J Comp Physiol (1981) 142:501–507

Journal
of Comparative
Physiology · B

Effects of Arginine Phosphate and Octopine on Glycolytic Enzyme Activities from *Sepia officinalis* Mantle Muscle

Kenneth B. Storey*
The Laboratory, Citadel Hill, Plymouth PL1 2PB, England

Accepted January 31, 1981

Summary. 1. Effects of arginine phosphate, arginine, and octopine as modulators of enzyme activity were tested on several partially purified glycolytic enzymes from *Sepia officinalis* mantle muscle.

2. Arginine phosphate, at concentrations within its physiological range in resting muscle, was found to be an inhibitor of hexokinase, phosphofructokinase, α-glycerophosphate dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate kinase, and pyruvate kinase.

3. Octopine, the glycolytic end product of mantle muscle, activated pyruvate kinase and phosphoglucomutase but inhibited hexokinase and phosphofructokinase. Arginine affected only one enzyme, slightly inhibiting phosphoglycerate kinase.

4. The effects of arginine phosphate and octopine were indicated to be direct effects on the glycolytic enzymes themselves as the purification procedures used removed any potential artifact-producing, contaminating enzymes.

5. The potential roles of arginine phosphate and octopine in the regulation of mantle muscle metabolism in resting versus active muscle are discussed.

Introduction

Muscle metabolism in the mantle of cephalopods is based on the aerobic or anaerobic catabolism of glycogen (Storey and Storey 1978, 1979). During burst muscular work or hypoxia in *Sepia officinalis*, the production of energy via anaerobic glycolysis is supplemented by the breakdown of arginine phosphate reserves which can drop from 34 μmol/g wet wt. in the muscle at rest to less than 4 μmol/g wet wt. during stress (Storey and Storey 1979). The depletion of arginine phosphate is met by a quantitative increase in arginine and octopine levels. Octopine levels are less than 0.1 μmol/g wet wt. in resting muscle of *Sepia* but can reach 13 μmol/g wet wt. after exercise (Storey and Storey 1979). In cephalopods, the formation of octopine as an anaerobic end product (octopine dehydrogenase reaction: pyruvate + arginine + NADH $\rightleftharpoons$ octopine + NAD^+) links the products of anaerobic glycogenolysis and phosphagen breakdown. An inverse relationship between the levels of arginine phosphate and octopine is generally found and the large percentage changes in the concentrations of these compounds under different metabolic states in muscle may indicate a potentially important role for these compounds in regulating mantle muscle metabolic rate.

Previous studies have indicated a regulatory role for arginine phosphate in inhibiting invertebrate glycolysis, specifically as an inhibitor of phosphofructokinase (Storey 1976) and pyruvate kinase (Wu et al. 1978). However, some recent studies have produced conflicting reports about the nature of arginine phosphate effects. While some studies show that arginine phosphate has an apparent kinetic effect on pyruvate kinase (competitive inhibition) (Guderley et al. 1976a, b), others indicate that this compound may act via enzyme phosphorylation (Wieser and Lackner 1977). Still other studies have demonstrated that the inhibitory effects of arginine phosphate on pyruvate kinase can, in some cases, be adequately accounted for by the presence of contaminating enzyme activities in unpurified pyruvate kinase preparations (deZwaan and Ebberink 1978; Wu et al. 1979). In the present study, the investigation of arginine phosphate effects on enzymes from *Sepia* mantle muscle was made only after purification of the enzymes to remove all potentially contaminating enzyme activities.

Although several studies have now addressed the role of changing arginine phosphate levels in inverte-

* Permanent address: Institute of Biochemistry, Carleton University, Ottawa, Ontario, Canada K1S 5B6

0174-1578/81/0142/0501/$01.40

brate muscle metabolic control, the role of octopine as a potential regulator of enzyme activities has been ignored. In this study, we have, for the first time, attempted to quantitate the effects of octopine on glycolytic enzymes from *Sepia* mantle muscle. Arginine phosphate was found to have inhibitory effects on a number of glycolytic enzymes, in particular hexokinase and phosphofructokinase. The magnitude of the effect was sufficient to severely reduce the activities of these enzymes at the levels of arginine phosphate found in resting muscle. Octopine was found to inhibit hexokinase and phosphofructokinase while activating pyruvate kinase and phosphoglucomutase. These effects might indicate a regulatory role for octopine in a) potentiating its own synthesis in working muscle, or b) inhibiting glycolytic flux when muscle returns to rest.

Materials and Methods

Chemicals and Animals. All substrates, cofactors, reagents, and coupling enzymes were from Sigma Chemical Co. except for arginine phosphate and creatine phosphate which were from CalBiochem Corp.. Ampholines, pH 3.5–10, were purchased from LKB Products. Cuttlefish, *Sepia officinalis*, were caught by vessels of the Marine Biological Association of the U.K. off Plymouth, England and were held in running seawater for 1–2 weeks and fed prawns until use.

