The Activation and Inhibition of 5-Nucleotidase

By Z. AHMED and J. L. REIS
Department of Chemical Pathology, Postgraduate Medical School, London, W. 12

(*Received* 27 *August* 1957)

The activation and inhibition of 5-nucleotidase have not been much studied. According to previous papers by Reis (1937, 1950) magnesium activates this enzyme to a much smaller extent than it does the non-specific phosphomonoesterase 'alkaline phosphatase'. Kaye (1955) mentions that zinc inhibits 5-nucleotidase, but his evidence is scanty.

We have limited our study to human tissues, and have used aorta wall as a source of 5-nucleotidase, where it is free from non-specific phosphatase activity, and placenta, which has both enzymes.

EXPERIMENTAL

pH. The enzyme-activity measurements were carried out at pH 7·5 (Reis, 1951). This pH is near the optimum for 5-nucleotidase (pH 7·8), whereas the non-specific phosphatases display very low activity at this hydrogen-ion concentration.

Substrates. By using phenyl phosphate as substrate we obtained values for the non-specific phosphatase activity. With 'muscle' adenylic acid (adenosine 5'-monophosphate) the values represent the sum of 5-nucleotidase and non-specific phosphatase activities. By subtraction of the latter the probable 5-nucleotidase activity is obtained.

Enzyme solutions. Human aorta was taken at autopsy about 24 hr. after death. The extracts were prepared from the media after removal of the intima and adventitia. Human placenta was obtained within 2 hr. of its delivery, and cleaned and perfused with tap water. The tissues were homogenized in a blender with 10 vol. of water and left for 3 days at room temperature with a few drops of chloroform. After centrifuging, the supernatant was used as tissue extract.

Estimations. Centrifuge tubes containing 1·4 ml. of 0·05 M-veronal buffer of pH 7·5, 0·2 ml. of 0·01 M-substrate (sodium phenyl phosphate or adenylic acid, adjusted to pH 7·5) and 0·2 ml. of activator or inhibitor solution (pH 7·5) were placed in a water bath for about 5 min. at 38°. Then 0·2 ml. of enzyme solution was added (tissue extract) and the sample incubated. To stop enzyme activity 4 ml. of 5% (w/v) trichloroacetic acid was added. After 5 min. the sample was centrifuged and the free phosphate estimated by the method of Fiske & Subbarow (1925).

Table 1. *Activation and inhibition of abrtic 5-nucleotidase*

The results are represented as μg. of P liberated. They correspond to the activity of 20 mg. of tissue during 20 min., at pH 7·5 and 38°. The phenyl phosphate hydrolysis under these conditions was only 0·2 μg. of P, so it was ignored and the adenylic acid hydrolysis regarded as due only to 5-nucleotidase activity.

	Ions added (final concn. mM)							
	None	Mg^{2+}	Mn^{2+}	Zn^{2+}	Co^{2+}	Ni^{2+}	PO_4^{3-}	Adenosine
Adenylic acid hydrolysis (5-nucleotidase activity)	9·1	10·2	14·5	1·0	2·2	0·3	8·6	8·9

Table 2. *Activation and inhibition of placental phosphatases*

The results are represented as μg. of P liberated. They correspond to the activity of 10 mg. of tissue during 20 min., at pH 7·5 and 38°.

	Ions added (final concn. mM)										
	None	Mg^{2+}	Mn^{2+}	Ca^{2+}	Ba^{2+}	Co^{2+}	Cu^{2+}	Zn^{2+}	Ni^{2+}	CN^-	F^-	PO_4^{3-}
Phenyl phosphate hydrolysis (non-specific phosphatase activity)	9·0	9·7	10·2	9·7	10·2	10·2	9·2	9·0	9·0	9·0	10·0	4·8
Adenylic acid hydrolysis (non-specific phosphatase and 5-nucleotidase activity)	22·3	22·8	27·1	22·0	25·6	14·6	14·6	9·4	9·0	22·8	23·5	18·9
Hydrolysis due to 5-nucleotidase activity (calc. by subtraction)	13·3	13·3	16·9	12·3	15·4	4·4	5·4	0·4	0·0	13·8	13·5	14·1

RESULTS

In Tables 1 and 2 the results obtained with different ions are shown. The influence of different ions on the 5-nucleotidase from aorta and from placenta (where it occurs together with phosphatase) is the same, which points to the identity of aortic and placental 5-nucleotidase. Magnesium, the well-known activator of phosphatases, has only a slight action on 5-nucleotidase. The highest activation of 5-nucleotidase was caused by manganese (60 %).

