New Studies on Histochemical Demonstration of Peptidase

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Peptidase is, as is well known, a generic term of enzyme series which split the linkage of peptic bonding. In this group, carboxypeptidase, aminopeptidase, dipeptinase, prolinase and prolidase are classified. For histochemical study on the substrate specificity of peptidases still more new method needed to be developed. At the second general meeting of the association, we reported a new method¹⁾, regarding capture of naphthylamine from naphthylaminic substrate of soluble salts. Insoluble compounds are not available as a substrate of enzyme in this method.

We synthesied palmityl-alpha-naphthylamine, palmityl-beta-naphthylamine and palmityl-anilide. These substances are not soluble in water. We can make a relatively stable emulsion of the substances by use of non-ionic emulsifier as we described in the following mehtod.

In the new method, released palmitic acid was precipitated by calcium ion as is in the method of lipase histochemistry. The calcium was replaced by lead in a lead nitrate solution as is in the Gomori method for lipase. The lead was visualized by staining with Alizarin Red according to the method of Takamatsu, Ozawa and Izaki²⁾.

Method

Preparation of substrate solution.

Solution 1 ; Saturated solution of substrate in warm acetone and keep at 37°C.

Solution 2 ; 3% water solution of sterox SK (Monsant Chem. Comp)	2.5 cc
Tris HCl Buffer Solution (pH. 7.4)	20.0 cc
2% water solution of Calcium chloride	3.0 cc
Aq. dest.	15.0 cc

Solution 2 is kept at 37°C. Substrate mixture was made by adding of 3 cc of the solution 1 dribbly in solution 2 under continuous shaking.

Procedure of histochemical demonstration.

- 1) Prepare acetone-fixed paraffin sections according to a routine method. Remove paraffin from the slide through xylene, pass to water via alcohols.
- 2) Incubate for about 20 hours in the substrate mixture at 37°C.
- 3) Wash in distilled water thoroughly.
- 4) Immerse in 2% aqua solution of lead nitrate for 5 to 10 minutes.

- 5) Wash in distilled water thoroughly.
- 6) Stain in 0.5% Alizarin Red in diluted ammonia solution for 20 minutes. Preparation of the diluted ammonia solution; Dilute 0.5 cc of ammonia water (Japanese pharmacopoeia) with distilled water and make it up to 100 cc.
- 7) Wash in the diluted ammonia with several changes until no more fading.
- 8) Wash in tap water.
- 9) Counterstain in hematoxylin solution, if desired.
- 10) Dehydrate in alcohol, clear in xylene and mount in Canada balsam.

Result and Comment

The sites of the enzyme activity stain in red colour. The substrates which we used in this experiment are naphthylamine peptide or aniline peptide with palmitic acid. The activity which is responsible for the resolution of the peptic linkage of the substrates should be a carboxypeptidase. Even in this method, only diluted substrate emulsion can be obtained. Therefore, the staining of the tissues appears usually slightly after this method. If the solubility of the substances could be improved the staining pattern will become better.

References

- 1) H. Takamatsu, and M. Takezawa; Proc. Jap. Histochem. Ass. vol. 2, 17. 1961.
- 2) H. Takamatsu, S. Ozawa and H. Izaki; (In Japanese). The Histochem. Rec., vol. 1. No. 8, 10. 1951.

Studies on the Histochemical Specificity of Esterase.

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Introduction

This study is designed to investigate the specificity of enzyme activity. we separated the enzymes by paper chromatographic method and identified individual enzyme by means of histochemical method, applied to the paper strip directly.

By this method, we have successfully separated and identified esterase and