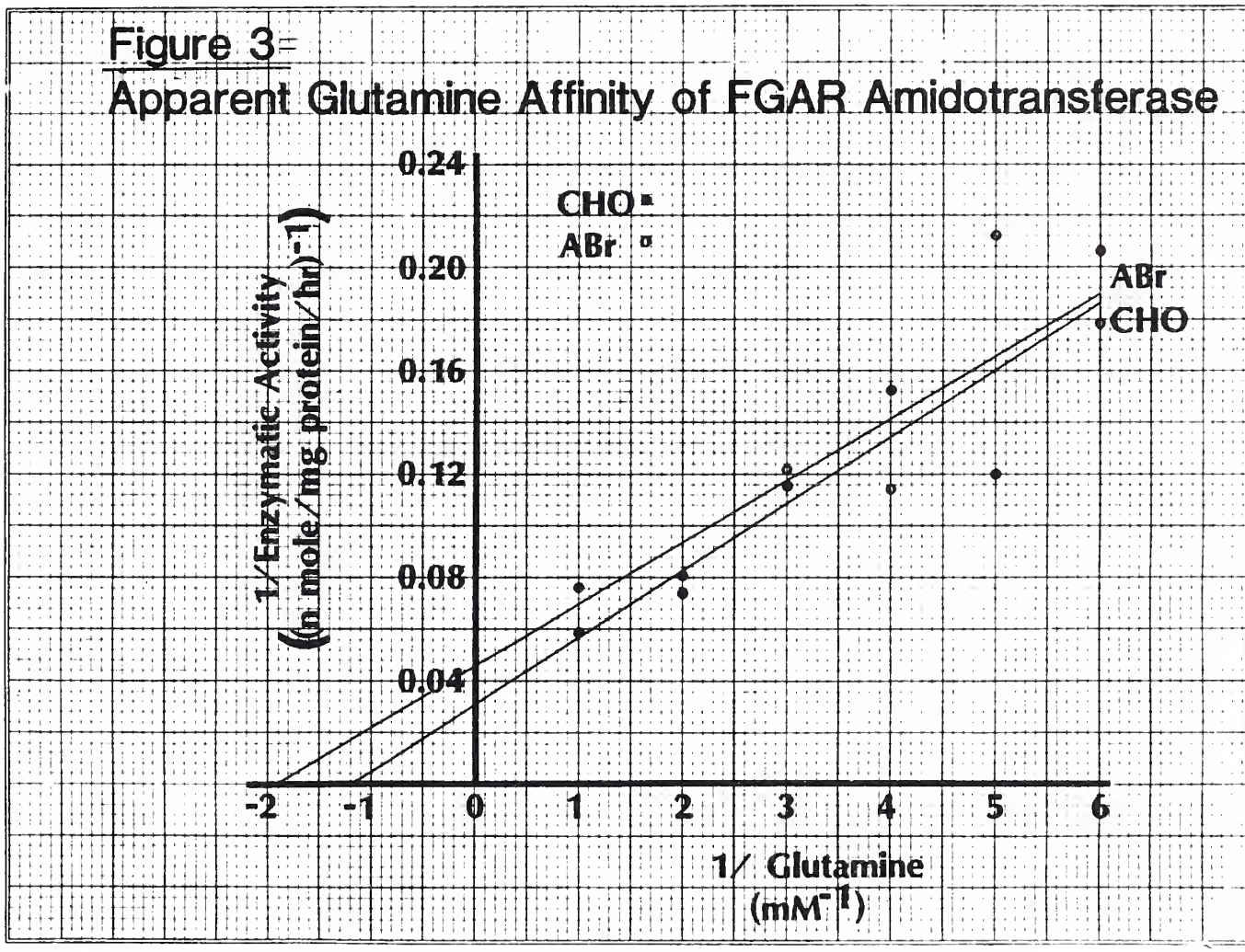
*Activity is measured at 4mM glutamine.



