Biochemical Pharmacology, Vol. 31, No. 23, pp. 3879–3884, 1982.
Printed in Great Britain.

0006-2952/82/233879-06 $03.00/0

PHOSPHORYLATION OF ACYCLOVIR DIPHOSPHATE BY CELLULAR ENZYMES

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(*Received* 16 *February* 1982; *accepted* 21 *April* 1982)

Abstract—Acyclovir diphosphate (acyclo-GDP) is a metabolite of the antiviral drug acyclovir [9-(2-hydroxyethoxymethyl)guanine]. Seven enzymes capable of catalyzing the phosphorylation of GDP and dGDP (nucleoside diphosphate kinase, pyruvate kinase, creatine kinase, phosphoglycerate kinase, succinyl-CoA synthetase, phosphoenolpyruvate carboxykinase and adenylosuccinate synthetase) also catalyzed the phosphorylation of acyclo-GDP. In general, acyclo-GDP had a lower V'_{max} and a higher K'_m than either GDP or dGDP. None of these enzymes showed significantly higher rates of phosphorylation with GDP or acyclo-GDP in herpes simplex virus-infected Vero cells as compared to uninfected Vero cells. The contribution of each enzyme to the phosphorylation of acyclo-GDP *in vivo* was estimated from the kinetic data from the partially purified enzymes, the level of each enzyme in Vero cells, and the physiological concentrations of both substrates and inhibitors. The relative order of estimated rates of acyclo-GDP phosphorylation in Vero cells was phosphoglycerate kinase ≫ pyruvate kinase > phosphoenolpyruvate carboxykinase > nucleoside diphosphate kinase > succinyl-CoA synthetase > creatine kinase > adenylosuccinate synthetase. The calculated potential for acyclo-GDP phosphorylation by these enzymes was adequate to account for the amounts of acyclo-GTP formed in cell culture.

Acyclovir† is a new antiviral drug with therapeutic usefulness in humans for treatment of herpes simplex virus infections [1, 2]. It appears to exert its antiviral action as the nucleoside triphosphate analog, acyclo-GTP, by inhibiting viral DNA replication [3]. Acyclovir is selectively phosphorylated by an HSV-coded thymidine kinase [4], and the resultant monophosphate, acyclo-GMP, is phosphorylated to acyclo-GDP by host cell GMP kinase [5].

This study examined the identity of the enzymes involved in the phosphorylation of acyclo-GDP (Fig. 1). Several enzymes capable of phosphorylating GDP (Table 1) were studied, and the contribution of each to intracellular acyclo-GTP formation was estimated.

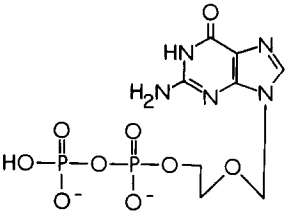

Fig. 1. Structural formula for acyclo-GDP.

EXPERIMENTAL PROCEDURES

Materials. Vero cells, a line derived from the kidney of the African green monkey (ATCC CCL 81), and Vero cells infected with HSV-1 (T_1H29 strain) were grown and infected as described previously [6]. Sprague–Dawley rats were obtained from Harlan Industries, Indianapolis, IN. Adenylosuccinate synthetase (EC 6.3.4.4) was prepared from rabbit muscle [7]. Acyclo-GDP and acyclo-GTP were prepared by the method of Furman *et al.* [3]. [8-^{14}C]Acyclo-GMP was prepared as described previously [5]. [8-^{14}C]GDP (60 mCi/mmole) and [U-^{14}C]dGMP (482 mCi/mmole) were purchased from Amersham, Arlington Heights, IL. [8-^{14}C]GTP (43.2 mCi/mmole), [8-^{14}C]ADP (49.6 mCi/mmole) and Aquasol-2 were purchased from the New England Nuclear Corp., Boston, MA. Other nucleotides and agarose-hexane-guanosine-5′-triphosphate Type 4 (ribose linkage) were obtained from P-L Biochemicals, Milwaukee, WI. Boehringer Mannheim, Indianapolis, IN, was the source for NADH, NAD^+, phosphoenolypyruvate, creatine phosphate, GMP kinase (EC 2.7.4.8), lactate dehydrogenase (EC 1.1.1.27), glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.12), malate dehydrogenase (EC 1.1.1.37), pyruvate kinase from rabbit muscle, creatine kinase from rabbit muscle, phosphoglycerate kinase from yeast, nucleoside diphosphate kinase from beef liver and succinyl-CoA synthetase from pig heart. Schwarz/Mann, Orangeburg, NY, was the source for ultrapure Tris, ultrapure sucrose, and bovine serum albumin. Succinyl coenzyme A, DL-glyceraldehyde-3-phosphate, adenylosuccinic acid,

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† Abbreviations: acyclovir, 9-(2-hydroxyethoxymethyl)-guanine, ZOVIRAX; acyclo-GMP, -GDP and -GTP, the mono-, di- and triphosphates of acyclovir; HSV, herpes simplex virus; Pipes, 1,4-piperazinediethanesulfonic acid; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; and HPLC, high pressure liquid chromatography.

