Physico-Chemical Poperties of Chromatin Isolated from *Vicia Faba* Embryonic Axes

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ABSTRACT

Faba bean (Vicia faba L.), an old-world grain legume, is grown approximately in 3 m/ha area world-wide from temperate. In favorable conditions, it gives very high yields. The present work was carries out to produce a good preparation of chromatin isolated from embryonic axes of *Vicia faba, Chromatin* which can be isolated as a chemically defined entity including DNA, RNA, histone and non- histone protein. Vicia faba variety white and Brassica oleracea variety botrytis as two unrelated species.

The activity of chromatin can be also measured by its stabilization degree against thermal denaturation. Chromatin in 1 M NaCl was purified by chromatography on Sephadex G- 25 Fine/ Pharmacia fine chemicals AB, Sweden. The purity of chromatin prepartions was estimated, Extraction of histones was carried out from purified chromatin with 0.4 HCl by using 10 ml chromatin in each analysis.

The products of genetic activity vary greatly in amount in different cell types and in the same cell types and in the same cell type at successive stages of differentiation

Key words: Vicia faba, faba bean, Chromatin Isolated, composition of chromatin

INTRODUCTION

There have been a considerable number of attempts There have been a considerable number of attempts to fractionate and characterize the active and inactive fractions of chromatin. The active fraction is generally characterized by several properties such as a high transcriptional activity, a higher solubility, an apparent absence of satellite DNA, attachment of nascent RNA chains, an increased amount of non-histone proteins and a weaker stabilization against thermal denaturation. Comparison of chromatin properties in different organs, developmental stages and species has been a fruitful approach for studying transcription activity.

The present work was carries out to produce a good preparation of chromatin isolated from embryonic axes of *Vicia faba* and to study the physico- chemical properties of chromatin. The genetic material of eukaryotic organisms is a nucleoprotein complex, chromatin. Chromatin which can be isolated as a chemically defined entity including DNA, RNA, histone and non- histone protein (Smart and Bonner, 1971).

Chromatin isolated from a given organ has a specific template activity for the RNA synthesizing system (Bonner *et al*.,1968. Paul and Gilmour, 1968; Tan and Miyagi, 1970; Fukasawa and Hamada 1973; Bonner 1976; Yoshida and Sasaki 1977; Wielgat *et al*.,1979 ; Seehy 1980; Seehy *et al*., 1990 and seehy and khatab .,2012).

Fukasawa and Hamada (1973) showed that the apical part of the cauliflower inflorescence has a higher nucleic acid content than the axis segments; the DNA amount of the former is about five times as much as that of the latter. They found that the RNA amounts in the apical part were about twice as much as in the axis segments. while Wielgat *et al* (1979) reported that chromatin isolated from various plant species (maize ane pea) shows differences in its template activity.

The activity of chromatin can be also measured by its stabilization degree against thermal denaturation. Ultraviolet hypo-chromatin provides the simplest and most widely utilized means of observing the denaturation of chromatin. Bonner and Huang (1963), and Bonner and Bonner *et al.* (1968b) studied the properties of chromatin and nucleohistone and they characterize the transition range by the temperature, Tm, at which half of all hyperchromicity has manifested itself.

Huang *et al.* (1964) showed that the Tm- value of soluble reconstituted nucleohistone I b of thymus is 81˚C. they concluded that the histone I b contributing the greatest stabilization to DNA, histone IV the least, while histones II b and III are intermediate.

Murray 1969 found that the removal of histones from native deoxyribnucleoprotein was accompanied by a drop in melting temperature and sedimentation rate, by an increase in susceptibility to hydrolysis by pancreatic deoxyribnuclease, and by an improved efficiency in the support of RNA synthesis by the polymerase of *E. coli*.

Olins and Olins (1971) found that complexes of T 7 DNA with F 1 histone, reconstituted from urea- NaCl resulted in structures with different solubility, thermal stability, sedimentation and electron microscopic properties than those of F 2 a 1- DNA complexes formed under the same conditions. Organ and /or developmental stage- specific properties of chromatin were discussed by several workers.

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Myczkowski (1974) during his studies on winter wheat chromatin, found a gradual decrease of the total protein content and an increase of the non- histone protein content in the course of wheat germination. These changes were accompanied by a decrease of chromatin- thermal stability. He also counts that the changes in chemical composition of the chromatin of vernalized embryos of wheat consisted of an increase of the total protein content, especially that of non- histone protein and a decrease of the DNA content. At the same time the thermal stability of the chromatin decreased after the full induction of generative development and it increased after 7 days of vernalization.

Fellenberg and Schomer (1975) reported that the Tm of chromatin isolated from etiolated pea seedlings is 71.2˚C. they as well as Fellenberg (1969) stated that this Tm- value can be changed by IAA treatment.

