EFFECTS OF SOLVENTS, CONCENTRATION OF BIOPOLYMER AND DOSE OF γ IRRADIATION ON THE X-PEAK AND Y-PEAK OF NUCLEOHISTONE

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UNBOUND DNA in solution shows X-peak and Y-peak at 258–260 nm and 185–210 nm respectively[1-6]. Relative sensitivity of Y-peak to ionic change compared to X-peak for DNA has been reported[4]. The dependency of γ-irradiated Y-peak on the concentration of DNA, change of ionic strength and change of solvent system has also been studied[6]. To understand the nature of the chromophoric groups responsible for the X-peak and Y-peak, spectrophotometric observations on the changes in the Y-peak of actinomycin D complexed to DNA on γ irradiation have also been made[7]. Recently the characteristics of nucleohistone in the unirradiated and irradiated states have been reported[8]. The aim of the present work is to understand the mode of action of ionizing radiation on nucleohistone under different dissociated states as inside the living system DNA always exists in conjunction with proteins.

Nucleohistone (DNH) (Lot. No. 100790) from Calbiochem was dissolved in ECA and different molarities of sodium chloride from 0.002 M to 0.20 M by keeping the solutions in a refrigerator and stirred magnetically overnight. The pH of solutions of deoxyribonucleohistone (DNH), ECA (10^{-3} M ethylene diamine tetraacetic acid, 10^{-3}M sodium cacodylate, pH 8) and different molarities of sodium chloride were 7.24 ± 0.10 and 6.30 ± 0.10 respectively.

The UV spectra of all solutions were recorded in the wavelength range of 190–300 nm by a Carl Zeiss spectrophotometer PMQ II (accuracy of absorbance 0.005 units). Full transmittance was achieved for all blanks with double-distilled water as reference.

Gamma cell 220 (Atomic Energy of Canada) was used as the source of γ-irradiation. The dose rate (6.915 Gy/min) was ascertained by Fricke dosimetry[9]. The solutions after irradiation were kept at 0°C for 10 min to avoid renaturation[6]. The irradiated samples were then allowed to attain room temperature at which absorbance was measured against the respective blanks (different solvents). Equal volumes of DNH solutions were taken in test tubes of the same diameter. The experimental solutions were subjected to different doses of γ-irradiation approximately from, 100–500 Gy.

Table 1 shows that the increase in the molarity of sodium chloride results in red-shift which is also supported by earlier studies[6]. The increase in intensity with increasing molarity of the solvent (alkali halides in water) was reported earlier[10].

Nucleohistone when dissolved in the same molarities of sodium chloride and ECA retain the same absorption maxima values but the intensities are greater than the corresponding solvent absorbance intensity. Depending upon the molarity of sodium chloride, different fractions of histones are dissociated[11,12]. In the case of peptides[13] the $U \rightarrow A$ transition is completely hidden by the stronger $\pi \rightarrow \pi^*$ transition near 190 nm. In addition to this there are $n \rightarrow \pi^*$ transitions for the $-$CONH groups at 200 nm[13].

Table 1 *Wavelength of maximum absorption (λ max) and optical absorption values of Y-peak for different molarities of sodium chloride and ECA and nucleohistone (Conc^n 30 μg/ml) in the same solvents.*

Molarities of sodium chloride (M)	λ max for solvents and DNH(nm)	Absorbance values of solvents	Absorbance values of DNH	Percentage increase in absorbance value of DNH compared to solvent
0.002	191	1.15	1.51	31.3
0.01	194	1.95	2.15	10.3
0.05	197	2.18	2.35	7.8
0.10	198	2.19	2.40	9.5
0.20	199	2.38	2.45	2.9
ECA	201	2.50	2.75	10.0

Table 2 *X-Peak absorption values of nucleohistone in ECA when subjected to different doses of gamma irradiation at different concentrations.*

