Phosphatase Activity in Plant Cells with Special Reference to Those Treated with Colchicine and Gammexane

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Introduction

The study of chemical nature of chromosomes and specially of plants is quite recent though the inception of this line of investigation was made by Meischer in 1874. Recently, dessertations have been published from different centres of research, (Kaufmann et al 1947–1954, Kaufmann, Gay and Mcdonald 1951, Sharma, Mookerjea and Ghosh 1953, Sharma and Roy 1956a and 1956b) which have given some idea of the chemical nature of plant chromosomes.

A chromosome can be visualized, as is well known, as a giant complex chemical molecule made up of several less complex small molecules known as genes. Leaving aside the exact chemical differences accounting for gene to gene diversity, the chromosomes consist of, at least, several well defined segments whose differences are easily detectable under the microscope. Euchromatin, heterochromatin as well as secondary constriction regions can be identified by staining differences. If the coloration is considered to be an index of the chemical cycle, specially with regard to nucleic acid, then the chemical difference of these defined segments is implied. The chemistry of heterochromatic segments, to some extent (Darlington 1942), is already known. About the other segments the data is deplorably meagre.

The importance of phosphatase activity in nucleic acid metabolism has been elaborately discussed by a number of authors, and in chromosomes, its existence is regarded as a criterion of identifying nucleic acid at its site of occurrence (Danielli and Catcheside 1945, Bhattacharjee and Sharma 1951, etc.).

It needs no introduction that differential phosphatase activity, if obtained in the chromosomes, would give an idea of the presence of differential segments differing in their chemistry.

The present scheme of work was taken up to investigate the differential nature of chromosome segments on the basis of their alkaline phosphatase activity. At the same time a test for acid phosphatase too has been incorporated within the scope of this investigation. While dealing with the study

of phosphatase activity in the chromosomes, a study of the nucleoli and cytoplasm too has been performed and this data has been included in this paper.

Materials and methods

The investigations were carried out on the roots of *Vicia faba* belonging to the family Leguminosae. Seedlings were grown in earthenware pots.

The seedlings were pretreated in 0.002 M solution of oxyquinoline for three hours at a temperature of 14°–16° C. The root tips were then cut and fixed in acetic alcohol (1 : 1) for 45 minutes and hydrolysed in nHCl for fifteen minutes at a temperature of 40°C. This was followed by rinsing in water for ten minutes and squashing on a dry slide. The slides were then kept inverted in 70% alcohol for overnight.

The cover glasses were detached from the slides and the latter were brought down to water after passing through 50% and 30% alcohol. One set of slides was treated with boiling water for fifteen minutes to kill the tissues. Both the sets were tested for alkaline or acid phosphatase activity, as the case might be.

In order to induce polyploidy in the roots, one set of seedlings were treated in 0.5% colchicine solution for six hours and another set in 0.25 saturated solution of gammexane (hexachlorocyclohexane) for over night. These were then kept in Knop's culture solution for two days and were passed through the above process to render them ready for testing Phosphatase activity.

Test for alkaline phosphatase. 1. To study alkaline phosphatase activity within the tissues, Gomori's revised method was applied (Glick 1949). This consists of the following steps :

The slides were incubated in a medium containing,

Sodium glycerophosphate	–2% solution	–25 c.c.
Sodium barbitol	–2% solution	–25 c.c.
Distilled water	–	–50 c.c.
Calcium chloride	–2% solution	– 5 c.c.
Magnesium sulphate	–2% solution	– 2 c.c.
Chloroform	–	a few drops,

at 37°–40° C. for four hours.

2. They were rinsed in distilled water and kept in 2% cobalt chloride solution for 15 to 20 minutes.

3. After rinsing, they were treated with yellow ammonium sulphide solution (a few drops in 100 c.c. of distilled water) for 15 to 20 seconds.

4. The slides were again rinsed in distilled water and gradually passed through the different grades of alcohol and alcohol xylol mixture (1 : 1) keeping in each for one to two minutes.

5. Finally these were kept in pure xylol for five minutes and mounted in Canada balsam.

Test for acid phosphatase. To test for the activity of acid phosphatase in the tissues, Glick and Fisher's adaptation of Gomori's method was applied (Glick 1949). It comprises of the following steps :—

1. The slides, after coming down to water were transferred to the following substrate medium :

Acetate buffer (pH 5.1)	–0.1 M solution···	40 c.c.
Lead nitrate	–0.1 M solution···	10 c.c.
Distilled water	– ···	6 c.c.
Sodium glycerophosphate	–3.2 % solution···	4 c.c.