Enzyme Purification. Mantle muscle was homogenized in 5 vol 10 mM Tris-HCl buffer, pH 7.5 containing 20 mM β-mercaptoethanol and 0.1 mM EDTA using a Brinkmann Polytron. The homogenate was centrifuged at 35,000 *g* for 20 min and the pellet discarded. The supernatant was dialyzed against 5 mM Tris-HCl buffer, pH 7.5 containing 20 mM β-mercaptoethanol for 3 h. Portions of the dialyzed supernatant were then loaded onto a 110 ml LKB isoelectrofocusing column and run at pH 3.5–10 at 700 V for 18 h at 4 °C (Vesterberg 1971). The column was then drained, 0.33 ml fractions collected, and assayed for enzyme activities. Peak fractions for each enzyme activity were pooled and dialyzed for 2 h against homogenization buffer. Each partially purified enzyme was then further purified and desalted by gel filtration on a Sephacryl S-200 (40 cm × 0.7 cm) column equilibrated in homogenization buffer.

Mantle muscle hexokinase did not survive isolelectrofocusing and was purified instead by first passing the 35,000 *g* supernatant down the Sephacryl S-200 column and then chromatographing the peak fractions on a Sephadex G-100 column. Peak fractions from this column gave a hexokinase activity free of contaminating enzymes.

The partially purified enzymes were tested for contamination by other enzyme activities which would interfere with the individual coupled assays or with the testing of arginine phosphate, arginine or octopine effects on the enzymes. All enzyme preparations were found to be free of arginine phosphokinase, adenylate kinase, ATPase and octopine dehydrogenase activities. Individual enzyme preparations were also tested and found to be free of: phosphoglucoisomerase (for phosphoglucomutase); phosphoglucoisomerase, aldolase, pyruvate kinase, fructose diphosphatase, and NADH oxidase (for phosphofructokinase); fructose diphosphatase, α-glycerophosphate dehydrogenase, glyceraldehyde-3-P dehydrogenase and NADH oxidase (for aldolase); triosephosphate isomerase, and NADH oxidase (for α-glycerophosphate dehydrogenase); triosephosphate isomerase, phosphoglycerate kinase and NAD reductase (for glyceraldehyde-3-P dehydrogenase); lactate dehydrogenase, and NADH oxidase (for pyruvate kinase).

Enzyme Assays. All assays were done at 23 °C and in 20 mM imidazole buffer, pH 7.5 to facilitate comparisons. The enzyme preparations used contained no contaminating enzymes that would interfere with the enzyme under study or remove or interconvert any of the added metabolites. In experiments involving inhibition of enzyme activity, the product-sampling assay of Newsholme et al. (1970) was employed to ensure that the metabolite effects were specific for the enzymes under investigation. Coupled enzyme assays were essentially those of Storey and Bailey (1978) with enzyme substrate concentrations as follows: *Hexokinase:* 1 mM glucose, 2 mM ATP, 5 mM Mg^{2+}; *phosphoglucomutase:* 2 mM glucose-1-P, 0.04 mM glucose-1,6-diphosphate; *phosphofructokinase:* 5 mM fructose-6-phosphate, 2 mM ATP, 5 mM Mg^{2+}; *aldolase:* 0.5 mM fructose-1,6-diphosphate; *glyceraldehyde-3-phosphate dehydrogenase:* 35 mM P_i, 35 mM KCl, 3 mM dihydroxyacetonephosphate, 2 mM NAD^+, and excess triosephosphate isomerase; *phosphoglycerate kinase:* 2 mM ATP, 2 mM 3-phosphoglycerate; *pyruvate kinase:* 1 mM ADP, 1 mM phosphoenolpyruvate; *α-glycerophosphate dehydrogenase:* 1 mM dihydroxyacetonephosphate, 0.2 mM NADH. Initial tests for metabolite effects on enzymes were made at 20 mM arginine phosphate, 15 mM creatine phosphate, 20 mM arginine, or 10 mM octopine.

Results

Isoelectrofocusing of Mantle Muscle Glycolytic Enzymes

The isolelectric points (pI) of *Sepia* enzymes are summarized in Table 1. The enzymes under study had pI's of 5.30 to 7.00 and occurred as single peaks except for phosphoglucomutase (one major and one minor peak) and α-glycerophosphate dehydrogenase (one major and two minor peaks). For these last two enzymes, only the major peaks were further purified for kinetic study. Octopine dehydrogenase, hexokinase, and ATPase activities did not survive isoelectrofocusing.

