# NUCLEOTIDE ACTIVATION OF PHOSPHORYLASE b IN THE PRESENCE AND ABSENCE OF SALMINE

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Phosphorylase b is activated to its maximum velocity by $1x10^{-3}$M AMP (Cori et al, 1953). Similar concentrations of IMP activate the enzyme to about 10%, however IMP or less than saturating concentrations of AMP completely activate the enzyme in the presence of salmine (Krebs, 1954). The following work on the activation of phosphorylase b by structural analogues of AMP is part of a study designed to explore the chemical nature of the nucleotide binding site and clarify the action of salmine on activation. Preliminary studies of nucleotide activation indicate a high degree of specificity for AMP which is markedly altered in the presence of salmine. Nucleotide activation of phosphorylase b in the presence of salmine has manifested the critical importance of the 5'-phosphate and less rigid requirements for the pyrimidine and imidazole rings of the purine base.

## Materials and Methods

Phosphorylase b was isolated from rabbit skeletal muscle by the method of Fischer and Krebs (1958). Third crystals were dissolved in a pH 6.8, 0.04M glycerophosphate, $5x10^{-4}$M EDTA buffer prior to AMP removal by Norite treatment.

Enzymatic activity was measured according to the method of Illingsworth and Cori (1953) with the following changes. The enzymatic reaction was run in the previously described buffer using 0.1% glycogen. The compounds examined for activation were $9.4\pm0.2x10^{-4}$M in the final reaction media. All activity measurements were made against a blank containing substrate, enzyme and salmine

(where applicable) in order to correct for residual phosphorylase activity under these conditions. As noted by Cori et al. (1943), assay of phosphorylase under the conditions described above does not yield zero or first order kinetics. For this reason, all assay procedures were identical except for structural analogues and enzyme concentrations employed. The ability of structural analogues to activate the enzyme is expressed as the percent of AMP mediated activity, calculated from the moles of inorganic phosphate released in five minutes, with the same concentration of phosphorylase b.

Inosine 5'-chloromethylphosphonate and inosine 5'-phosphofluoridate were gifts of Dr. Alexander Hampton, University of Alberta. 3-β-D-ribofuranosyl-adenine 5'-monophosphate (iso-AMP) was a gift from Dr. Nelson Leonard, University of Illinois. The additional nucleotides were commercial samples of highest available purity. Due to limited availability of some of the compounds further purification was not attempted unless indicated.

## Results and Discussion

The percent activation data for the structural analogues of AMP on phosphorylase b is reproducible to within ± 5 percent activity units. Consistent data was obtained for different enzyme preparations and was reproducible over a four fold range of enzyme concentration.

In view of the enhanced activation of phosphorylase b by AMP and IMP in the presence of salmine, structural analogues of these nucleotides were used for the data in Table I. The failure of adenosine to activate phosphorylase b demonstrates the importance of the phosphate group on AMP. An examination of the results in Table I indicates a high degree of specificity of the enzyme site which binds the phosphate moiety. Examination of the structural formula for those analogues which failed to activate the enzyme suggests the possible importance of a hydrogen on the 5'-phosphate group which could readily hydrogen bond to the enzyme.

The chemical nature of the enzyme-adenine ring interaction which leads to active phosphorylase b in the presence and absence of salmine is not readily

apparent from the data of Table II. A number of general characteristics, however,

Table I

STRUCTURAL ANALOGUES OF AMP AND IMP

Modification on the Ribose 5-phosphate Moiety[a]

| compound | % activity without salmine | % activity with salmine |
|---|---|---|
| adenosine 5'-monophosphate (control) | 100 | 132 |
| adenosine 5'-phosphoramidate | trace[b] | 83 |
| adenosine 5'-acetate | 0 | 0 |
| adenylic acid, 3'(2') mixed isomer | 0 | 0[c] |
| adenosine 3',5'-cyclic phosphate | 0 | 0 |
| adenosine 5'-triphosphate | 0 | 0 |
| adenosine | --- | 0[c] |
| inosine 5'-monophosphate (control) | trace | 89 |
| inosine 5'-phosphofluoridate | 0 | 0 |
| inosine 5'-chloromethylphosphonate | 0 | 0 |

[a] Ribose-5'-phosphate failed to activate phosphorylase b with or without salmine (Krebs, 1954).

[b] Values from 5 to 12 percent.

[c] E. G. Krebs (1954).

are suggested. In the absence of salmine, little activity is observed for any of the analogue compounds except iso-AMP. In the presence of salmine this nucleotide is again unique, showing even greater ability than AMP to activate phosphorylase. Specificity at the AMP binding site is apparent from the activation data for modifications of the purine ring structure. Enzyme activation is sensitive to alteration (8-azaguanosine 5'-phosphate and iso-AMP) and complete deletion (uridine 5'-monophosphate and cytidine 5'-monophosphate) of the imidazole ring.

Until the mechanism by which salmine alters the high specificity for

Table II

STRUCTURAL ANALOGUES OF AMP

Modifications on the Adenine Ring

| compound | % activity without salmine | % activity with salmine |
|---|---|---|
| adenosine 5'-monophosphate (control) | 100 | 132 |
| 3-β-D-ribofuranosyladenine 5'-monophosphate | 87 | 151 |
| 6-mercaptopurine riboside 5'-phosphate | trace | 94 |
| inosine 5'-monophosphate[a] | trace | 89 |
| guanosine 5'-monophosphate[a] | trace | 61 |
| uridine 5'-monophosphate[a] | trace | 41 |
| 8-azaguanosine 5'-monophosphate | trace | 39 |
| cytidine 5'-monophosphate[a] | trace | 25 |
| 6-azauridine 5'-monophosphate | 0 | 0 |
| xanthosine 5'-monophosphate | 0 | 0 |

[a] Purified by Dowex 1 column chromatography.

nucleotide activators of phosphorylase b is elucidated, the significance of the chemistry of activation in the presence of salmine relative to the requirements to activate phosphorylase b in its absence will remain obscure. It should be noted that only those compounds which do not activate the native enzyme are not affected by salmine. This suggests that salmine is enhancing an activating ability inherent to certain nucleotide structures rather than instigating a different activation mechanism. Krebs (1954) reported a decrease of the $K_M$ values for IMP and AMP in the presence of salmine. Investigation of the effect of salmine on the dissociation constants of various analogues is currently in progress to determine if there is a parallel between structural specifications of the nucleotides for binding and their requirements for activation.

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