	Concentration of DNH(μg/ml)				
	15	30	45	60	75
Dose(Gy)	Absorbance	Absorbance	Absorbance	Absorbance	Absorbance
Unirradiated	0.23	0.34	0.57	0.74	1.04
97.90	1.18	1.36	1.61	1.67	1.83
195.80	1.18	1.37	1.50	1.65	1.86
293.70	1.20	1.38	1.50	1.68	1.83
391.60	1.17	1.38	1.51	1.67	1.84
489.50	1.17	1.35	1.52	1.65	1.86

Table 2 shows that for any particular concentration the absorbance values in the X-peak are independent of the dose upto an approximate maximum value of 500 Gy. As the concentration is increased the X-peak absorption values also increase. Table 2 shows that in the unirradiated DNH the O.D values of the X-peak increase with concentration[8]. But when the dose is 97.90 Gy, the absorption value considerably increase for all the concentrations indicating a gross alteration in nucleohistone. But when the dose is increased still further upto 500 Gy, there is practically no change in the absorbance. It has also been reported[14] that below 12 Gy, the susceptibility of nucleohistone to radiation seems to depend on the proportion of native DNH in equilibrium with its denatured components in solution. The resistance below 12 Gy is due to the shielding effect of the histone molecules.

In figure 1, the optical absorption values of X-peak of nucleohistone in ECA with increasing concentration has been plotted. A linear increase is observed in the values with increasing concentration but this is independent of radiation dose upto 500 Gy. Moreover, when the optical absorbance versus dose is plotted the former is constant for all the experimentally-absorbed doses for a particular concentration.

Hence within this does range no damage is caused to the purine and pyrimidine bases which are responsible for absorption in the X-peak region[15]. Moreover, in ECA, cacodylic acid is present which prevents denaturation of DNH thus giving a shielding effect[14]. The optical absorption values of Y-peak of nucleohistone increase linearly with increasing concentration both for unirradiated and irradiated states. But it does not show any regular trend.

In figure 2, the absorbance values of Y-peak of DNH in the unirradiated and irradiated states are plotted as a function of different molarities of sodium chloride at a concentration of 30 μg/ml. It is observed that upto

