Histochemical Studies on the Role of Magnesium in Phosphatase Activities

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Introduction

The magnesium ion has long been known as an activator of alkaline phosphatases. However, the role of the magnesium ion in phosphatase activities seems to be rather complex. It is well known that the elimination of magnesium ions from enzyme materials by dialysis will cause loss of enzyme activity and that the addition of magnesium ions will permit recovery of activity. The binding of magnesium in the enzyme is apparently not firm.

In the histochemical demonstration of phosphatase first devised by H. Takamatsu in 1938¹⁾²⁾³⁾, the substrate mixture does not contain magnesium. In Takamatsu's method, a prolongation of the incubation time or the presence of a high concentration of substrate causes an intense precipitation of the reaction product. The distribution of the positive sites obtained by the method was compared with the results obtained using a substrate solution containing magnesium. The data were reported from Takamatsu's laboratory in 1952 by Shinoto⁴⁾⁵⁾. The following results were noted:

- (1) Certain sites in various tissues were positive in the experiments regardless of the presence of magnesium in the substrate mixture. We called the enzyme in these sites as Active Form.
- (2) Some other sites were positive only in the presence of added magnesium. This should be called the Inactive Form. Of course there are other sites which were positive but become more strongly positive upon the addition of magnesium. The sites in the latter case may be mixtures of the active and the inactive forms. It is well known that other important factors are involved. By decalcification in neutral media with ethylene diamine tetraacetate solution⁶⁾⁷⁾ magnesium as well as calcium will be carried away from the hard tissues and the phosphatase activity becomes depressed. Therefore, it is necessary to add magnesium to the substrate mixture for histochemical experiments. The loss of enzyme activity by elimination of magnesium and recovery of the activity upon addition of magnesium suggest that magnesium is not an activator in the simple sense but something like a co-enzyme. The experiments reported in this meeting deal with the effects of pretreatments of tissue sections with certain magnesium reagents on histochemical phosphatase activities.

Experiments

The following reagents were tested for pretreatment of tissue sections on

glass slides.

(1) Titan Yellow. This is a salt of the diazoamino-anhydrothio-p-toluidine-sulfonic acid. This reagent gives colored lakes with aluminium, cadmium, calcium, cobalt, magnesium, nickel, tin and zinc. The addition of potassium cyanide prevents the reaction between the dye and the cations, cadmium, cobalt, nickel, and zinc. K. Okamoto and others⁶⁾ reported a histochemical test for magnesium by use of this dye.

(2) Alizarin. 1, 2-dihydroxyanthraquinone.

(3) Alizarin Orange. 1, 2-Dihydroxy-3-nitroanthraquinone.

(4) Alizarin red, or Sodium Alizarinsulfonate. 1, 2-Dihydroxy-3-sulfoanthraquinone.

(5) Quinalizarin. 1, 2, 5, 8-tetrahydroxyanthraquinone.

Many of the anthraquinone dyes are known to form colored lakes with magnesium and other metals in alkaline media. There are reports on the use of anthraquinone dyes for histochemical detection of magnesium in plant tissues.

(6) Magneson. p-nitrobenzene-azo-resorcinol. This is a brownish-red to dark-red powder which is yellow to red in alkaline solution and is known to form a yellow-brown to blue lake with magnesium. It also forms colored lakes with other metals, but the precipitations of these lakes are prevented by the addition of potassium cyanide. K. Okamoto tested the dye for the histochemical detection of magnesium.

(7) Diphenylcarbazide. This reagent is very slightly soluble in water but freely in ethyl alcohol and benzene. It gives colored compounds with magnesium and other metals and, therefore, has been used for the detection of magnesium.

(8) 8-Hydroxyquinolin (oxine, o-oxychinoline). This is a well known reagent which is soluble in acids, acetone, and ethyl alcohol, but is almost insoluble in water. It forms insoluble co-ordination complexes with many metals. Oxine will precipitate magnesium in ammoniacal alkaline solution.

(9) Para-phenylenediamine. This is a familiar compound which has been used for detecting oxidizing ions. It has been known to react with magnesium.

(10) Alkali Hypoiodite. The so-called alkali-hypoiodite test depends upon the formation of a reddish brown, insoluble absorption compound of $Mg(OH)_2$ and iodine. This is a very sensitive test which has been used for the detection of magnesium. K. Okamoto and his collaborators applied this principle for the histochemical detection of magnesium in tissue sections.

In addition to the reagents mentioned above, para-Nitorbenzol-azo-alpha-naphthol was also tested for detection of Mg. After pretreatment of deparaffinized tissue sections on glass slides with one of the reagents mentioned above, the sections were washed first and then soaked in a substrate solution. The sections were then incubated for various periods according to Takamatsu and Nishi's new method⁷⁾. Subsequent procedures were carried out in the usual way.

Results and Summary

The principle of the tests for magnesium mentioned above is primarily based

upon the characteristic binding of the reagents with magnesium hydroxide. Therefore, magnesium contained in tissue sections needs to be converted to magnesium hydroxide in strong alkaline solution. For this purpose, K. Okamoto has recommended that the tissue sections be soaked in 2% NaOH slution for at least 2 hours. Treatment of tissue sections in such a strong alkaline solution destroys tissue and cell structures.

The staining patterns of tissue sections by high concentrations of reagent solutions mentioned above in slightly alkaline media revealed a wide variety of results. Generally, the sites of staining seemed to be more widely distributed than those of the alkaline phosphatase reaction. This suggests that non-specific staining may occur. Pretreatments of tissues with the magnesium reagents generally depressed phosphatase activity. This effect was rather small with anthraquinone dyes whereas oxine and titan yellow greatly depressed the phosphatase activity. These results were not satisfactory for our histochemical purpose ; therefore further studies on alternate methods are in progress.

References

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Demonstration of Acid Phosphatase Activity in the Normal Rat Jejunum by Means of Electron Microscopy*

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By and large results obtained in the electron histochemical observation were in good agreement with those obtained in the light histochemical study.

Detailed results have been published elsewhere (Ogawa et al., J. Histochem. Cytochem., 10, 228, 1962; Ogawa et al., Okajimas Fol. Anat. Jap, 38, 355, 1962), and papers cited are referred by the author.

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