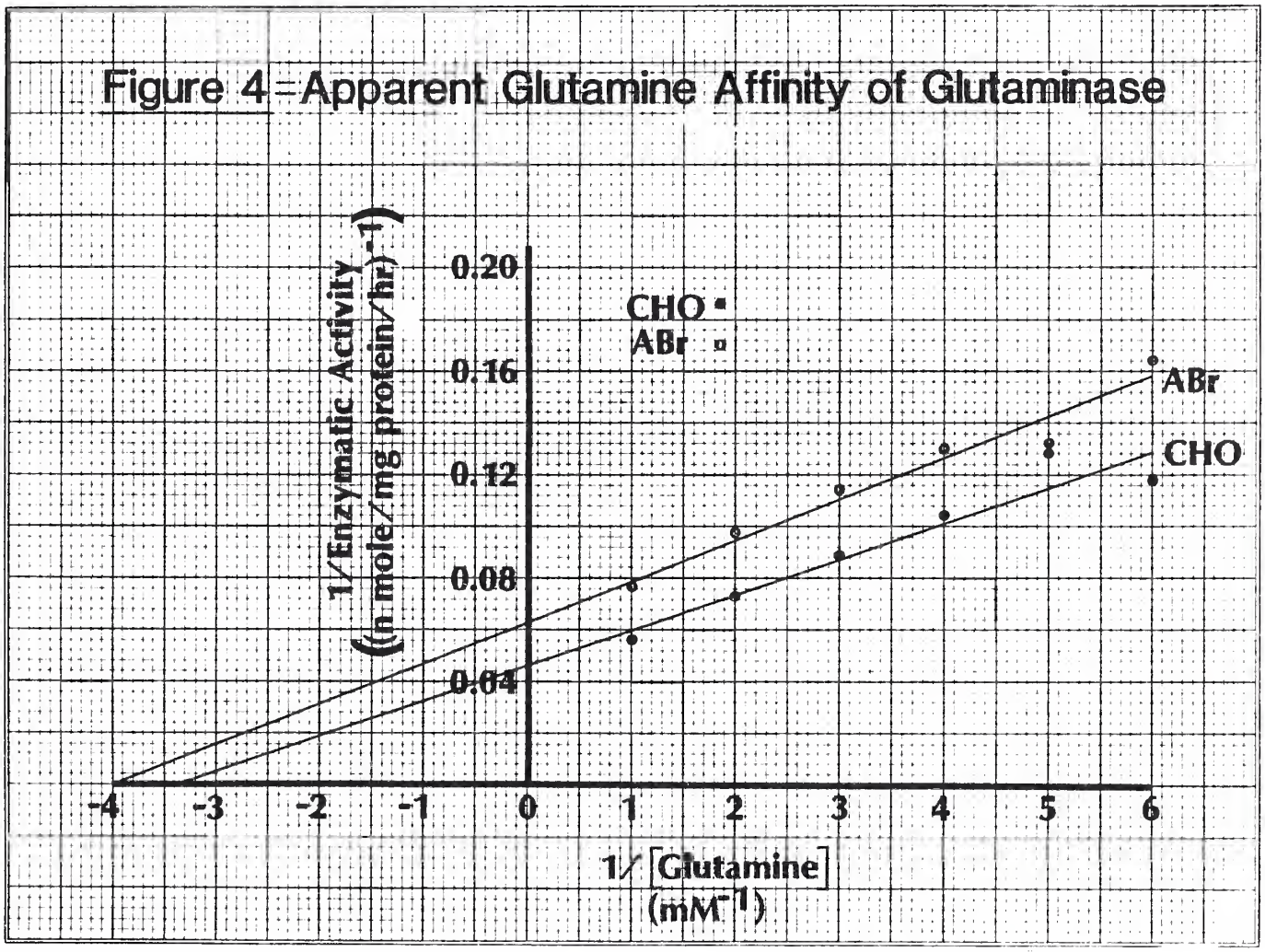
Thermal Inactivation of FGAR ATase: ABr (o) and CHO (•) are each the averaged result of two separate experiments. Dialyzed enzyme extracts from ABr and CHO are simultaneously heated to 50°C in open glass test tubes for 0.25 minutes to 4 minutes and immediately placed on an ice-water bath. Interpolation of the data yields the time required for the loss of 50% of FGAR ATase activity for ABr as 0.9 minutes and for CHO as 1.7 minutes.