Table 1. Enzymes tested for catalysis of acyclo-GDP phosphorylation

Enzyme	EC Number	Catalyzed reaction
Nucleoside diphosphate kinase	2.7.4.6	ATP + nucleoside diphosphate = ADP + nucleoside triphosphate
Pyruvate kinase	2.7.1.40	ATP + pyruvate = ADP + phosphoenolpyruvate
Creatine kinase	2.7.3.2	ATP + creatine = ADP + phosphocreatine
Phosphoglycerate kinase	2.7.2.3	ATP + 3-phospho-D-glycerate = ADP + 1,3-diphospho-D-glycerate
Succinyl-CoA synthetase (GDP-forming)	6.2.1.4	GTP + succinate + CoA = GDP + P_i + succinyl-CoA
Phosphoenolpyruvate carboxykinase (GTP)	4.1.1.32	GTP + oxaloacetate = GDP + phosphoenolpyruvate + CO_2
Adenylsocuccinate synthetase	6.3.4.4	GTP + IMP + L-aspartate = GDP + P_i + adenylosuccinate

Hepes, and Pipes were obtained from the Sigma Chemical Co., St. Louis, MO. DEAE-cellulose (DE52) was obtained from Whatman Inc., Clifton, NJ. Polyethyleneimine cellulose thin-layer chromatography plates were purchased from Brinkmann Instruments, Westbury, NY. Mätrex Gel Red A and PM-10 ultrafiltration membranes were purchased from the Amicon Corp., Danvers, MA. The Sephadex products were purchased from Pharmacia Fine Chemicals, Piscataway, NJ. Other chemicals were obtained from Fisher Scientific, Raleigh, NC.

Enzyme assays. Unless noted, all enzyme assays were performed at 37° in a volume of 100 μl. After a 3-min preincubation period at 37°, reactions were initiated by addition of enzyme appropriately diluted into 50 mM Tris-acetate (pH 7.5) containing 1 mg/ml bovine serum albumin. The reactions were terminated by spotting a 10-μl aliquot onto a polyethyleneimine thin-layer chromatography plate which had been spotted previously with 0.02 μmole each of GMP, GDP and GTP as carriers. The chromatography plate was developed in 1.6 M LiCl/1.6 M formic acid (1:1). The R_f values for the acyclovir nucleotides and the deoxyguanosine nucleotides were identical to the R_f values of the guanine nucleotides (R_f: GMP, Guo = 0.7; GDP = 0.4; GTP = 0.1). For assays of ATP formation, 1 M LiCl/1 M formic acid (1:1) was used as a solvent (R_f: AMP = 0.6; ADP = 0.3; ATP = 0.05). The spots containing radioactivity were cut out and placed in Aquasol-2, and radioactivity was determined in a scintillation counter. Before assaying, Vero cell extracts were desalted on a Sephadex G-25 column equilibrated with 20 mM Tris-acetate (pH 7.5). Unless specified, assay mixtures of Vero cell extracts contained GDP or acyclo-GDP at a concentration equal to their K'_m value, as determined for the purified enzymes (Table 3). Each enzyme reaction mixture contained 0.03 μCi of ^{14}C-labeled nucleotide in addition to various concentrations of unlabeled nucleotide.

Nucleoside diphosphate kinase assay mixtures contained 50 mM Tris-acetate (pH 7.5), 5 mM $MgCl_2$, 4 mM ATP, and the appropriate concentrations of nucleoside diphosphate.

Phosphoglycerate kinase assay mixtures contained 50 mM Tris-acetate (pH 7.5), 5 mM $MgCl_2$, 10 mM sodium phosphate (pH 7.5), 4 mM NAD^+, 4 mM DL-glyceraldehyde-3-phosphate, 8 I.U./ml glyceraldehyde-3-phosphate dehydrogenase and nucleoside diphosphate. These reaction mixtures were incubated for 20 min at room temperature before starting the assays so that the concentration of 1,3-diphosphoglycerate would reach a maximum.