Voges and Fellenberg (1976) studied the properties of chromatin isolated from three different organs selected from pea plants. They found that the Tm-values, estimated at 260 nm, are 75.7 for apical zone chromatin, 73.2 for stem chromatin and 64.3 for chromatin isolated from root system. Seehy et al (1990) mentioned that ± 0.5°c in tm- value is considered that significant

Myczkowski (1979) reported that- in the investigation on pea chromatin in four successive germinations stages- a decrease in the relative protein content, an increased proteolytic activity, and a lower temperature of melting were found in the chromatin of seedlings developing new organs. He stated that these changes are correlated with the course of morphogenetic processes during germination.

MATERIALS AND METHODS

The material used in the present study consisted of Vicia faba variety white and Brassica oleracea variety botrytis as two unrelated species. Three different developmental stages of Vicia faba variety white were selected as follows:

1- First stage: germs or embryonic axes after 4 days of germination.

Growth of plants: seeds of Vicia faba varitus white were washed for 2 hours in running tap water, soaked overnight in tap water, washed the second time for 2 hours in running tap water, transferred into petri-dishes on wet filter paper, the moisture was kept constant by adding daily new redistilled water to the petri- dishes, and allowed to germinate at 25˚ C **in** the dark. The beginning of the seeds-washing period was taken as the onset of germination. Embryonic axes were removed from the germinated seeds, when they were about 1 cm long. These embryonic axes were frozen at 18˚C until usage /2-3 days/ or used immediately.

Isolation of chromatin

Chromatin was isolated by the method described by (Mirsky and Pollister (1946), Pollister and Mirsky (1946) and modified by Fellenberg (1967) and described lastly by Fellenberg and Schomer (1975).Frozen or fresh tissue about 100g were ground well in a chilled mortar using the following buffer:

0.075 M NaCl

0.01 M Na- citrate

0.01 M Tris- HCl, Ph 8.0

Then they were homogenized for 2 min. at 6000 r.p.m. by using an electrical homogenizer /3 ml buffer for each gram material/ and filtered successively through three layers of nylon mesh. The filtrate was saved while the residue was again homogenized in the same buffer and filtered. The residue was discarded and the combined filtrates were centrifuged for 30 min. at 7.000 xg/ at 0˚C. The supernatant was discarded and the pellet was suspended in the same buffer and centrifugation was carried out at 15.000 xg for 10 min. at 0˚C this last procedure was repeated four times by using the same buffer and centrifugation. Then the pellet was suspended in 0.3 M NaCl, 0.01 M Tris – HCl, Ph 8.0 and centrifuged for 10 min. at 15.000 xg. This procedure was repeated.

From the pellet chromatin was extracted with 1 M NaCl, 0.01 M Tris- HCl, pH 7.2, by adding 1 ml buffer for 50 g of fresh weight of material in the case of chemical composition etermination and estimation of thermal stability, while 3 ml buffer was added for 50 g of fresh weight of material in the case of extraction of histones. Then the pellet was gently stirred for 90 min. by an electrical homogenizer using glass rod while the tube containing pellet was embedded in ie, stored at 2˚C overnight, and then was centrifuged at 23, 000 xg for 30 min. at 0˚C.

Purification of chromatin:

Chromatin in 1 M NaCl was purified by chromatography on Sephadex G- 25 Fine/ Pharmacia fine chemicals AB, Sweden. The purity of chromatin prepartions was estimated:

1- Firstly by their ultraviolet absorption spectrum. The following coefficients were calculated; absorbance by:

a- 260/280, it is assumed that the value of this coefficient for the preparation of purified chromatin should be 1.5 at least.

b- 250/260, between 0.8 to 0.9.

c- 280/260, between 0.5- 0.6.

d- 320/260, should be less than 10% (Fellenberg ., 1974)

2- Secondly by their hyperchromicity during heating at 260 nm as well as at 280 nm as it will be discussed later.

Thermal stability and chemical composition

When purified chromatin showe adequate ultraviolet absorption spectrum, it was then dialyzed three times at 0 to 2˚ C against the following buffer one liter per 10 ml in each time: 0.0014 M NaCl, 0.00025 M Tris- HCl, PH 7.2.

Estimation of Tm- value:

Melting temperature of the chromatin was recorded at 260 nm as well as 280 nm by PYE Unicam SP 1750 ultraviolet spectrophotometer with pogramme controller Unicam SP 1805, while heating was done by temperature programme controller at 876 series 2, giving a temperature rate of 1˚C/2 min.