Thermal Inactivation of Glutaminase: ABr (o) and CHO (•) are each the combination of three separate experiments. All points are compiled from two independent studies except at 0.5, 1, and 3 minutes, which are each from one study. Dialyzed enzyme extract is heated to 50°C in an open glass test tube for 0.5 minutes to 10 minutes and then immediately placed on an ice-water bath. Results are drawn from the control data which are the reaction mixtures without FGAR present. The 3 minute point does not lie on a smooth curve of the other data points. Since it is also from one experiment and not as reliable, it is left out of the curve. By interpolation, both ABr and CHO lose 50% of their glutaminase activities in approximately 3 minutes.



Apparent Glutamine Affinity of FGAR ATase: Reciprocal plot of FGAR ATase specific activity and glutamine concentration. ABr (o) is the result of three experiments and CHO (•) is the result of two experiments. Glutamine concentrations vary while all other enzyme assay conditions remain constant. Glutamine concentrations greater than 1mM approach saturation and are therefore not in these plots. A least-squares plot for ABr yields an apparent glutamine Km of 0.54mM and a V_{max} of 22.2 nmol/mg protein/hr, with a correlation coefficient of 0.86. Similar analysis for CHO produce an apparent glutamine Km of 0.86mM and V_{max} of 33.0 nmol/mg protein/hr, with a correlation coefficient of 0.90.



Apparent Glutamine Affinity of Glutaminase: ABr (o) is the result of three experiments and CHO (•) is the result of two experiments. These results are extracted from the controls of the glutamine binding study which are enzyme assays without FGAR. A least-squares plot for ABr yields an apparent glutamine K_m of 0.25mM and V_{max} 15.8 nmol/mg protein/hr, with a correlation coefficient of 0.98. The results for CHO are an apparent glutamine K_m of 0.30mM and V_{max} of 21.8 nmol/mg protein/hr, with a correlation coefficient of 0.95.

FOOTNOTES

1. Oates, D.C., Vannais, D., Patterson, D. A mutant of CHO-K1 cells deficient in two nonsequential steps of de novo purine biosynthesis. Cell, Vol. 20, 797-805, July 1980.

2. Holmes, E.W. In Handbook of Experimental Pharmacology, edited by Kelley, W.N. and Weiner, I.M. Published by Springer-Verlag, Berlin, Heidelberg, 1978.

3. Itakura, M., Sabina, R.L., Heald, P.W., Holmes, E.W. Basis for the control of purine biosynthesis by purine ribonucleotides. J. Clin. Invest. 67: 994-1002, April, 1981.

4. Taylor, M.W., Olivelle, S., Levine, R.A., Cog, K., Hershey, H., Gusta, K.C., Zawistowich, L. Regulation of de novo purine biosynthesis in Chinese hamster cells. J. Biol. Chem. 257: #1, 377-380, January 10, 1982.

5. Welch, M.M., Rudolph, F.B. Regulation of purine biosynthesis and interconversion in the chick. J. Biol. Chem. 257, #22, 13253-13256, November 25, 1982.

6. Rosiers, C. des, Lalanne, M., Willemot, J. Effect of glycerol and fructose on purine synthesis de novo and on PP-ribose-P availability in rat liver cells. Can. Jo. Biochem. 60: 1109-1115, 1982.

7. Bernlohr, D.A., Switzer, R.L. Reaction of Bacillus subtilis glutamine phosphoribosylpyrophosphate amidotransferase with oxygen: Chemistry and regulation by ligands. Biochem. 20: #20, 5675-5681, 1981.

8. Ruppen, M.E., Switzer, R.L. Degradation of Bacillus subtilis glutamine phosphoribosylpyrophosphate amidotransferase in vivo. J. Biol. Chem. 258: #5, 2843-2851, March 10, 1983.