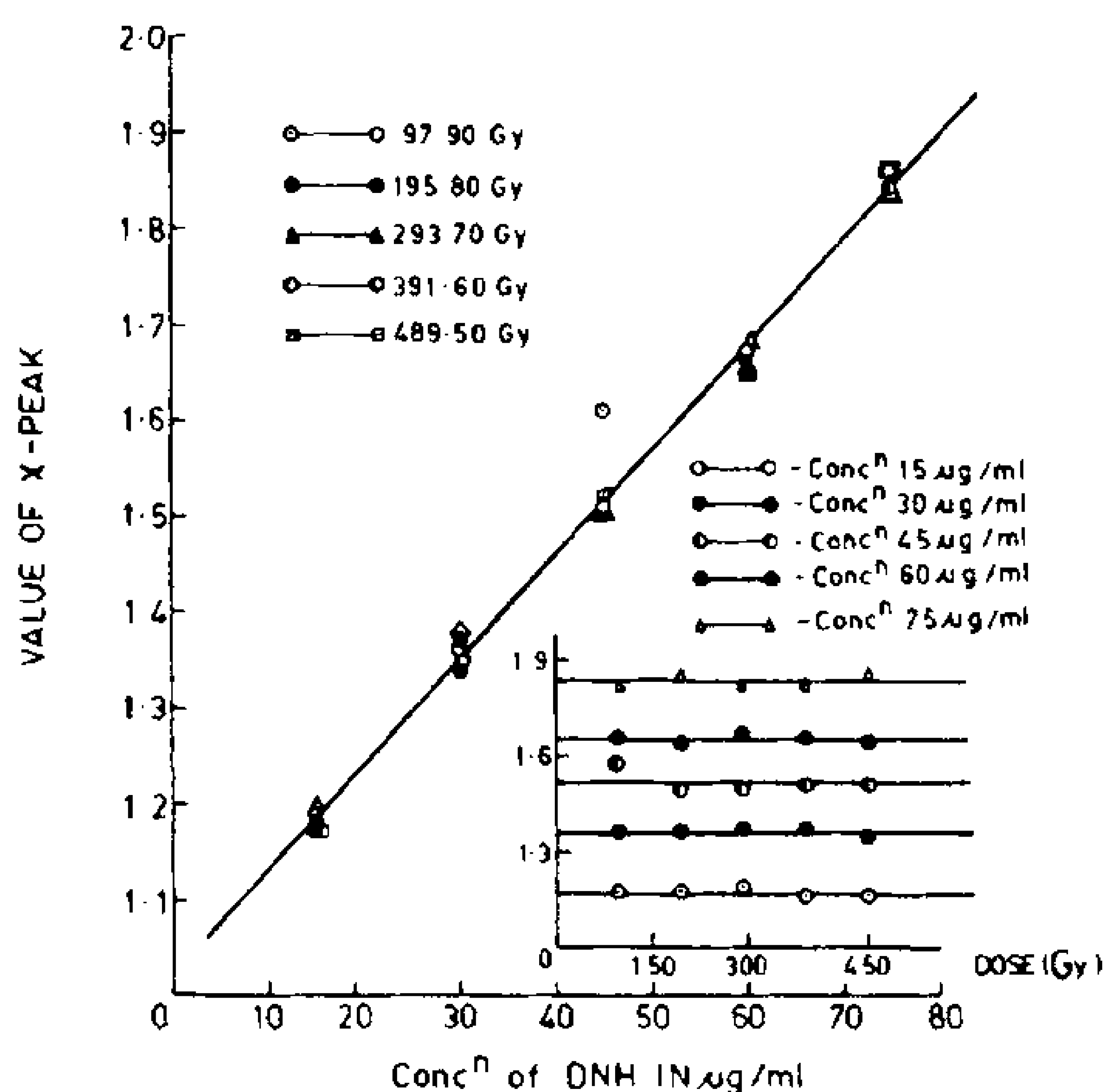

Figure 1. Dependency of X-peak of nucleohistone in ECA on different concentrations when subjected to different doses of γ-irradiation.

0.05 M sodium chloride the absorbance values increase sharply but subsequently it flattens off, up to a sodium chloride molarity of 0.20 M for all absorbed doses. Here sodium chloride plays the role of ligand and γ-radiation is the moderator. Na^+ ions per se exist in close electrostatic interaction with negatively-charged phosphate groups of DNA which is necessary for close packing of DNA helix[6]. With low molarity of sodium chloride the damage can easily be caused to DNH as it is in the dissociated state. Hence the increase in Y-peak. The minimum solubility is at a molarity of 0.15. At this point there is a little more of protein in solution than DNA. Moreover there will be altered electrostatic interaction between Na^+ ions and DNH at high mo-