To estimate the transition range by the temperature, Tm, at which half of all hyperchromicity has manifested itself, the procedure was the same as that described by Benner *et al*., (1968b). From each extraction two estimations were calculated, while three separate extractions were made for each organ or tissue.

The absorbance of all investigated samples was recorded every minute at 260 nm as well as 280 nm. The relative absorbance was calculated as described by Spang and Platt 1970. Relative absorbance= AG/A 50˚ C where AG is the absorbance at a given temperature and A 50˚C is the absorbance at 50˚C. Averages of Tm- values and standard errors were calculated.

Chemical composition of chromatin:

Chromatin fractions were purified by chromatography on Sephadex G-25 and displayed adequate ultraviolet absorption spectra, then they were mixed together and used for estimation of chemical composition of chromatin.

Deoxyribonucleic acid, total protein, histones and non- histone protein were determined as ug/1 ml chromatin and then, the different proteins were calculated as relative to DNA.

1- DNA estimation

The diphenylamine method of Giles and Myers (1965) was used for determination of DNA concentration. For 1 ml purified chromatin,2 ml of diphenylamine 1g diphenylamine + 100 ml glacial acetic acid +3 ml H_2SO_4 were used, measured at 600 nm by spectrophotometer " Spekol" Carl Zeiss, Jena, using calf thymus DNA as standard. From each extraction three estimations were done and three chromatin extractions were made from each organ or tissue.

2- Total protein:

Total protein was determined by the method of Lowry *et al*. (1951) using albumin from bovine serum crystallized and lyophilized Sigma chemical company as standard.

3- Histones

Extraction of histones was carried out from purified chromatin with 0.4 HCl by using 10 ml chromatin in each analysis. Chromatin solution was acidified to 0.4 NHCl with 5 NHCl + 2˚ C for 12 h, centrifuged at 6.000 xg for 10 min. at 0˚C . the supernatant was saved, while the sediment was washed the second time by 0.4 NHCl for 30 min. at +2˚C , then it was centrifuged at 6.000 xg for 10 min. at 0˚C. the comined supernatants were neutralized with NaOH and pH adjusted at 7.2 then, determination of histone was done by the method of Lowry *et al* .(1951).

Gohen and Gotchel (1971) studied the histones of polytene and non polytene nuclei, and they found that the histones of the two types of nuclei yield identical patterns on gel electrophoresis.

Marushige and Dixon (1971) reported that nucleohistones is completely transformed into nucleoprotamine as aresult of the replacement of histones.

Panyim et al. (1971) reported that the lysine – rich group of histones shows considerable variation in electrophoretic mobility which is reflected in changes in amino acid composition.

Rall and Cole (1971) suggested that the lysine – rich histone has been redesigned from the fundamental structure of the other histones in order to carry out different function .in light of the fact that arginine – rich histones are almost invariant in structure from tissue to tissue, while lysine – rich histones exhibit a much greater degree of variability, it seems reasonable to assume the two classes have diverged in both structure and function.

4- Non-histone protein:

non-histone protein was calculated as the difference between the total protein and histones.

DNA isolation

To a purified chromatin solution in 1 M NaCl, NaCl was added to afinal concentration OP 2.5 M, then it was gently shaken, stored at + 2˚C overnight, and separated from chromosomal proteins, i.e. purified by chromatography on a Sephadex G- 200 Column G -200 was from Pharmacia Fine Chemicals AB, Uppsala, Sweden.

The purity of this DNA was estimated by:

1- Their ultraviolet absorption spectrum at wavelengths from 230 nm to 20 nm. The spectrum of DNA contaminated with protein was distinguished from that of deproteinized DNA most strikingly by its greater absorption at wave- length 230 nm due to the peptide bonds.

2- Their hyperchromicity during heating at 260 nm 280 nm.

3- DNA solution was tested for the presence of protein by the method of Lowry *et al* 1951. all DNA used samples were optically clear at wavelengths greater than 305 nm. When purified DNA showed adequate ultraviolet absorption spectrum, DNA sample in 2-5 M NaCl, 0.01 M Tris – HCl, Ph 7.2 was dialyzed at + 2˚C firstly against 1 M NaCl 0.01 M Tris – HCl, Ph 7.2 and secondly against 0.0014 M NaCl, 0.00025 M Tris- HCl, Ph 7.2.

Estimation of Tm- value:

Melting temperature of DNA was recorded at 260 nm as well as 280 nm as previously described in estimation of Tm- value of chromatin. From each organ or tissue six samples were estimated at 260 nm and at 280 nm, while three separately prepared extractions were done/ two samples/ each chromatin extraction. from the composition of histone and of DNA, one can calculate that in a nucleohistone in which DNA is fully complexed with basic protein, the mass ratio of histone to DNA should be approximately 1.35 to 1/ Bonner 1965. By this way the repressed fraction of genome was calculated as follows:

$$\text{Repressed fraction of genome, \%} = \frac{HG}{1.35} \times 100$$

Where HG is the ratio of histone / relative to DNA / obtained from a given organ or tissue.