This mixture was centrifuged and the slides were incubated in the clear supernatant liquid at 37°–40° C. for three hours.

2. They were washed with distilled water, dipped in 2% acetic acid and rinsed in distilled water.

3. They were kept in yellow ammonium sulphide solution (a few drops in 100 c.c. of distilled water) for 2 to 3 minutes.

4. They were then passed through the same steps as in case of alkaline phosphatase test and mounted in Canada balsam.

Observations were made at a magnification of ×900, using UCE. compensating eye piece of ×12 and 1.3 apochromatic objective with an aplanatic condenser of 1.4 N. A. Photomicrographs have been taken at the above magnification and have been enlarged to double the size approximately.

Observations

Alkaline phosphatase activity in normal tissue

General appearance of the tissue—Bright, with different cellular components well differentiated.

Cytoplasm—Less bright than chromosomes but positive, granular, granules being positive, with negative areas sparsed throughout, area adjoining the metabolic nucleus phosphatase negative.

Nucleus—Nuclear wall brighter than cytoplasm and nucleus itself. Nuclear precipitation much denser than that of cytoplasm.

Nucleolus—(in the metabolic stage)—Perinucleolar zone sharply positive in the form of a ring, intranucleolar zone almost negative. In mature nucleus, nucleolus throughout its surface is positive.

Chromosomes : Prophase—Chromosomes distinctly positive.

Metaphase—Chromosomes distinctly positive. End portions sharper. Positive areas alternating with negative zones in certain chromosomes. One satellited chromosome throughout positive, except a gap at the secondary constriction region. Chromosomes apparently discontinuous due to the presence of negative areas in between the positive sites. Cytoplasmic activity low.

Anaphase—Chromosomes similar to those of metaphase but much fainter.

Acid phosphatase activity in normal tisssue

General appearance of tissue—Very faint, only a slight yellowish tinge

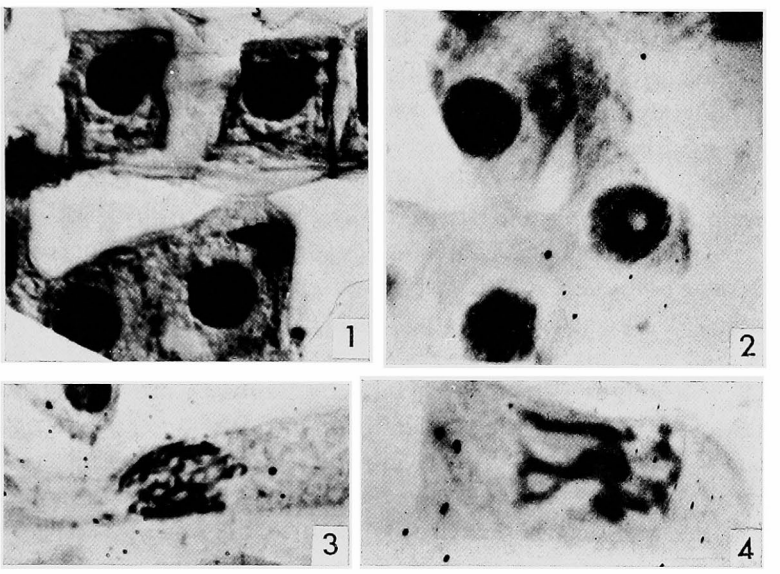
present.

Cellular components—Not distinguishable

Alkaline phosphatase activity in colchicine induced polyploid tissue

General appearance of the tissue—Faint, different cellular components not clearly differentiated.

Cytoplasm—Faintly positive, granular, granules being positive with negative areas sparsed throughout, area adjoining the metabolic nucleus is phosphatase negative.



Figs. 1-4. Alkaline phosphatase activity in normal roots of *Vicia faba* showing nuclei, nucleolus, prophase chromosomes, and metaphase chromosome.

Nucleus—Nuclear precipitation much denser than that of cytoplasm, nuclear wall is not differentiated from its inner region.