9. Bernlohr, D.A. and Switzer, R.L. Regulation of Bacillus subtilis glutamine phosphoribosylpyrophosphate amidotransferase inactivation in vivo. J. Bacteriol. 153: #2, 937-949, February, 1983.

10. Itakura, M., Holmes, E.W. Regulation of amidophosphoribosyltransferase activity in Eukaryotic cells by oxygen inactivation, (in press), personal communication.

11. Holmes, E.W., McDonald, J.A., McCord, J.M., Wyngaarden, J.M., Kelley, W.N. Human glutamine phosphoribosylpyrophosphate amidotransferase: Kinetic and regulatory properties. J. Biol. Chem. 248: #1, 144-150, January 10, 1973.

12. Kovarsky, J., Evans, M.C., and Holmes, E.W. Regulation of human amidophosphoribosyltransferase: Interaction of orthophosphate, PP-ribose-P, and purine ribonucleotides. Can. Jo. Biochem. 56: #5, 334-338, 1978.

13. Meyer, E. and Switzer, R.L. Regulation of Bacillus subtilis glutamine phosphoribosylpyrophosphate amidotransferase activity by end products. J. Biol. Chem. 254: #6, 5397-5402, June 25, 1979.

14. Leff, R.L., Itakura, M., Udom, A., and Holmes, E.W. Amidophosphoribosyl transferase: A possible role for oxygen inactivation in the regulation of amidophosphoribosyltransferase activity in mammalian cells. Advances in Enzyme Regulation 22: 403-411, 1984.

15. Holmes, E.W., Wyngaarden, J.B., and Kelley, W.N. Human glutamine phosphoribosylpyrophosphate amidotransferase: Two molecular forms interconvertible by purine ribonucleotides and phosphoribosylpyrophosphate. J. Biol. Chem. 248: #17, 6035-6040, September 10, 1973.

16. Singer, S.C. and Holmes, E.W. Human glutamine phosphoribosylpyrophosphate amidotransferase: Hysteretic properties. J. Biol. Chem. 252: #22, 7959-7963, November 25, 1977.

17. King, G.L., Bounous, C.G. and Holmes, E.W. Human placental amidophosphoribosyltransferase: Comparison of the kinetics of glutamine and ammonia utilization. J. Biol. Chem. 253: #11, 3933-3938, June 10, 1978.

18. Itakura, M. and Holmes, E.W. Human amidophosphoribosyltransferase: An oxygen-sensitive iron-sulfur protein. J. Biol. Chem. 254: #2, 333-338, January 25, 1979.

19. Wong, J.Y., Meyer, E., and Switzer, R.L. Glutamine phosphoribosylpyrophosphate amidotransferase from Bacillus subtilis: A novel iron-sulfur protein. J. Biol. Chem. 252: #2 7424-7426, November 10, 1977.

20. Averill, B.A., Dwivedi, A., Debrunner, P., Vollmer, S.J., Wong, J.Y., and Switzer, R.L. Evidence for a tetranuclear iron-sulfur center in glutamine phosphoribosylpyrophosphate amidotransferase from Bacillus subtilis. J. Biol. Chem. 255: #13, 6007-6010, July 10, 1980.

21. Vollmer, S.J., Switzer, R.L., Hermodson, M.A., Bower, S.G. and Zalkin, H. The glutamine-utilizing site of Bacillus subtilis glutamine phosphoribosylpyrophosphate amidotransferase. J. Biol. Chem. 258: #17, 10582-10585, September 10, 1983.

22. Messenger, L.J. and Zalkin, H. Glutamine phosphoribosylpyrophosphate amidotransferase from Escherichia coli: Purification and properties. J. Biol. Chem. 254: #9, 3382-3392, May 10, 1979.

23. Mizobucki, K. and Buchanan, J.M. Purification and properties of formylglycinamide ribonucleotide amidotransferase from chicken liver. J. Biol. Chem. 243: #18, 4842-4852, September 25, 1968.