RESULTS AND DISCUSSION

The fundamental unit of development, as of life itself, is the cell, and perhaps as many as a thousand distinct cell types arise from a single cell- the fertilized egg- during the development of complex organism.

How does a single cell-the fertilized egg- give rise to the many different kinds of cells? this is the fundamental problem of developmental biology. Phrased in genetic terms it becomes the central question of developmental genetics: how does one cellular genotype give rise to many hundreds of different cellular phenotypes?

Most of the genetic information of cells, encoded in DNA, is embedded in complex organelles, the chromosomes. Chromosomes contain, in addition to DNA, several classes of protein and some RNA.

Chromosomal material, i.e. chromatin, is that fraction of a cell remaining after removel of tissue fragments, membranes, and other non-nuclear components. The chemistry of chromatin must be comprehended if the structure and function of chromomsomes is to be understood.

Table 1. Properties of chromatin isolated from embryonic axes of *Vicia faba*

Property	Chromatin	DNA
1.Tm 260 in °C	78.58±0.38	69.58±0.37
2. Tm 280 in °C	79.92±0.15	70.92±0.15
3. Tm 280-260	1.34	1.34
4. Tmc- Tmd at 260	9 °C	
5. Tmc-Tmd at 280	9 °C	
6. Fraction of total DNA which melts with Tm= 70°C at 260 nm.	12%	
7. Fraction of total DNA which melts with Tm= 72°C at 280 nm.		
8. % of hyperchromaticity at 260	36.72±0.45	40.38±0.51
9.% of hyperchromicity at 280	34.82±1.15	36.68±0.80
10. absorbance by:		
260/280	1.79	1.93
250/260	0.84	0.83
280/260	0.60	0.58
320/260	0.02	0.01
11. DNA : Total proteins	1:1.13±0.05	
12. DNA : Histones	1:0.85±0.02	
13. DNA: Non-histone	1:0.28±0.04	
14. Non- histones: % of total proteins	24±2.08	
15. repressed fraction of genome	63%	

Tmc: is the Tm – value of chromatin Tmd: is the Tm – value of DNA

Table 2.Amino acid composition of whole histones isolated from embryonic axes of Vicia faba

Amino acid	• Moles/100 moles	Amino acid	Moles/100 moles
Lysine	15.33	Half cystine	ND
Histidine	1.48	Valine	6.01
Arginine	9.99	Methionine	ND
Aspartic acid	6.02	Isoleucine	4.94
Threonine	5.29	Leucine	7.91
Serine	4.89	Tyrosine	2.02
Glutamic acid	9.18	Phenylalanine	2.82
Proline	4.48		
Glycine	8.51	Basic: acidic	1.76
		Lysine to arginine ratio	1.53

The products of genetic activity vary greatly in amount in different cell types and in the same cell types and in the same cell type at successive stages of differentiation. Thus, genes must be differentially expressed, with respect to both time and place in the organism.

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الملخص العربي

الخواص الفيزيوكيماوية للكروماتين المعزول من المحاور الجنينيه للفول البلدي

عزة مرجان ومحمد الصيحي

تم الحصول علي بذور الفول البلدى المستخدمه في هذه التجربه واخذ منها جزء وتم انباته ومن ثم عزل منه المحاورالجنينيه وتم استخلاص الكروماتين من محاور الاجنه وتنقيته على عمود الغربله الجزيئيه في وجود Sephadex G-25 fine

وقياس معاملات الامتصاص بالاشعه الفوق بنفسجيه وعندما وجد ان المعاملات مقبوله تم الدفع بهذه العينات لعمل وتقدير درجه الانصهار وكذلك تقدير المكونات الكيميائيه للكروماتين النقي علاوة على تقدير الاحماض الامينيه للهستون واظهرت النتائج التالى أن:

Tm 260 in °c for chromatin 78.58 ± 0.38 for DNA 69.58 ± 0.37

Tm 280 in °c for chromatin 79.92 ± 0.49 for DNA 70.92 ± 0.15

واظهر تحليل الاحماض الامينيه للهستونات انها تراوحت من ١,٥٣ إلي ١,٧٦ جلايسين واظهر البحث الحالى ان الفول البلدي مصدر جيد لدراسه خواص الكروماتين، ال DNA حيث انه في الدراسه المستقبليه التي ستظهر بالرساله سوف يتضح اثر الاصابه بالفطر على هذه الخواص.