Nucleolus—Not sharply differentiated from the nucleus. Perinucleolar zone comparatively slightly positive in the form of a ring in the diploid cells, but in polyploid cells it is absent. Intra-nucleolar zone less positive. In the mature nucleus, nucleolus indistinguishable.

Chromosomes: Prophase—Chromosomes more positive than cytoplasm, precipitation not uniform throughout the chromosomes.

Metaphase—Chromosomes positive, clearly differentiated from other cellular components. End regions sharper. In some chromosomes positive areas alternate with negative zones. Chromosomes apparently discontinuous due to the presence of negative zones in between the positive sites. Chromosomes sharper in diploid cells than in polyploid cells.

Anaphase—Chromosomes give the same reaction as in metaphase but the precipitation is much lower.

Alkaline phosphatase activity in Gammexane induced polyploid tissue

General appearance of tissue—Faint, with cellular components not clearly differentiated. Polyploid cells more faint as compared to diploid ones.

Cytoplasm—Faintly positive, granular, granules being positive with negative areas scattered throughout, area adjoining the metabolic nucleus hyaline

but this differentiation is not so sharp in polyploid cells. Cytoplasm of polyploid cells show very low positive activity.

Nucleus—In diploid cells nuclei quite distinct from cytoplasm and have much denser precipitation. In polyploid cells nuclei not sharply differentiated from cytoplasm, precipitation lesser than in diploid cells and fairly homogeneous with no distinction between inner and outer regions.

Nucleolus—In diploid cells the nucleolus is placed in the middle of a hyaline zone of nucleus. Perinucleolar zone sharply negative in the form of a ring. Intranucleolar region phosphatase positive. In mature nuclei, nucleoli not visible. In polyploid cells nucleoli practically invisible, only a faint perinucleolar ring and an inner hyaline zone visible.

Chromosomes : Prophase—In diploid cells chromosomes more positive than cytoplasm. Positive areas alternate with negative or faint areas giving a beaded appearance. In polyploid cells the chromosomes are same as in diploid cells but the phosphatase activity is much lower.

Metaphase—In diploid cells chromosomes are clearly distinguished from the cytoplasm and sharply positive. Positive areas alternate with negative zones but majority of the segments are positive. End regions sharper. In polyploid cells the chromosomes give the same reaction as in diploid cells but the phosphatase activity is much lower.

Anaphase—The chromosomes have the same appearance as in metaphase. But they are thinner and phosphatase activity is much diminished.

Discussion

In general, activity of alkaline phosphatase has been found to be quite high as compared to that of acid phosphatase. This is likely specially in view of the previous literature existing on the alkaline phosphatase activity of the chromosomes. Danielli and Catcheside (1945) demonstrated the activity of this enzyme in salivary gland chromosomes of *Drosophila* and correlated the nucleic acid positive areas as indicated by Feulgen staining. It was however pointed out that in spite of a general correlation, not all the phosphatase positive areas are nucleic acid positive. This differential behaviour has been pointed out by later authors (Sharma and Roy 1956a and 1956b) as possibly due to the fact that alkaline phosphatase is present even in the protein skeleton. The latter experiments were carried out on roots of *Allium cepa* and it was emphasized that in salivary glands too investigation should be carried out in this direction. It was noted that even after digestion of one of the proteins i.e., either basic or non-basic, and the nucleic acid the chromosomes remained phosphatase positive. In view of these observations, the high alkaline phosphatase activity as noted in the present series of experiments can be explained. The activity of acid phosphatase though present in the tissue is very low and the precipitation is so uniform that it is difficult to distinguish between the cytoplasm and the nuclei.

Differential activity in the chromosomes is marked. The secondary and primary constriction regions remain as gaps indicating that these regions do not contain significant amount of alkaline phosphatase. In several of the metaphase plates the end segments have been found to show very high alkaline phosphatase activity, indicating possibly the presence of this enzyme in these regions.

It may be noted that though in all the cases secondary constriction segments have been found to be negative, satellite stalk shows positive behaviour in some of the metaphase plates. In majority of the plates, on the other-hand, satellites do not come out clearly and as such this positive behaviour could not be detected. It is well known that in *Allium cepa* one pair of satellited chromosomes, which characterizes the complement, is very difficult to clarify in metaphase plates. As in some of the plates positive alkaline phosphatase activity has been noted, it is obvious that such behaviour would have been expected in case the constrictions remained clarified in other plates too.