Table 1. Isoelectric points of glycolytic enzymes from *Sepia* mantle muscle. Isoelectrofocusing was carried out as outlined in Materials and Methods. The pI's given are the mean values of 3 isofocusing runs and are reproducible within ±3%. The major peak of enzyme activity (when more than one peak was found) is underlined

Enzyme	pI
Arginine phosphokinase	3.20
Adenylate kinase	6.50
Phosphoglucomutase	6.30, 6.77
Phosphofructokinase	3.90
Aldolase	5.30
α-Glycerophosphate dehydrogenase	7.00, 6.75, 7.15
Glyceraldehyde-3-P dehydrogenase	5.75
Phosphoglycerate kinase	6.20
Pyruvate kinase	6.60

Table 2. Summary of arginine phosphate, arginine, and octopine effects on *Sepia* mantle muscle enzymes

Enzyme	Effector	Type of effect	Comments; apparent inhibition constants
Hexokinase	Arginine-P	Negative	Mixed competitive wrt[a] ATP; $K_i = 5$ mM Non-competitive wrt glucose; $K_i = 45$ mM
	Octopine	Negative	Mixed competitive wrt ATP; $K_i = 7$ mM Non-competitive wrt glucose; $K_i = 50$ mM
Phosphoglucomutase	Octopine	Positive	Increases V_{max} activity
Phosphofructokinase	Arginine-P	Negative	Competitive wrt fructose-6-P; $K_i = 5$ mM
	Octopine	Negative	Competitive wrt fructose-6-P; $K_i = 5$ mM
Aldolase	Arginine-P	Negative	Non-competitive wrt fructose-1,6-diP; $K_i = 30$ mM
α-Glycerophosphate dehydrogenase	Arginine-P	Negative	Non-competitive wrt dihydroxyacetone-P; $K_i = 20$ mM
Glyceraldehyde-3-P dehydrogenase	Arginine-P	Negative	Competitive wrt glyceraldehyde-3-P; $K_i = 32$ mM Competitive wrt NAD^+; $K_i = 35$ mM
	Octopine	Negative	Non-competitive wrt glyceraldehyde-3-P; $K_i = 50$ mM
Phosphoglycerate kinase	Arginine-P	Negative	Competitive wrt ATP; $K_i = 15$ mM
	Arginine	Negative	Non-competitive wrt ATP; $K_i = 100$ mM
	Octopine	Negative	Non-competitive wrt ATP; $K_i = 105$ mM
Pyruvate kinase	Arginine-P	Negative	Non-competitive wrt ADP; $K_i = 47$ mM Mixed competitive wrt P-enolpyruvate; $K_i = 20$ mM
	Octopine	Positive	Increases V_{max} activity

[a] wrt = with respect to; K_i's given are apparent K_i's

Enzyme Purification

The use of isoelectrofocusing and gel chromatography techniques in this study resulted in individual enzyme preparations which were purified free of the activities of any of the other enzymes under study. Each preparation was also tested for, and found to be free of, other enzyme activities which would interfere in the testing of the effects of arginine phosphate, arginine, or octopine on enzyme activities. Especially crucial to the interpretation of the metabolite effects seen in this study was the complete removal of the contaminant activities, octopine dehydrogenase, arginine kinase, and adenylate kinase from all of the partially purified enzyme preparations.

Effects of Arginine Phosphate, Arginine, Creatine Phosphate and Octopine on Glycolytic Enzymes

Arginine phosphate affected all but one of the glycolytic enzymes studied and was an inhibitor in all cases. Octopine affected fewer enzymes in total but was an inhibitor of some enzymes and an activator of others. Phosphoglycerate kinase showed some inhibition by arginine but otherwise arginine had no effect on any of the enzymes studied. The effects of creatine phosphate (at 15 mM) on the V_{max} activities of *Sepia* enzymes were tested. Creatine phosphate had no effect on any of the enzymes.

Recently, two laboratories have demonstrated that it is a contaminant present in creatine phosphate preparations, and not creatine phosphate itself, which is responsible for reported enzyme inhibitions by this compound (Fitch et al. 1979; Tornheim and Lowenstein 1979). In order to test the possibility that a similar inhibitor was present in arginine phosphate preparations, the effects of arginine phosphate (at 20 mM) were tested on rabbit muscle phosphofructokinase and pyruvate kinase (purchased from Sigma Chemical Co.). Both of these enzymes are known to be inhibited by the inhibitor present in commercial preparations of creatine phosphate. However, the arginine phosphate solutions produced only minimal inhibition (V_{max} decreased by less than 10%) of either of these enzymes. This indicates that commercial arginine phosphate preparations do not contain a potent, unidentified enzyme inhibitor.