Creatine kinase assay mixtures contained 60 mM Tris-acetate (pH 7.5), 5 mM magnesium acetate, 20 mM creatine phosphate (pH 7.5), and nucleoside diphosphate.

Succinyl-CoA synthetase activity was determined in 50 mM Tris-acetate (pH 7.5), 5 mM $MgCl_2$, 10 mM sodium phosphate (pH 7.5), 2 mM succinyl-CoA, and a nucleoside diphosphate.

Pyruvate kinase activity was determined using a spectrophotometric assay at 340 nm ($\Delta\varepsilon$ = 6.22 $mM^{-1}\,cm^{-1}$). A 250-μl reaction mixture contained 50 mM Tris-acetate (pH 7.5), 5 mM $MgCl_2$, 100 mM KCl, 2.5 mM phosphoenolypyruvate, 4.4 I.U./ml lactate dehydrogenase, 0.15 mM NADH, and nucleoside diphosphate. With assays using radioactive substrates, lactate dehydrogenase and NADH were omitted.

Adenylosuccinate synthetase activity was assayed with 30 mM sodium Hepes (pH 7.0), 5 mM $MgCl_2$, 10 mM sodium phosphate (pH 7.0), 2 mM adenylosuccinate, and nucleoside diphosphate.

Phosphoenolpyruvate carboxykinase assay mixtures contained 100 mM sodium Pipes (pH 6.8), 50 mM $NaHCO_3$, 5 mM $MnCl_2$, 2 mM dithiothreitol, 2.5 mM phosphoenolpyruvate and ^{14}C-nucleoside diphosphate (final pH = 7.0). Spectrophotometric assays were performed at 340 nm during the preparation of phosphoenolpyruvate carboxykinase from rat liver. In addition, these assay mixtures also contained 0.15 mM NADH, 24 I.U./ml malate dehydrogenase, and 0.10 mM GDP. Phosphoenolpyruvate carboxylase activity was determined with the same reaction mixture components with the omission of GDP.

Nucleoside triphosphate hydrolysis was assayed in reaction mixtures containing 50 mM Tris-acetate (pH 7.5), 5 mM $MgCl_2$, and either [8-^{14}C]GTP or [8-^{14}C]acyclo-GTP.

Preparation of phosphoglycerate kinase from human erythrocytes. The erythrocyte hemolysate was prepared and dialyzed as described by Brownson and Spencer [8]. After the retentate was adjusted to pH 7.1 with NaOH, hemoglobin precipitation and phosphoglycerate kinase precipitation by ethanol

were performed as described by Yoshida and Watanabe [9]. The precipitate was suspended in 20 mM Tris-acetate (pH 7.5) and dialyzed against 200 vol. of the same buffer. The phosphoglycerate kinase activity was further purified by chromatography on Mātrex Gel Red A [10]. This purification procedure resulted in a 9400-fold purification, 55% yield, and a specific activity of 1000 μmoles GTP formed per min per mg protein.

Preparation of phosphoenolpyruvate carboxykinase. The preparation of rat liver supernatant fraction, ammonium sulfate fractionation, calcium phosphate gel titration and DEAE-cellulose chromatography, in that order, were as described by Ballard and Hanson [11]. Exceptions were that 20 mM Tris-acetate (pH 7.5) containing 1 mM dithiothreitol and 1 mM EDTA was used as the buffer, and the ammonium sulfate precipitate was dialyzed against 100 vol. of buffer before titration with calcium phosphate gel. The fractions from DEAE-cellulose chromatography were concentrated in an Amicon concentration cell equipped with a PM-10 membrane. A further purification step on agarose-hexane-GTP (ribose linkage) was performed as described by Colombo *et al.* [12], except that the phosphoenolpyruvate carboxykinase was eluted with 0.5 M NaCl. This preparation resulted in a 400-fold purification, 50% yield, and a specific activity of 4.1 μmoles GTP formed per min per mg protein. Pyruvate kinase and phosphoenolpyruvate carboxylase activities were present at 2% and less than 2%, respectively, of the phosphoenolpyruvate carboxykinase activity.

Preparation of nucleotides. An ion exchange HPLC method [6] was used to separate the various nucleotides for product identification, for verification of substrate purity, and for purification of nucleotides on a preparative scale. Acyclo-GTP was identified as the product of each enzyme reaction by a comparison of authentic acyclo-GTP to the radioactive product using this HPLC system which was capable of separating the phosphorylated products of both guanosine and acyclovir.