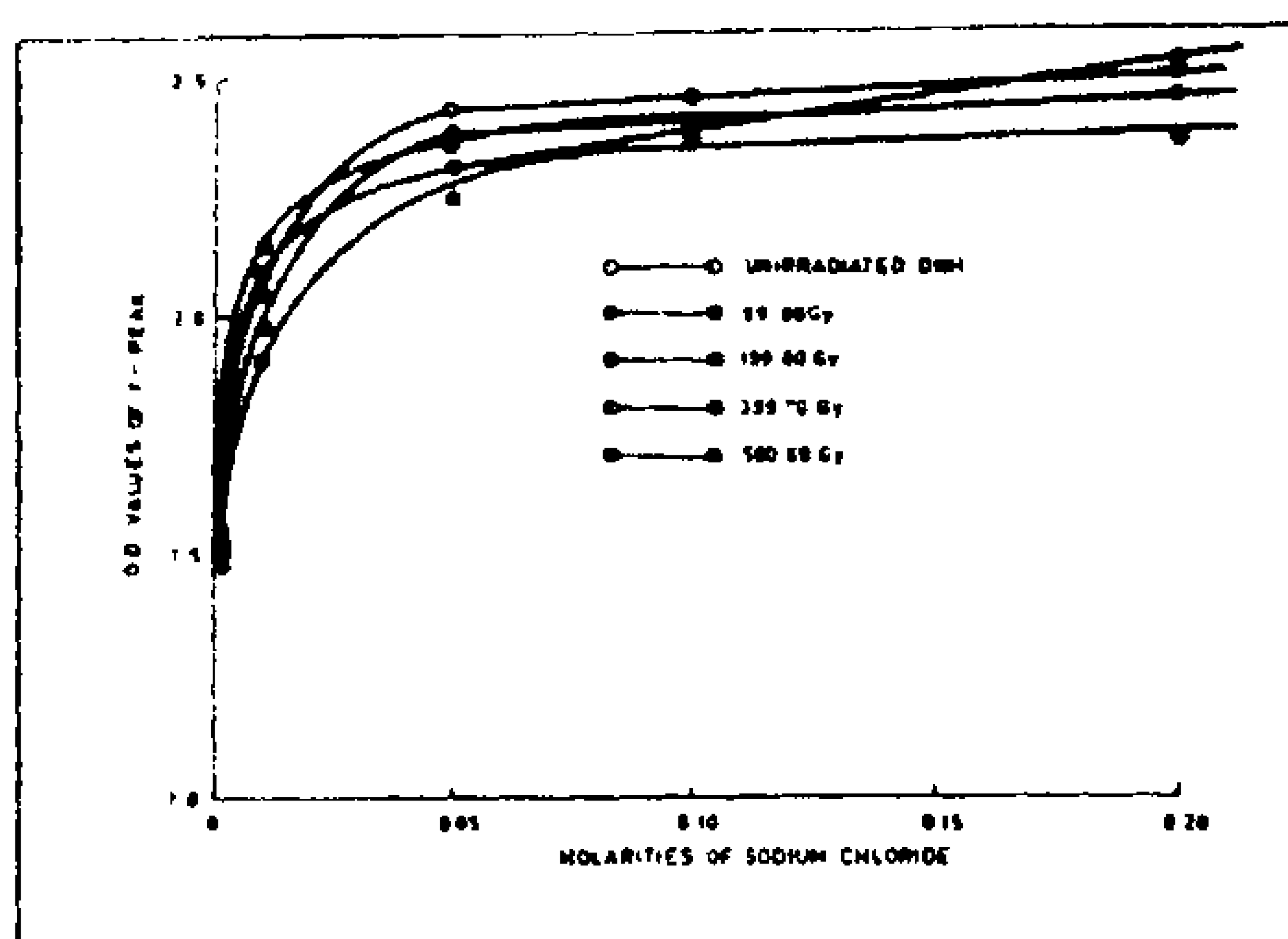

Figure 2. Changes in the O.D. values of Y peak of nucleohistone in the unirradiated and irradiated states as function of different molarities of sodium chloride.

larity giving a shielding effect against γ-irradiation. All these have been reflected in the flattening of the Y-peak value with increase in molarity of sodium chloride.

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A MODIFIED STAINING METHOD FOR GLUTAMATE OXALOACETATE TRANSAMINASE ISOZYMES

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GLUTAMATE oxaloacetate transaminase (GOT), also known as aspartate amino transferase (AAT), is an important isozyme that shows considerable polymorphism in electrophoretic studies[1]. Its role in the reactions in metabolism together with its physiological functions has been demonstrated[2,3]. This is a dimeric enzyme and in electrophoretic studies in fish, in general, two systems appear to work in staining schemes[1]; the anodally migrating one, GOT_{-2} is assumed to represent a supernatant form and the slower migrating one, the cathodal form GOT_{-1} is the mitochondrial form. Genetic variations were found to occur in both these two systems[1]. However, because of its poor resolution in usual staining procedure, its usage in routine polymorphic screening has been considered unsuitable.

In the present study enzyme variability was assayed electrophoretically in some guppy strains (*Poecilia reticulata*), and GOT at first showed very poor activity with usual staining schemes to be of any use in genetic variability studies. However, on the basis of trial and error, repeated attempts of staining were made and the activity of the enzyme could be enhanced with only a slight alteration in the stage of mixing of one recipe. Therefore, in contrary to earlier findings[1,4], the enzyme produced very clear resolution with the appearance of high polymorphism in the GOT_{-2} system with the heterozygotes with three bands. The following is the buffer system and the staining schemes employed for this enzyme.