The effects of arginine phosphate, arginine and octopine on individual enzymes from *Sepia* mantle muscle are summarized in Table 2 and were as follows:

Hexokinase. Both arginine phosphate and octopine inhibited hexokinase while arginine had no effect on the enzyme activity. The inhibitors were mixed competitive with respect to ATP, the apparent K_m for ATP rising from 0.092 mM to 0.186 mM in the presence of 10 mM octopine and to 0.32 mM in the presence of 20 mM arginine phosphate. Apparent K_i values (from Dixon plots) were 5 mM for arginine phosphate and 7 mM for octopine. The inhibitors acted non-competitively with respect to glucose (K_m = 0.05 mM) with apparent K_i's of 45 mM for arginine phosphate and 50 mM for octopine.

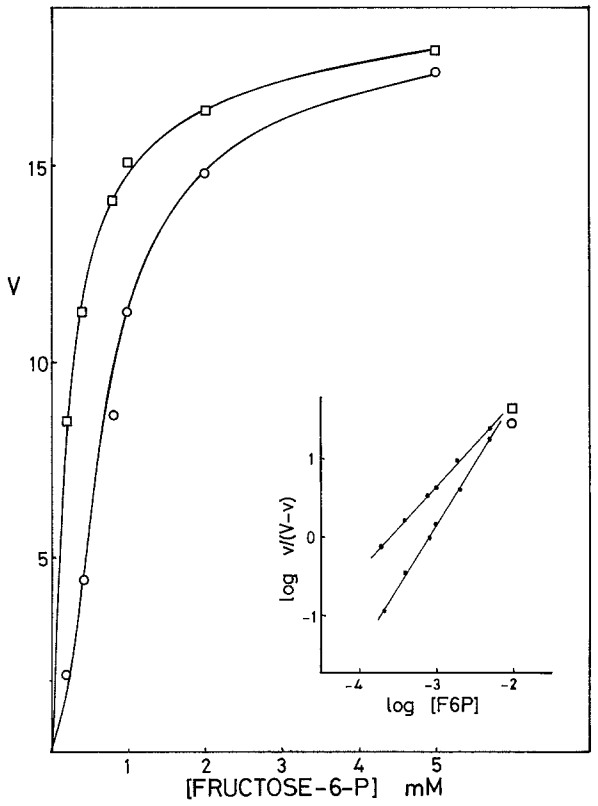

Fig. 1. Effect of AMP on phosphofructokinase from *Sepia* mantle muscle. Assay conditions are as in Materials and Methods. Reaction velocity versus fructose-6-phosphate concentration for: ○, control; □, plus 0.1 mM AMP. Inset is a Hill plot of the data

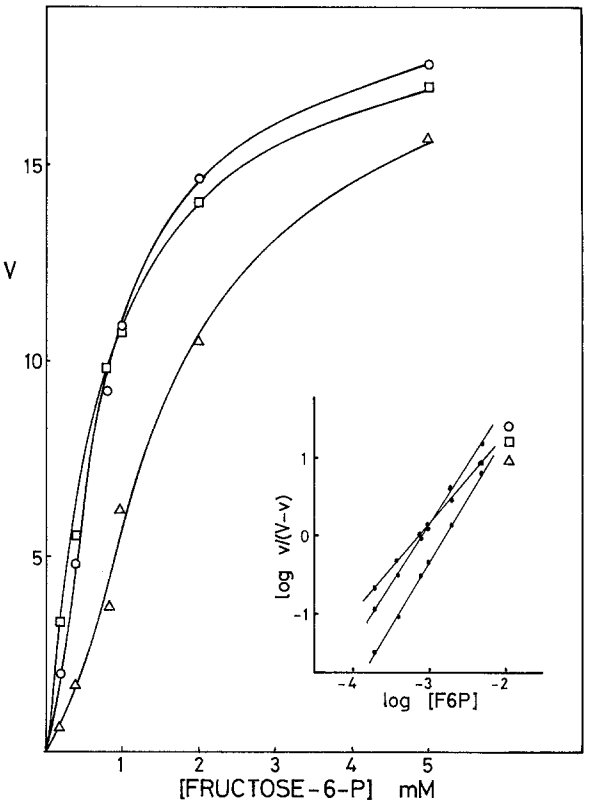

Fig. 2. Effects of octopine and AMP on *Sepia* mantle muscle phosphofructokinase. Assay conditions are as in Materials and Methods. Reaction velocity versus fructose-6-phosphate concentration for: ○, control; △, plus 10 mM octopine; □, plus 10 mM octopine + 0.1 mM AMP. Inset is a Hill plot of the data

Phosphoglucomutase. Arginine phosphate and arginine did not affect phosphoglucomutase activity but octopine, at 10 mM, increased the maximum velocity of the enzyme by 50%. Octopine did not significantly affect either the K_m for glucose-1-phosphate (0.086 mM) or the K_m for glucose-1,6-diphosphate (0.015 mM).