Nucleotides were purified as follows. The nucleotide solution was applied to a column containing DEAE-Sephadex A-25 equilibrated with 50 mM ammonium bicarbonate. The volume of gel was chosen to have 20- to 100-fold excess charge capacity compared to milliequivalents of nucleotide. A linear gradient (20 column volumes) of 50–600 mM ammonium bicarbonate was used to elute the nucleotide. To remove ammonium bicarbonate from solution, the pooled nucleotide fractions were dried in a rotary evaporator at 37°, dissolved in water, and dried again. The purity was 99% as judged by HPLC.

GMP kinase was used to produce [8-^{14}C]acyclo-GDP (11 mCi/mmole) and [U-^{14}C]dGDP (482 mCi/mmole) from the corresponding nucleoside monophosphate. The reaction mixtures (200 μl) contained 25 mM Tris-acetate (pH 7.5), 25 mM KCl, 5 mM $MgCl_2$, 1 mM ATP, 0.2 I.U./ml GMP kinase and 1 mM [8-^{14}C]acyclo-GMP or 0.04 mM [U-^{14}C]dGMP. The acyclo-GMP reaction mixture also contained 2 mM phosphoenolpyruvate and 2 I.U./ml pyruvate kinase. The reaction mixtures used to synthesize [8-^{14}C]acyclo-GTP contained 20 mM Tris-acetate (pH 7.5), 10 mM sodium phosphate, 5 mM $MgCl_2$, 4 mM NAD, 4 mM DL-glyceraldehyde-3-phosphate, 8 I.U./ml glyceraldehyde-3-phosphate dehydrogenase, 4.5 I.U./ml phosphoglycerate kinase, and 1 mM [8-^{14}C]acyclo-GDP. The ^{14}C-nucleotides were purified by HPLC, and the potassium phosphate was removed by means of the DEAE-Sephadex procedure described above.

Other procedures. Preparation of Vero cell extracts was performed using the method of Cheng and Ostrander [13]. Protein determinations were performed using the Coomassie reagent as described by Spector [14]. Enzyme kinetic analyses were performed as described by Wilkinson [15] and Cleland [16].

RESULTS

Assays of Vero cell extracts. The activity of each of seven enzymes capable of catalyzing the phosphorylation of GDP and acyclo-GDP was determined in Vero cell extracts from both uninfected cells and cells infected with herpes simplex virus type 1 (Table 2).* In no case were the virus-infected cell extracts found to have significantly higher rates of phosphorylation for either GDP or acyclo-GDP than were the uninfected cell extracts. In addition, the ratio of rates of acyclo-GDP to GDP phosphorylation for each enzyme was similar in extracts from both infected and uninfected cells. Thus, it appeared that none of the enzymes assayed was induced by herpes simplex virus infection and that a virus-induced enzyme was not necessary for acyclo-GDP phosphorylation.

Enzyme kinetics. Kinetic constants were determined for each of the seven partially purified enzymes using GDP, dGDP and acyclo-GDP as substrates (Table 3). The enzymes were relatively non-specific. In general, acyclo-GDP was a less efficient substrate with a lower maximum velocity and a higher K'_m value than either of the natural substrates, GDP or dGDP. The K'_m values for GDP and dGDP were similar to those previously reported [12, 17–22].†

Estimates of acyclo-GTP formation. Estimates of the intracellular acyclo-GDP phosphorylation contributed by each enzyme are presented in Table 4. These estimates take into account [23] the subsaturating concentrations of both acyclo-GDP and the phosphate donor as well as the presence of ADP and GDP which could be alternative substrate inhibitors.

* The rates of GTP or acyclo-GTP hydrolysis were equivalent in extracts from both uninfected Vero cells and HSV-infected cells. In these extracts, GTP had a K'_m of 0.009 mM and a V'_{max} of 25 nmoles/(min · g cells) and acyclo-GTP had a K'_m of 0.2 mM and a V'_{max} of 55 nmoles/(min · g cells). These hydrolysis rates, while insignificant relative to the phosphoglycerate kinase rate, were about the same as the phosphorylation rate observed with some of the other enzymes (Table 2). However, because only initial velocity measurements were obtained, the rates of nucleoside triphosphate hydrolysis had a negligible effect on the rates of GTP and acyclo-GTP formation reported in Table 2.

