EFFECT OF MUSTINE AND SARCOLYSIN ON THE STRUCTURAL OF TOTAL HISTONE

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The effect of sarcolysin and mustine on the structure of total histone was studied by the methods of optical rotatory dispersion, spectrophotometry, and viscosimetry. Both agents caused an increase in viscosity, while sarcolysin altered the parameters of optical rotatory dispersion of histone. The writers suggest that the observed effects are due to the aggregating and, in the case of sarcolysin, the possible spiralizing action of the agents.

When mutagens belonging to the family of alkylating compounds penetrate into the cell they react with all the components of the nuclear chromatin. The effect of these agents on DNA structure has been adequately investigated [4], but the writers know of no investigations into the action of alkylating compounds on the structural organization of the protein components of chromatin. At the same time, the principal nuclear proteins, the histones, have a substantial influence on the macromolecular organization of DNA and on its functions in the nucleoprotein complex [2, 7]. These functions of the histones are evidently dependent on their structural state [6, 8].

The object of the present investigation was to study the action of two classical bifunctional alkylating compounds – mustine (nitrogen mustard) and sarcolysin (phenylalanine mustard) [3] – on the structural organization of total histone in solution by the methods of optical rotatory dispersion, spectrophotometry, and viscosimetry.

EXPERIMENTAL METHOD

Total histone from the calf thymus was isolated by extraction with 0.25 N HCl. The resulting preparation was freeze-dried. Amino acid analysis, using the Hitachi analyzer, showed that the total protein

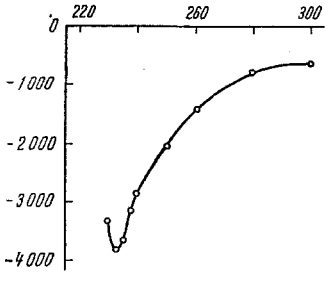

Fig. 1. Optical rotatory dispersion of histone. Abscissa, wavelength (in nm); ordinate, specific rotation.

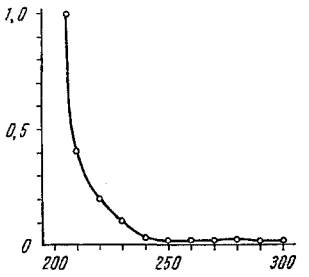

Fig. 2. Absorption spectrum of histone. Abscissa, wavelength (in nm); ordinate, optical density.

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TABLE 1. Level of Significance (P) of Changes in $([\eta]_{sp}/C)$ and b_0 on Treatment of Histone with Phenylalanine, Mustine, and Sarcolysin

Agent	Incubation time							
	15 min		18 h		42 h		120 h	
	η_{sp}/c	b_0	η_{sp}/c	b_0	η_{sp}/c	b_0	η_{sp}/c	b_0
Phenylalanine	>0,05	—	>0,05		>0,05	—	—	—
Mustine	0,02	>0,05	<0,01	>0,05	<0,01	>0,05	<0,01	>0,05
Sarcolysin	<0,01	<0,01	<0,01	0,03	<0,01	>0,05	<0,01	—

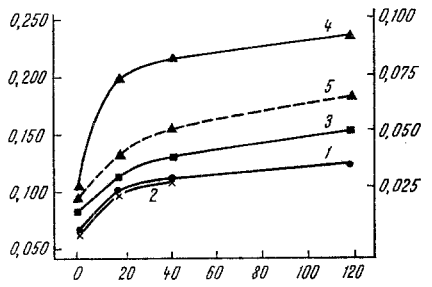

Fig. 3. Kinetics of changes in reduced viscosity and nonspecific absorption of histone preparations on incubation with phenylalanine, mustine, and sarcolysin: 1) native histone; 2) histone + phenylalanine; 3) histone + mustine; 4, 5) histone + sarcolysin. 1-4) Changes in reduced viscosity; 5) changes in nonspecific absorption at wavelength 340 nm. Abscissa, time (in h) of interaction with agents (O represents initial measurement after reaction between histone and agents for 15 min); ordinate: on the left — reduced viscosity, on the right — nonspecific absorption at wavelength 340 nm.