Phosphofructokinase (Figs. 1–3). Both arginine phosphate and octopine inhibited mantle muscle phosphofructokinase while arginine had no effect on enzyme activity. Both inhibitors decreased the affinity of phosphofructokinase for fructose-6-phosphate and also had a small effect on substrate cooperativity (as indicated by the n_H values) (Table 3). The apparent $S_{0.5}$ for fructose-6-phosphate more than doubled in the presence of 10 mM octopine or 20 mM arginine phosphate. AMP, an important activator of phosphofructokinase activity (Uyeda 1979; Storey and Hochachka 1975) reduced the apparent $S_{0.5}$ for fructose-6-phosphate by two-thirds and also greatly reduced n_H. The addition of AMP to the octopine or arginine phosphate-inhibited enzyme reversed the inhibitory effects of these metabolites, returning the $S_{0.5}$ for fructose-6-phosphate to near control levels as well as strongly reducing n_H. However, by comparison with the AMP-activated enzyme, AMP did not completely override the effects of the inhibitors on $S_{0.5}$.

Aldolase. Aldolase was inhibited by arginine phosphate but not affected by either arginine or octopine. Arginine phosphate inhibition was non-competitive with respect to fructose-1,6-diphosphate (K_m = 0.02 mM) with an apparent K_i of 30 mM. This K_i value closely resembles the arginine phosphate levels in resting mantle muscle indicating that aldolase may be about 50% inhibited in the muscle at rest.

α-Glycerophosphate Dehydrogenase. α-Glycerophosphate dehydrogenase was inhibited at high levels of arginine phosphate but was unaffected by octopine or arginine. Arginine phosphate was a non-competitive inhibitor with respect to dihydroxyacetonephosphate (K_m = 0.08 mM) with an apparent K_i of 20 mM.

Glyceraldehyde-3-Phosphate Dehydrogenase. Arginine phosphate was a competitive inhibitor of glyceraldehyde-3-phosphate dehydrogenase with respect to both NAD^+ (apparent K_i = 35 mM) and glyceraldehyde-3-phosphate (apparent K_i = 32 mM). Octopine was a

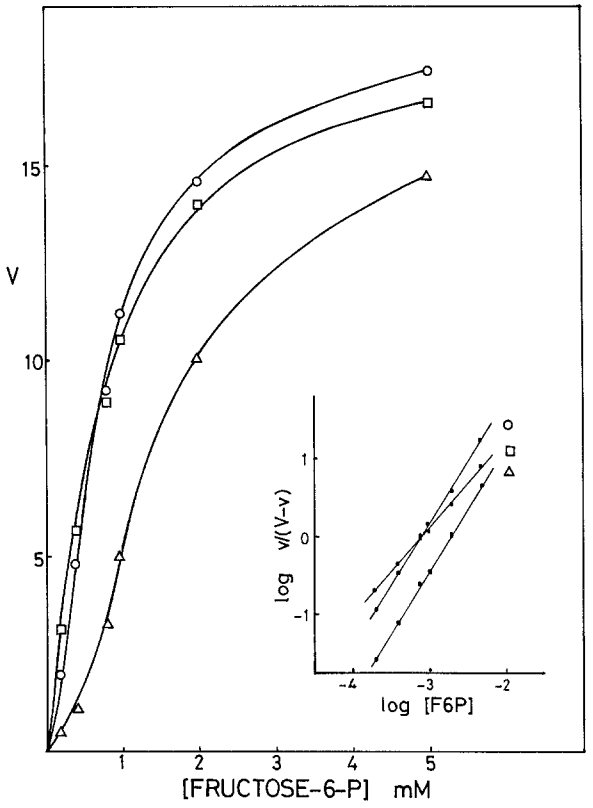

Fig. 3. Effect of arginine phosphate and AMP on *Sepia* mantle muscle phosphofructokinase. Assay conditions are as in Materials and Methods. Reaction velocity versus fructose-6-phosphate concentration for: ○, control; △, plus 20 mM arginine phosphate; □, plus 20 mM arginine phosphate + 0.1 mM AMP. Inset is a Hill plot of the data

non-competitive inhibitor with respect to glyceraldehyde-3-phosphate (apparent $K_i = 50$ mM) while arginine had no effect on enzyme activity. The addition of 20 mM arginine phosphate raised the apparent K_m for glyceraldehyde-3-phosphate from 0.087 (control) to 0.143 mM and increased the apparent K_m(NAD) from 0.21 to 0.33 mM.

