INVESTIGATIONS INTO THE CROSS REACTIVITY AND INHIBITION OF POTENTIAL GUANIDINE SUBSTRATES FOR 'ATP'-GENERATING KINASES

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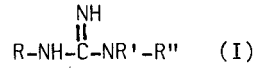
N-Phosphorylated guanidines (phosphagens (I,R=H_2PO_3)), found in living tissues, are natural substrates for those kinases which regulate the levels of adenosine triphosphate (ATP). Vertebrates possess phosphocreatine (I,R=H_2PO_3,R'=Me,R"=CH_2CO_2H) and creatine kinase as their sole phosphagen and kinase, whereas in invertebrates, phosphoarginine (I,R=H_2PO_3,R'=H,R"=$(CH_2)_3CH(NH_2)CO_2H$) and its corresponding kinase predominate. In certain helminths (Annelida), novel phosphagens and kinases exist (Thoai and Robin, 1969), the phosphagen's structure being based on a phosphodiester, exemplified by phospho-opheline (I,R=H_2PO_3,R'=H,R"=$(CH_2)_2OP(O)OHOMe$).



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Since many parasites possess non-creatine based phosphagen kinases it seemed appropriate to perform fundamental biochemical investigations in order to determine the specificity of these kinases towards potential substrates. This may help to elucidate important structural requirements needed for substrate-enzyme recognition, which must be known if possible inhibitors are to be developed. Initially, research hasconcentrated on arginine kinase owing to its availability. The assessment of cross reactivity and/or inhibition by the addition of various possible substrates (see Table) has been investigated by a continuous spectrophotometric assay (adaptation of Barrett and Lloyd's method, 1981), which utilises a coupled NADH-NAD^+ dependent enzyme indicator reaction to monitor phosphate transfer.

TABLE: Effect of potential substrates on the degree of inhibition of arginine phosphorylation and cross reactivity of arginine kinase.

Potential substrate (10mM)	Cross reactivity	% Inhibition
Creatine	none	22±8
Glycocyamine	"	13±7
Taurocyamine	"	41±9
2-Guanidinoethylphosphate	"	60±1
Opheline	"	4±8
2-Guanidinoethyl-neopentyl-phosphate	"	10±9
2-Guanidinopropionic acid	"	11±12

Arginine kinase has been shown to be very specific for its natural substrate arginine, but not significantly inhibited by potential substrates with the exception of taurocyamine (I,R=R'=H, R"=$(CH_2)_2SO_3H$) and 2-guanidinoethylphosphate (I,R=R'=H, R"=$(CH_2)_2OP(O)(OH)_2$). For the latter, Km (apparent) for arginine increases by 38% and Vmax decreases by 44%. A different pattern of inhibition is observed for pyruvate kinase (coupled indicator reaction) where Km (apparent) for ADP is increased by 300% and Vmax decreased by 33%. This indicates that the 2-guanidinoethyl-phosphate inhibition observed in the arginine kinase assay was in part due to inhibition of the coupled reaction.

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Thoai, N.V. and Robin, Y. (1969) Chemical Zoology 4: 163-203.
Barrett, J. and Lloyd, G.M. (1981) Parasitology 82: 11-16.