thus isolated was virtually identical in its amino acid composition with that described in the literature [8].* A 0.5% solution of the histone was incubated with the agent at 25°C. The concentration of the agents and phenylalanine in every case was $3 \cdot 10^{-2}$ M. Measurements of the optical rotatory dispersion (ORD) and viscosity also were made at 25°C without dilution of the preparations. The ionic strength of the incubation medium was 0.71 (0.7 M NaCl + 0.01 M HCl), pH 2. An acid pH was chosen to minimize aggregation of the histone, which is unavoidable at this high ionic strength. The ORD was measured with a Soviet SPU-M spectropolarimeter in the wavelength range 600-230 nm in a 1-cm cell (Fig. 1). To calculate b_0 (a coefficient proportional to the content of α-helical structures in the protein) by the Moffit–Yang [9] equation, λ_0 was taken as 212 nm; b_0 was calculated by the method of least squares from the ORD curves measured in the range 600-400 nm. The viscosity of the preparations was measured on a viscosimeter of the Ostwald type with a solvent flow fime of 280 sec. Measurements of the ORD and viscosity began 15 min after equal volumes of solutions of histone and agents had been mixed. The level of significance (P) of the changes in b_0 and $[\eta]_{sp}/C$ on treatment of the histone with phenylalanine, mustine, and sarcolysin was estimated from Student's table from a sample of not less than 5 experimental measurements. Spectrophotometry of the histone preparations treated with the agents was carried out on a Hitachi model 356 spectrophotometer (Fig. 2). The density of the solution of the control histone and agent, incubated separately for the corresponding time, was subtracted from the optical density of the experimental solutions at 340 nm, measured against the solvent.

EXPERIMENTAL RESULTS

The viscosity of the experimental preparations increased much more rapidly than the viscosity of the control (Fig. 3). The significance of the differences is shown in Table 1. The difference was greatest during the first 42 h of interaction, and thereafter the increase in viscosity took place more slowly. The observed increase in viscosity of the preparations was most probably due to the aggregating action of the agents, as is confirmed by the finding of an increase in the nonspecific absorption at 340 nm of solutions of total histone treated with sarcolysin (Fig. 3), and it is in agreement with the effects of bifunctional

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alkylating agents on bovine serum albumin [5]. It is clear from Fig. 3 that sarcolysin causes greater changes in the viscosity of the histone than mustine. The possibility is not ruled out that the more marked action of sarcolysin on the viscosity of histone is due to replacement of the inactive methyl radical or mustine by the phenylalanine radical of sarcolysin.

To verify this hypothesis an experiment was carried out with phenylalanine. The viscosity of the histones in the presence of phenylalanine was found to be the same as the viscosity of the control preparations (Fig. 3, Table 1). The higher activity of sarcolysin than of mustine was therefore evidently due entirely to properties peculiar to this molecule and not to the additive properties of its individual components. The greater activity of sarcolysin on molecules of total histone was also demonstrated by the results of spectrophotometry and ORD. Whereas the value of b_0 for histone preparations treated with mustine was virtually indistinguishable from the control (−150), the negative value of b_0 for histone treated with sarcolysin was significantly greater than b_0 for the control preparations (Table 1) during the first hours of the reaction (−230). The increase in the negative value of b_0 during the action of sarcolysin is evidence of a change in the native conformation of the histone molecules and, in particular, of an increase in the content of α-helical segments [9]. The ultraviolet absorption spectra and ORD of the histone preparations treated with mustine likewise were indistinguishable from the controls shown in Figs. 1 and 2. Unfortunately, measurements of the optical characteristics of histone preparations treated with sarcolysin in the ultraviolet region are rendered difficult by absorption of the agents in this region of the spectrum ($E_{260\,nm} = 23.1 \cdot 10^3$) [1].

These results show that the structural organization of the total histone molecule is sensitive to the action of both alkylating agents, although their sensitivity is different. Clearly sarcolysin, in this case, has the much greater activity. This conclusion is of fundamental interest for the elucidation and classification of the disturbances produced by bifunctional alkylating agents in the molecules of deoxyribonucleoprotein, the principal genetic substrate of cells.

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