Phosphoglycerate Kinase. Phosphoglycerate kinase was the only enzyme studied found to be affected by all three metabolites: arginine phosphate, arginine, and octopine. However, the effects of arginine and octopine occurred at levels of these compounds much higher than their concentrations in vivo. Arginine (apparent $K_i = 100$ mM) and octopine (apparent $K_i =$ 105 mM) were non-competitive inhibitors with respect to ATP. However, arginine phosphate showed inhibitory effects within its physiological concentration range. Arginine phosphate inhibition was competitive with respect to ATP (apparent $K_i = 15$ mM), 20 mM arginine phosphate increasing the apparent K_m(ATP) from 0.138 to 0.34 mM.

Pyruvate Kinase. Pyruvate kinase activity was inhibited by arginine phosphate, activated by octopine, and unaffected by arginine. Arginine phosphate was a mixed competitive inhibitor with respect to phosphoenolpyruvate (apparent $K_i = 20$ mM), 20 mM arginine phosphate raising the apparent K_m for phosphoenolpyruvate from 0.07 to 0.11 mM. Fructose-1,6-diphosphate, a feed-forward activator of pyruvate kinase, could reverse, but not completely override, the inhibitory effects of arginine phosphate. The addition of 0.02 mM fructose-1,6-diphosphate reduced the K_m for phosphoenolpyruvate of the unihibited enzyme to 0.055 mM and lowered the K_m of the arginine phosphate-inhibited enzyme to 0.07 mM. Fructose-1,6-diphosphate also increased the apparent K_i for arginine phosphate to 82 mM, a 4-fold increase. With regard to ADP kinetics (K_m(ADP) = 0.143 mM), arginine phosphate was a non-competitive inhibitor, the apparent K_i being 47 mM. The effect of octopine on pyruvate kinase was to increase the V_{max} activity of the enzyme, the addition of 10 mM octopine increasing activity to 125% of control levels. Octopine, however, did not alter the K_m's for either phosphoenolpyruvate or ADP.

Table 3. *Sepia* mantle muscle phosphofructokinase: Effects of arginine phosphate, octopine, and AMP on substrate affinity and cooperativity

Addition	$S_{0.5}$(Fructose-6-P)	n_H
Control	0.80	1.53
+0.1 mM AMP	0.26	1.07
+20 mM Arginine-P	1.92	1.60
+20 mM Arginine-P/0.1 mM AMP	0.82	1.13
+10 mM Octopine	1.62	1.63
+10 mM Octopine/0.1 mM AMP	0.75	1.15

Assay conditions are: 20 mM imidazole buffer, pH 7.5, 2 mM ATP, 5 mM Mg^{2+}, 0.1 mM NADH, varying fructose-6-P levels, and excess aldolase, triose-P isomerase, and α-glycero-P dehydrogenase

Discussion

The results of this study show that both arginine phosphate and octopine have kinetic effects on a number of glycolytic enzymes in the mantle muscle of *Sepia*. The arginine phosphate and octopine effects occurred, for several of the enzymes tested, at levels well within the normal physiological concentration range of these metabolites in mantle muscle. Between the resting and active metabolic states in mantle muscle, the changes in the levels of these two metabolites (on both an absolute and a percentage basis) are the largest seen in *Sepia* muscle (Storey and Storey 1979). Effects of these two compounds on the activities of glycolytic enzymes could, therefore, be of great importance in the regulation of glycolytic flux in vivo.

By contrast, arginine and creatine phosphate had no effects on the enzymes tested (with the exception of an arginine effect on phosphoglycerate kinase) despite their close structural similarity to arginine phosphate and octopine. These compounds, then, effectively act as 'controls' against which to compare the effects of the other two metabolites. In the one case where arginine affected enzyme activity, the slight inhibition of phosphoglycerate kinase activity occurred only at very high arginine concentrations, well above the physiological range of arginine in mantle muscle.

Certain recent studies have argued that the observed arginine phosphate inhibition of invertebrate pyruvate kinase is not due to a direct effect of this metabolite upon enzyme activity but stems instead from the presence of contaminating enzymes in unpurified pyruvate kinase preparations (deZwaan and Ebberink 1978; Wu et al. 1979). For example, in the presence of contaminating arginine kinase, added arginine phosphate will swiftly utilize ADP and pyruvate kinase activity will be inhibited by a lack of ADP as substrate. In the present study, the techniques of isoelectrofocusing and gel filtration used in purifying the enzymes climinated all enzyme activities (specifically ATPase, arginine kinase, adenylate kinase, and octopine dehydrogenase) which could interfere with the determination of arginine phosphate, arginine, or octopine effects on enzymes. Therefore, the metabolite effects seen in this study cannot be linked to the presence of contaminating enzymes. In addition, the use of the product sampling method of Newsholme et al. (1970) ensured that the observed effects were not effects upon coupling enzymes but were specific effects of arginine phosphate (or octopine) upon the enzymes under study. Another recent report on the effects of arginine phosphate on snail pyruvate kinase suggested that arginine phosphate may modify enzyme activity via a protein kinase-mediated phosphorylation mechanism (Wieser and Lackner 1977). However, the purification procedures used in this study have likely eliminated this possibility for the *Sepia* mantle muscle enzymes. All evidence in the present study indicates that the effects of arginine phosphate or octopine on mantle muscle enzyme activities are 'direct' effects, i.e. competitive or non-competitive inhibitions or activations of enzymes.