Following Feulgen staining the satellite thread in *Allium cepa* shows positive reaction. It is therefore not unusual that the region will show positive activity too. In that case the problem arises as to what should be the nature of the secondary constriction segments. Such segments, as are generally regarded, are similar to satellite segments in the sense that they perform a common function, that is, they represent loci of nucleolar origin. On the basis of this function, it appears that the two are identical in their chemical constitution and the term satellite is generally applied to those secondary constrictions which represent the end segment, the ultimate chromatin matter being reduced to a minute droplet.

If their chemical constitution is considered to be similar then the phosphatase positive activity should have been present in both. The negative behaviour of secondary constriction segments may however be due to the phosphatase activity in these regions being too low to be detected under the microscope. In that case this differential behaviour is dependent on the degree of phosphatase positive substances present in both. In any case, this suggestion is yet to be confirmed following observation under much more high magnification. In this connection it may be mentioned that Soumalainen (1947) has noted different types of secondary constriction segments in species of *Polygonatum*. In some of these the intervening threads show positive activity, whereas in others they are negative. How far this hehaviour is indicative of their qualitative difference or is just dependent on the quantity of nucleic acid present in the two is not known. If phosphatase negative secondary constriction segments of *Allium cepa* are proved to be positive under high magnification and better resolution of the lenses than that would imply that all secondary constriction segments are fundamentally similar in nature though they may differ with respect to the quantity of nucleic acid

or phosphatase positive substances present in them.

Results of another set of experiments involving the effect of colchicine and gammexane on phosphatase activity show, on analysis, that diploid and polyploid cells differ markedly with regard to their response to phosphatase test.

The induced polyploid cells after colchicine and gammexane treatment, in general, show a low phosphatase activity as compared to that of diploids. No doubt, the chromosome differentiation remains the same and no significant difference could be worked out between those of diploid and polyploid cells. Only the differential nature of chromosome segments noticed in case of diploid cells is more pronounced than in those of polyploid cells. In contrast to diploid cells, here the nucleoli are practically indistinguishable from the surrounding medium. Cytoplasmic activity is low.

It appears therefore, that treatment in polyploidizing chemicals does not accelerate the alkaline phosphatase activity. Within an increased cell surface the enzymes naturally become more dispersed as compared to that of diploid cells and because of this dispersion the activity seems to be low.

The loss of distinction between the nucleoli and the nucleoplasm is rather interesting. The distinction between the two is, no doubt, dependent on their viscosity. Following chemical treatment the viscosity of the plasma is increased, becoming solidified to some extent. Because of this increase in viscosity, the identity of the nucleoli gradually becomes lost. The data clearly reveal therefore that nucleolus formation is markedly effected by treatment with polyploidizing chemicals. An interesting fact is that in adult nuclei too, this differentiation is rarely seen even in normal tissue. This is because of the fact that in adult nuclei the plasma is very much effected and in all probability the viscosity difference between the nucleoli and nucleoplasm is very low.

Summary

1. The investigation was made to study the differential nature of the chromosomes and other cell components on the basis of their phosphatase activity.

2. To test alkaline phosphatase activity, Gomori's revised method and for acid phosphatase activity, Glick and Fisher's adaptation of Gomori's method were applied.

3. Differential precipitation has been very high in all the cellular components in case of alkaline phosphatase, while with acid phosphatase the activity has been very low and uniform.

4. In polyploid cells the nucleoli are practically indistinguishable, other cellular components giving the same reaction at a much reduced rate.

5. The high alkaline phosphatase activity is due to the presence of this enzyme in both nucleic acid and protein components of the chromosomes.

6. End portions of chromosomes contain a higher dosage of the enzyme. In the constriction regions it is insignificant, but the satellite thread is positive in some of the plates. This implies that satellite stalks and secondary constriction segments which are functionally similar, may differ in the quantity of phosphatase positive substances or nucleic acid.

7. Decreased phosphatase activity in polyploid cells is due to the greater dispersion of the enzyme as a result of increase in cell surface.

8. Loss of distinction of nucleoli in polyploid cells is the result of diminution of viscosity difference between the nucleoli and nucleoplasm caused by chemical treatment.

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