24. Chu, S.Y. and Henderson, J.F. Kinetic studies of phosphoribosylformylglycineamide synthetase. Can. Jo. Biochem. 50: 490-500, 1972.

25. Mizobuchi, K. and Buchanan, J.M. Isolation and characterization of formylglycinamide ribonucleotide amidotransferase-glutamyl complex. J. Biol. Chem. 243: #18, 4853-4862, September 25, 1968.

26. Mizobuchi, K., Kenyon, G.L. and Buchanan, J.M. Binding of formylglycinamide ribonucleotide and adenosine triphosphate to formylglycinamide ribonucleotide amidotransferase. J. Biol. Chem. 243: #18, 4863-4877, September 25, 1968.

27. Li, H.C. and Buchanan, J.M. Catalytic properties of the glutamine site of formylglycinamide ribonucleotide amidotransferase from chicken liver. J. Biol. Chem. 246: #15, 4713-4719, August 10, 1971.

28. Levenberg, B., Melmick, I., Buchanan, J.M. The effect of Aza-L-serine and 6-diazo-5-oxo-L-norleucine on inosine acid biosynthesis de novo. J. Biol. Chem. 255: #1, 163-176, March, 1957.

29. Patterson, D., Kao, F.T. and Puck, T.T. Genetics of somatic mutant cells: Biochemical genetics of Chinese hamster cell mutants with deviant purine metabolism. Proc. Nat. Acad. Sci. USA 71: 2057-2061, 1974.

30. Patterson, D. Biochemical genetics of Chinese hamster cell mutants with deviant purine metabolism: Biochemical analysis of eight mutants. Somatic Cell Genet. 1: 91-110, 1975.

31. Oates, D.C. and Patterson, D. Biochemical genetics of Chinese hamster cell mutants with deviant purine metabolism: Characterization of Chinese hamster cell mutants defective in phosphoribosylpyrophosphate amidotransferase and phosphoribosylglycinamide synthetase and an examination of alternatives to the first step of purine biosynthesis. Somatic Cell Genet. 3: 561-577, 1977.

32. Irwin, M., Oates, D.C. and Patterson. D. Biochemical genetics of Chinese hamster cell mutants with deviant purine metabolism. Isolation and characterization of a mutant deficient in the activity of phosphoribosylaminoimidazole synthetase (E.C. 6.3.3.1). Somatic Cell Genet. 5: 203-216, 1979.

33. Holmes, E.W., King, G.L., Leyva, A. and Singer, S.C. A purine auxotroph deficient in phosphoribosylpyrophosphate amidotransferase and phosphoribosylpyrophosphate amidotransferase activities with normal activity of ribose-5-phosphate aminotransferase. Proc. Natl. Acad. Sci. USA 70: 2458-2461, 1976.

34. Kao, F.T. and Puck, T.T. Genetics of somatic mammalian cells. VII. Induction and isolation of nutritional mutants in Chinese hamster cells. Proc. Nat. Acad. Sci. USA 60: 1275-1281, 1968.

35. Methods in Enzymology II [78] pp. 508-509.

36. Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.S. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265-275, 1951.

37. Methods in Enzymology VI [96] pp. 678-681.

38. Nierlich, D.P., Magasanik, B. Control by feedback repression of the enzymes of purine biosynthesis in Aerobacter aerogenes. Biochim. et Biophys. Acta 230: 342-348, 1971.

39. Chu, S.Y., Henderson, J.F. Purification and properties of phosphoribosyl-formylglycinamide synthetase of Ehrlich ascites tumor cells. Can. J. Biochem. 50: 484-489, 1972.

40. Reem, G.H. Enzymatic synthesis of phosphoribosylamine in human cells. J. Biol. Chem. 249: #6, 1696-1703, March 25, 1974.

41. Patterson, D., Kao, F.T., Puck, T.T. Genetics of somatic mammalian cells: Biochemical genetics of Chinese hamster cell mutants with deviant purine metabolism. Proc. Natl. Acad. Sci. USA 71: #5, 2057-2061, May, 1974.