The effects of arginine phosphate on glycolytic enzyme activities fall into three groups. In the first group are enzymes unaffected by arginine phosphate (at 20 mM). In this study, only phosphoglucomutase falls into this group. In the second group are enzymes which show inhibition by arginine phosphate at high concentrations (with K_i's of 15 mM or greater). This group includes aldolase, glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate kinase, pyruvate kinase, and α-glycerophosphate dehydrogenase. These enzymes could be partially inhibited (by 50% or more) in resting muscle when arginine phosphate concentration is high. The effects on α-glycerophosphate dehydrogenase indicate a coordinated regulation by arginine phosphate of both glycolysis and the α-glycerophosphate cycle, the means used in cephalopod muscle to transfer cytoplasmic reducing equivalents into the mitochondria. In the third group are enzymes which are strongly inhibited by arginine phosphate. Hexokinase and phosphofructokinase are in this group. It is in this group that the effects of arginine phosphate could be of the greatest importance in regulating metabolism in vivo. These two enzymes are irreversible, rate-limiting steps in glycolysis in essentially all tissues and in cephalopod muscle have been shown to be 'cross-over' points in metabolism during the activation of muscle work (Storey and Storey 1978). Arginine phosphate inhibition of hexokinase could limit the phosphorylation and use of blood glucose as an energy source for basal metabolism under resting conditions in muscle thus sparing glucose for use by other tissues such as brain.

The inhibition of phosphofructokinase at high arginine phosphate concentrations will serve to effectively 'turn off' glycolysis in resting mantle muscle which performs only slow ventilatory movements. Indeed, when phosphofructokinase activity is monitored in vitro under substrate conditions simulating those found in resting mantle muscle (fructose-6-phosphate = 0.01 mM, ATP = 8.7 mM; Storey and Storey 1979), the addition of 20 mM arginine phosphate causes a 95% inhibition of enzyme activity (K. Storey, unpublished observations). However, during muscle work, several factors will combine to produce a rapid activation of phosphofructokinase leading to an increased glycolytic flux. In the initial moments of muscle work, ATP is rapidly hydrolyzed. This initiates two responses: a) a breakdown of arginine phosphate reserves to supplement ATP levels and b) a rapid increase in cellular AMP levels due to the action of adenylate kinase. Both of these responses will contribute to a rapid and complete activation of phosphofructokinase. Decreased arginine phosphate concentration would relieve the inhibition of phosphofructokinase while an elevation of AMP levels would not only activate phosphofructokinase via AMP effects alone but would also reverse residual arginine phosphate inhibition of the enzyme.

The effects of octopine on glycolytic enzyme activities are somewhat more varied than those of arginine phosphate but again three groups of effects can be identified. In the first group are those enzymes which are not inhibited by octopine or inhibited only at very high, non-physiological concentrations of octo-

pine. This group includes aldolase, glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate kinase, and α-glycerophosphate dehydrogenase. In the second group are enzymes which were strongly inhibited by octopine: hexokinase and phosphofructokinase. The third group contains enzymes whose activities were activated by octopine and in this study includes phosphoglucomutase and pyruvate kinase.

The inhibitory effects of octopine on hexokinase and phosphofructokinase activities could have important consequences in vivo. Octopine inhibition of hexokinase is competitive with ATP so that the combination of decreased ATP levels in working or hypoxic muscle with increasing octopine concentrations as glycolysis proceeds (Storey and Storey 1979) would result in progressive inhibition of hexokinase. The result of this would be to inhibit the further utilization of blood glucose (which drops quite dramatically during stress; Storey et al. 1979) by mantle muscle and promote instead muscle glycogenolysis. The inhibitory effects of octopine on phosphofructokinase might appear to result in an inhibition of this key glycolytic enzyme during muscular work. However, this inhibition can be reversed by modest levels of AMP, at concentrations lower than those found in vivo in working muscle (Storey and Storey 1978). The activity of phosphofructokinase in working muscle is unlikely, therefore, to be greatly affected by rising tissue octopine levels. However, octopine effects on phosphofructokinase activity could play a role in dampening glycolytic flux at the cessation of muscular work when octopine concentrations are highest in muscle and arginine phosphate levels have not yet been restored. Octopine inhibition of phosphofructokinase could replace arginine phosphate inhibition during the recovery period. In addition, octopine inhibition of phosphofructokinase and the consequent dampening of glycolytic flux could act indirectly to promote the aerobic oxidation of accumulated octopine during the recovery period in muscle.