A very strong inhibition was obtained with Zn^{2+} and Ni^{2+} ions. Zince sulphate precipitated the enzyme from solution, so it could not be excluded that this precipitation was the cause of inhibition. Nickel did not cause any precipitation and inhibited 5-nucleotidase sometimes to a greater extent than zinc did. A millimolar solution of nickel salts inhibited 5-nucleotidase almost totally, while not affecting at all non-specific phosphatase activity (Figs. 1, 2).

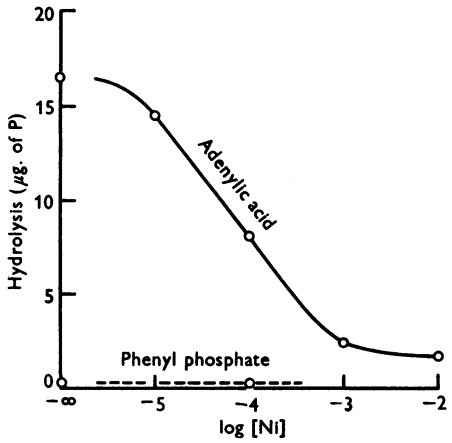

Fig. 1. Inhibition of aortic 5-nucleotidase by nickel. (20 mg. of tissue, 30 min., pH 7·5, 38°.)

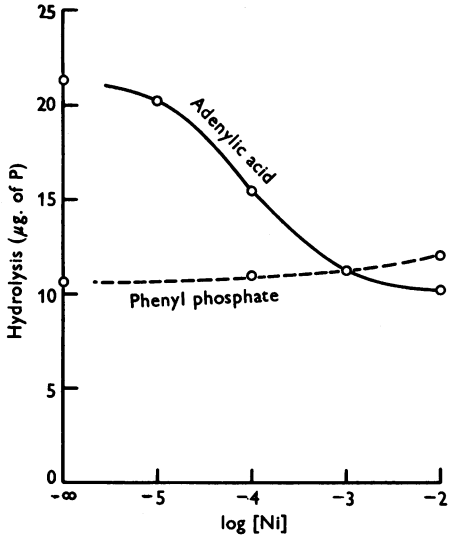

Fig. 2. Inhibition of placental 5-nucleotidase by nickel. (10 mg. of tissue, 20 min., pH 7·5, 38°.)

This inhibition by Ni^{2+} ions could be used for estimations of 5-nucleotidase activity. The present method consists in subtracting from the rate of hydrolysis of adenylic acid the rate of hydrolysis of phenyl phosphate at pH 7·5, on the assumption that adenylic acid is hydrolysed by 5-nucleotidase and non-specific phosphatase, whereas phenyl phosphate is hydrolysed by non-specific phosphatase only. Since the alkaline phosphatase hydrolyses phenyl phosphate more quickly than it hydrolyses many other phosphoric esters (King & Delory, 1939), including adenylic acid, an error is introduced by subtracting too high values, thus obtaining too low values for 5-nucleotidase activity. By using β-glycerophosphate for the same purpose an error is made in the opposite direction, as this ester is hydrolysed by alkaline phosphatase more slowly than is adenylic acid. What we should subtract is the rate of hydrolysis of adenylic acid due only to the non-specific phosphatase. A more direct estimation of 5-nucleotidase can be obtained by using inhibition of 5-nucleotidase by nickel.

Inhibition by phosphate has also been tried, as phosphate is a competitive inhibitor of phosphatases. Our results show that 5-nucleotidase is much less inhibited by phosphate than is the alkaline phosphatase. Adenosine in concentration equal to that of the substrate had no inhibitory action.

SUMMARY

1. 5-Nucleotidase is activated strongly by Mn^{2+} ions.

2. Nickel (Ni^{2+}) and Zn^{2+} ions inhibit 5-nucleotidase activity. Ni^{2+} in millimolar concentration almost completely inhibits the 5-nucleotidase, whereas non-specific phosphatase activity is not affected. The possibility of using nickel inhibition for 5-nucleotidase estimations is discussed.

3. Phosphate inhibits 5-nucleotidase to a much smaller extent than it does alkaline phosphatase.

The authors wish to thank Professor E. J. King for his advice.

REFERENCES

Fiske, C. H. & Subbarow, Y. (1925). *J. biol. Chem.* **66**, 375.
Kaye, M. A. G. (1955). *Biochim. biophys. Acta*, **18**, 456.
King, E. J. & Delory, G. E. (1939). *Biochem. J.* **33**, 1185.
Reis, J. L. (1937). *Enzymologia*, **2**, 110.
Reis, J. L. (1950). *Biochem. J.* **46**, xxi.
Reis, J. L. (1951). *Biochem. J.* **48**, 548.