42. Oates, D.C., Patterson, D. Biochemical genetics of Chinese hamster cell mutants with deviant purine metabolism: Characterization of Chinese hamster cell mutants defective in phosphoribosylpyrophosphate amidotransferase and phosphoribosylglycinamide synthetase and an examination of alternatives to the first step of purine biosynthesis. Somatic Cell Genetics 3: #6, 561-577, 1977.

43. Wyngaarden, J.B., Appel, S.H., Rowe, P.B. in Exploitable Molecular Mechanisms and Neoplasia, pp. 415-434, Williams and Wilkins Co., Baltimore, 1968.

44. Rowe, P.B., McCairns, E., Madsen, G., Sauer, D., and Elliott, H. De novo purine synthesis in Avian liver: Co-purification of the enzymes and properties of the pathway. J. Biol. Chem. 253: #21, 7711-7721, November 10, 1978.

45. McCairns, E., Fahey, D., Sauer, D., and Rowe, P.B. De novo purine synthesis in human lymphocytes: Partial co-purification of the enzymes and some properties of the pathway. J. Biol. Chem. 258: #3, 1851-1856, February 10, 1983.

46. Wong, J.Y., Bernlohr, D.A., Turnbough, C.L., and Switzer, R.L. Purification and properties of glutamine phosphoribosylpyrophosphate amidotransferase from Bacillus subtilis. Biochem. 20: 5669-5674, 1981.

47. Coleman, P.F., Suttle, D.P., and Stark, G.R. Purification from hamster cells of the multifunctional protein that initiates de novo synthesis of pyrimidine nucleotides. J. Biol. Chem. 252: #18, 6379-6385, September 25, 1977.

48. Padgett, R.A., Wahl, G.M., Coleman, P.F., and Stark, G.R. N-(Phosphonacetyl)-L-aspartate-resistant hamster cells overaccumulate a single mRNA coding for the multifunctional protein that catalyzed the first steps of UMP synthesis. J. Biol. Chem. 254: #3, 974-980, February 10, 1979.

49. Davidson, J.N. and Patterson, D. Alteration in structure of multifunctional protein from Chinese hamster ovary cells defective in pyrimidine biosynthesis. Proc. Natl. Acad. Sci. USA 76: #4, 1731-1735, April, 1979.

50. Patterson, D., Graw, S., and Jones, C. Demonstration by somatic cell genetics of coordinate regulation of genes for two enzymes of purine synthesis assigned to human chromosome 21. Proc. Natl. Acad. Sci. USA 78: 405-409, January, 1981.

51. Flaks, J.G., Erwin, M.J., and Buchanan, J.M. 5-Amino-1-ribosyl-4-imidazolecarboxamide 5'-phosphate transformylase and inosinicase. J. Biol. Chem. 229: 603-612, 1957.

52. Gots, J.S., Dalal, F.R., and Shumas, S.R. Genetic separation of the inosine acid cyclohydrolase-transformylase complex of Salmonella typhimurium. J. Bacteriol. 99: #2, 441-449, August, 1969.

53. Tso, J.Y., Hermodson, M.A., and Zalkin, H. Glutamine phosphoribosylpyrophosphate amidotransferase from cloned Escherichia coli pur F. J. Biol. Chem. 257: #7, 3532-3536, April 10, 1982.

54. Chu, E.H.Y. and Stanley, W. Assignment of the gene for phosphoribosylpyrophosphate amidotransferase to the pter → q21 region of human chromosome 4. Cytogenet. Cell Genet. 22: 228-231, 1978.

55. Kao, F.T. and Puck, T.T. Genetics of somatic mammalian cells: Demonstration of a human esterase activator gene linked to the AdeB gene. Proc. Natl. Acad. Sci. USA 69: #11, 3273-3277, November, 1972.

56. Kao, F.T. Chromosomal assignment of the gene for phosphoribosylformylglycinamidine synthetase (PFGS) to human chromosome 14. J. Cell Biology 87: Abstracts 291a, November, 1980.

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