† Phosphoglycerate kinase from yeast was also tested with results similar to those with the enzyme from human erythrocytes.

Table 2. Enzyme levels in extracts of uninfected and HSV-infected Vero cells*

Enzyme	Vero cells		HSV-infected Vero cells	
	GDP [nmoles · min^{-1} · (g cells)$^{-1}$]	acyclo-GDP [nmoles · min^{-1} · (g cells)$^{-1}$]	GDP [nmoles · min^{-1} · (g cells)$^{-1}$]	acyclo-GDP [nmoles · min^{-1} · (g cells)$^{-1}$]
Phosphoglycerate kinase	200,000	3,200	190,000	3,400
Nucleoside diphosphate kinase	100,000	30	120,000	22
Pyruvate kinase	38,000	46	22,000	16
Phosphoenolpyruvate carboxykinase	1,000	35	740	20
Creatine kinase	360	7.0	300	8.6
Succinyl-CoA synthetase	300	3.0	360	2.4
Adenylosuccinate synthetase	6.2	3.6	2.8	1.0

* The subtrate concentration equaled the K'_m value (Table 3). Values are the average of determinations on two cell extracts.

Table 3. Kinetic constants for nucleoside diphosphates obtained using partially purified enzymes

Enzyme	K'_m* (mM)			V'_{max} [μmoles · min^{-1} · (mg protein)$^{-1}$]			Relative V_{max} (acyclo-GDP)/GDP (%)
	GDP	dGDP	acyclo-GDP	GDP	dGDP	acyclo-GDP	
Adenylosuccinate synthetase	0.0031	0.017	0.31	0.24	0.043	0.10	43
Phosphoenolpyruvate carboxykinase	0.0032	0.032	0.23	4.1	6.5	0.58	14
Succinyl-CoA synthetase	0.0041	0.0081	0.66	8.8	11	0.48	5.4
Phosphoglycerate kinase†	0.33	1.3	2.4	1000	170	47	4.7
Pyruvate kinase	0.72	3.5	2.0	410	71	0.51	0.13
Creatine kinase	1.7	0.23	3.1	20	0.11	0.014	0.068
Nucleoside diphosphate kinase	0.068	0.12	3.1	240	270	0.083	0.035

* The determinations were performed at saturating concentrations of other substrates.
† ADP was also tested as a substrate with these results: K'_m = 0.43 mM, V'_{max} = 960 μmoles · min^{-1} · (mg protein)$^{-1}$, and relative V_{max} (GDP/ADP) = 1.04.

DISCUSSION

None of the seven enzymes investigated showed higher activity in herpes simplex virus-infected cells than in uninfected cells. It was reported previously that one of these enzymes, nucleoside diphosphate kinase, showed no increase in activity upon virus infection [38]. Thus, it appears that phosphorylation of acyclo-GDP is catalyzed by host cell enzymes, as was the phosphorylation of acyclo-GMP [5]. Unlike the other steps in the activation of acyclovir which are catalyzed by single enzymes [4, 5], the phosphorylation of acyclo-GDP may be catalyzed by several enzymes.

From the kinetic data in Table 3 no conclusion can be drawn as to which enzyme is most likely to phosphorylate acyclo-GDP *in vivo*. The estimates in Table 4 for cultured Vero cells were an attempt to account for various quantitative factors. Since substrate and inhibitor concentrations vary according to the metabolic state of cells and tissue, the acyclo-GDP phosphorylating activities of these enzymes could differ from those calculated. No attempt was made to account for the influence of reaction products or other metabolic regulations of enzyme activity. Each of the enzymes in this study catalyzes a reversible reaction. In general, the reactions of succinyl-CoA synthetase [39], pyruvate kinase [34] and phosphoglycerate kinase [18] would be expected to proceed in the direction of nucleoside triphosphate formation under physiological conditions. Nucleoside diphosphate kinase [17] and creatine kinase [19] catalyze freely reversible reactions whereas phosphoenolpyruvate carboxykinase [31] and adenylosuccinate synthetase [40] probably do not catalyze the formation of nucleoside triphosphate under physiological conditions. The salient feature of Table 4 is that one enzyme, phosphoglycerate kinase, has approximately one hundred times as much estimated activity as the next highest, pyruvate kinase. It is interesting to note that two glycolytic enzymes have the highest estimated activity of acyclo-GDP phosphorylation in Vero cells, whereas nucleoside diphosphate kinase, usually considered to be a non-specific nucleoside diphosphate–nucleoside triphosphate interconverting catalyst [17], has