The effects of octopine as an activator of enzyme activities were not dramatic, a 50% and 25% increase in activity for phosphoglucomutase and pyruvate kinase, respectively, but could be of some physiological significance in vivo. During anaerobic glycogenolysis in working muscle, octopine effects on pyruvate kinase could have a net effect of feed-forward activating the synthesis of octopine. Octopine activation of phosphoglucomutase could have its effect during the recovery phase in mantle muscle in promoting the resynthesis of glycogen stores.

In summary then, this study has demonstrated that both arginine phosphate and octopine can modulate the activities of mantle muscle glycolytic enzymes in vitro. The effects of these two compounds may be of major importance in regulating glycolytic flux in vivo. The inhibitory effects of arginine phosphate on *Sepia* muscle enzymes occurred in the absence of 'artifactual' contaminating enzymes which have marred the intrepretation of phosphagen effects on enzymes in several previous studies.

The author wishes to express his gratitude to the Marine Biological Association of the United Kingdom, and in particular to Dr. P. Dando, for providing animals, equipment, and laboratory facilities for this study. The work was supported by NSF grant no. PCM-77 19033 and an N.S.E.R.C. operating grant.

References

Fitch CD, Chevli R, Jellinek M (1979) Phosphocreatine does not inhibit rabbit muscle phosphofructokinase or pyruvate kinase. J Biol Chem 254:11357–11359

Guderley HÉ, Storey KB, Fields JHA, Hochachka PW (1976a) Catalytic and regulatory properties of pyruvate kinase isozymes from octopus mantle muscle and liver. Can J Zool 54:863–870

Guderley HE, Storey KB, Fields JHA, Hochachka PW (1976b) Pyruvate kinase from *Oplophorus gracilirostris*. Comp Biochem Physiol 55B:475–478

Newsholme EA, Sugden P, Opie L (1970) The apparent inhibition of phosphofructokinase by reduced nicotinamide-adenine dinucleotide: A problem of coupled enzyme assays. Biochem J 119:787–789

Storey KB (1976) Purification and properties of adductor muscle phosphofructokinase from the oyster, *Crassostrea virginica*. The aerobic/anaerobic transition: role of arginine phosphate in enzyme control. Eur J Biochem 70:331–337

Storey KB, Bailey E (1978) The intracellular distribution of enzymes of carbohydrate degradation in the fat body of the adult male cockroach. Insect Biochem 8:73–79

Storey KB, Hochachka PW (1975) Redox regulation of muscle phosphofructokinase in a fast swimming squid. Comp Biochem Physiol 52B:159–164

Storey KB, Storey JM (1978) Energy metabolism in the mantle muscle of the squid, *Loligo pealeii*. J Comp Physiol 123:169–175

Storey KB, Storey JM (1979) Octopine metabolism in the cuttlefish, *Sepia officinalis*. Octopine production by mantle muscle and role of the glycolytic end product as an aerobic substrate for non-muscular tissues. J Comp Physiol 131:311–319

Storey KB, Storey JM, Johansen K, Hochachka PW (1979) Octopine metabolism in *Sepia officinalis:* Effect of hypoxia and metabolite loads on the blood levels of octopine and related compounds. Can J Zool 57:2331–2336

Tornheim K, Lowenstein JM (1979) Creatine phosphate inhibition of heart muscle lactate dehydrogenase and muscle pyruvate kinase is due to a contaminant. J Biol Chem 254:10586–10587

Uyeda K (1979) Phosphofructokinase. Adv Enzymol 48:193–244

Vesterberg O (1971) Isoelectrofocusing of proteins. Methods Enzymol 22:389–412

Wieser W, Lackner R (1977) Inhibition of the pyruvate kinase of *Helix pomatia* L. by phospho-L-arginine. FEBS Lett 80:299–302

Wu SWN, Wong SC, Yeung D (1978) Comparative studies of vertebrate and invertebrate pyruvate kinases. Comp Biochem Physiol 61B:93–98

Wu SWN, Wong SC, Yeung D (1979) Apparent inhibition of pyruvate kinase by phosphocreatine and phosphoarginine. Comp Biochem Physiol 63B:29–34

Zwaan A de, Ebberink RHM (1978) Apparent inhibition of pyruvate kinase by arginine phosphate. A problem of kinetic studies on partially purified extracts. FEBS Lett 89:301–303