Table 4. Estimated rate of acyclo-GTP formation in cultured Vero cells*

Enzyme	HSV-infected Vero cells [pmoles · min^{-1} · (g cells)$^{-1}$]
Phosphoglycerate kinase	1400
Pyruvate kinase	14
Phosphoenolpyruvate carboxykinase	8.8
Nucleoside diphosphate kinase	6.9
Succinyl-CoA synthetase	2.8
Creatine kinase	0.70
Adenylosuccinate synthetase	0.62

* These values were estimated from the acyclo-GDP phosphorylation rates in Table 2 which were measured in Vero cell extracts at the K'_m concentration of acyclo-GDP. The equations of Segel [23] were used to calculate factors which accounted for non-V'_{max} rates at physiological conditions under which substrates were not saturating and ADP and GDP were present as inhibitors. The following values for *in vivo* levels of the substrates and inhibitors were used for substitution into the equations: 0.7 mM ADP [24–29], 0.04 mM GDP [15, 26–29], 0.01 mM acyclo-GDP [6], 0.0004 mM 1,3-diphosphoglycerate [30], 0.051 mM phosphoenolpyruvate [25, 26], 7 mM bicarbonate ion [31], 3.1 mM free inorganic phosphate [25], 2.5 mM ATP [25] and 4.0 mM creatine phosphate [24, 25]. Succinyl-CoA and adenylosuccinate levels were assumed to be equal to their K_m value [32]. The following values were used as the K'_m of the phosphate donor and other substrates: 0.0022 mM 1,3-diphosphoglycerate [18, 33], 0.08 mM phosphoenolpyruvate for pyruvate kinase [34], 0.13 mM phosphoenolpyruvate for phosphoenolpyruvate carboxykinase [31, 33, 35], 25 mM bicarbonate ion [33, 35], 0.030 mM succinyl-CoA [36], 0.6 mM phosphate for succinyl-CoA synthetase [36], 0.2 mM ATP [17], 0.004 mM adenylosuccinate [22], 8 mM phosphate for adenylosuccinate synthetase [22] and 17 mM creatine phosphate [19]. The K'_m values for GDP (Table 3) were assumed to equal its K_i value for each enzyme [37]. The following values for the K'_m of ADP were assumed to equal the K_i value: 0.35 mM for phosphoglycerate kinase [18, 33], 0.2 mM for pyruvate kinase [20], 0.04 mM for nucleoside diphosphate kinase [17] and 0.05 mM for creatine kinase [19].

far less estimated activity. Phosphoglycerate kinase is the most likely candidate for *in vivo* acyclo-GDP phosphorylation because of its high level in cells (Table 2) and relatively high maximum velocity for acyclo-GDP. Although pyruvate kinase and nucleoside diphosphate kinase are also present at high levels, their high K'_m and low V'_{max} values for acyclo-GDP are less favorable. An attempt was made to do a similar comparison of potential acyclo-GDP phosphorylation in tissues other than Vero cells using published values for enzyme activities in various tissues. Because of the variability in the published activities, a comparison of all seven enzymes from a single tissue could not be obtained. However, it is of interest that human erythrocytes, like Vero cells, possess high levels of phosphoglycerate kinase [9] and nucleoside diphosphate kinase [17] [48,000 and 75,000 nmoles/(min · g tissue), respectively] but relatively low levels of pyruvate kinase [3,100 nmoles/(min · g tissue)] [41]. The estimated rates of acyclo-GDP phosphorylation derived from these values for human erythrocytes are similar to those for Vero cells (results not shown).

The rate of acyclo-GTP formation in Vero cells infected with herpes simplex virus and treated with 100 μM acyclovir was 33 pmoles/(min · g cells) [6]. A comparison of this value to those in Table 4 shows that the theoretical acyclo-GDP phosphorylation potential greatly exceeds that which is actually formed. Since these cellular enzymes have a broad tissue distribution, it would appear that phosphorylation of acyclo-GDP is assured in acyclovir-treated HSV-infected cells.

Acknowledgements—We would like to thank Dr. P. A. Furman, M. H. St. Clair, and C. Lubbers for providing Vero cell cultures, Dr. T. Spector for the gift of adenylosuccinate synthetase, and Dr. J. L. Rideout for the synthesis of acyclo-GDP. We gratefully acknowledge Dr. G. B. Elion for continued support, interest and constructive criticism of this manuscript.

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