BINDING OF SARCOLYSIN WITH HISTONES OF YOSHIDA'S SARCOMA OF STRAINS SENSITIVE AND RESISTANT TO ALKYLATING AGENTS

G. A. Panosyan

UDC 616-006.3.04-092.9-085.277.3-07:616-006.3-008.962.2:615.277.3

Investigations by gel-filtration, spectrophotometry in the far ultraviolet region, and equilibrium dialysis using sarcolysin-H^3 and hydrolyzed sarcolysin-H^3 showed that sarcolysin binds with the histones of two strains of Yoshida's sarcoma. The histone of the strain sensitive to alkylating agents binds more sarcolysin than that of the resistant strain. A possible correlation is suggested between sensitivity to antitumor preparations and their interaction with histones.

Ujhazy and Winkler [7, 8] obtained a resistant line of Yoshida's ascites sarcoma by repeated injection of increasing doses of tris-(chloroethyl)-aminohydrochloride. This strain retained its resistance to other alkylating compounds also and was 100 times less sensitive to sarcolysin than the original tumor. Ball et al. [5, 6] investigated some properties of the original and resistant strains of this tumor and found no difference in the number of chromosomes, rate of growth, content of nucleic acids and proteins, number of free thiol groups, and kinetics of cell division.

Data showing that these two strains of tumors differ in the ability of their histones to bind sarcolysin are presented below.

EXPERIMENTAL METHOD

Binding of sarcolysin with histones of resistant and sensitive strains of Yoshida's tumor (R-histone and S-histone, respectively) was determined by gel-filtration, spectrophotometry in the UV region, equilibrium dialysis, and utilization of sarcolysin-H^3 (with the H^3 in the ortho position of the aromatic ring). The corresponding unfractionated histones were isolated as described previously [3]. The cells of both lines of tumors were obtained from Dr. Connors (Chester Beatty Institute, London). Spectrophotometry was carried out on a Perkin-Elmer spectrophotometer (model UV 137). Radioactivity was measured on a Packard Tricarb model 314 FX scintillation counter.

EXPERIMENTAL RESULTS AND DISCUSSION

For gel-filtration a column with Sephadex G-25 was used to separate protein and sarcolysin from the reaction mixture. Since sarcolysin has a higher absorption at 260 nm than at 280 nm, while histone behaves in the opposite way, the ability of sarcolysin to bind histones can be determined by gel-filtration if the quantity of sarcolysin bound is sufficient to affect the E_{280}/E_{260} ratio (where E represents the optical density). The R- and S-histones in the protein peak have an underestimated value of the E_{280}/E_{260} ratio, slightly more marked in the case of the S-histone. This effect could be the result of addition of the sarcolysin to the histones and its elution in the protein fraction.

Spectrophotometry in the far ultraviolet region (190-220 nm) reveals changes in the intensity of absorption during complex formation by histones with DNA and dyes [1, 2]. On mixing histones with sarcolysin

Department of Biophysics, Erevan University. (Presented by Academician of the Academy of Medical Sciences of the USSR S. S. Debov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 76, No. 12, pp. 67-70, December, 1973. Original article submitted February 24, 1972.

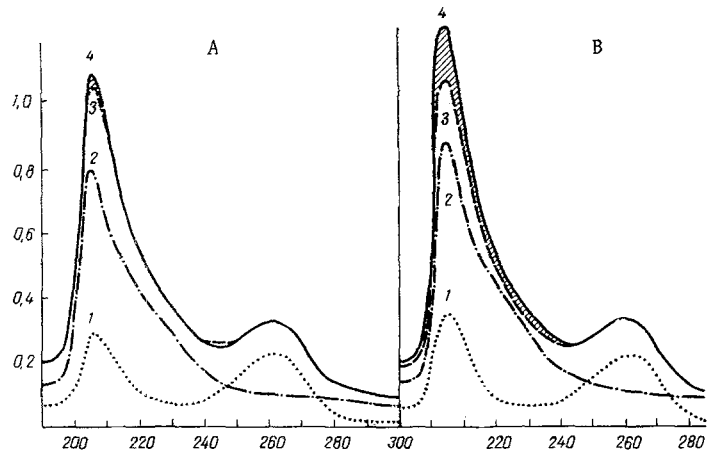

Fig. 1

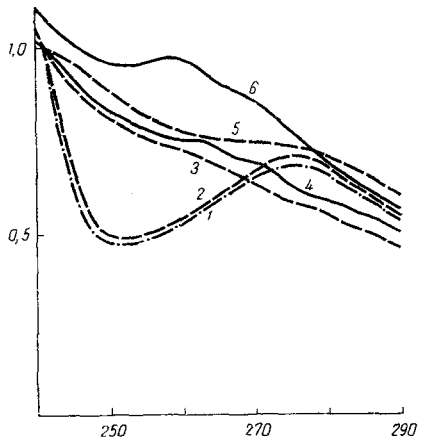

Fig. 2

Fig. 1. Absorption spectra in the UV region of R- and S-histones, sarcolysin, and their mixtures: A) R-histone, B) S-histone; 1) sarcolysin, 2) histone, 3) mixture of histone and sarcolysin, 4) calculated curve of some of absorptions of histone and sarcolysin measured separately. Abscissa, wavelength (in nm); ordinate, optical density (E). Shaded area represents hypochromic effect ($-\Delta D$). Medium: 0.1 M phosphate buffer, pH 6.8. Concentration: of histone 25 μg/ml, of sarcolysin 5 μg/ml.

Fig. 2. Absorption spectra of R- and S-histones after dialysis against sarcolysin and hydrolyzed sarcolysin. Before dialysis: 1) R-histone, 2) S-histone. After dialysis against hydrolyzed sarcolysin: 3) R-histone, 4) S-histone; against sarcolysin: 5) R-histone, 6) S-histone. Abscissa, wavelength (in nm); ordinate, optical density (E).

TABLE 1. Optical Characteristics of Mixture of R- and S-Histones with Sarcolysin

Histone	Hypochromic effect ($-\Delta D$)				
	H_2O	0.01M HCl	0.01M NaOH	0.1 M phosphate buffer, pH 6.8	0.1 M acetate buffer, pH 4.5
R	0,090—0,110	0,020—0,050	0	0,020—0,095	0,020—0,140
S	0,190—0,205	0,200—0,230	0	0,130—0,165	0,210—0,220

TABLE 2. Radioactivity of Histone after Dialysis for 24 h against Sarcolysin-H^3 and Hydrolyzed Sarcolysin-H^3 (in pulses/min per 0.4 ml solution)

	Sarcolysin-H^3	Hydrolyzed sarcolysin-H^3
R-histone	237	717
S-histone	371	744
Radioactivity of original solution (in pulses/min per 0.4 ml solution)	21 002	67 130

the sum of the absorptions of histone and sarcolysin does not correspond to the intensity of absorption of the mixture. A hypochromic effect ($-\Delta D$) was observed, and its value differed for R- and S-histones (Fig. 1). The value of $-\Delta D$ also depended on the medium in which the histone was mixed with the sarcolysin (Table 1).

In every case the S-histone had a higher value of $-\Delta D$ than the R-histone; i.e., more sarcolysin was bound with the S-histone. With an increase in the concentration of sarcolysin, the value of $-\Delta D$ increased much more for the R-histone than for the S-histone.

Equilibrium dialysis against sarcolysin and biologically inactive ("hydrolyzed") sarcolysin [4] showed that both these compounds penetrate into the dialysis sac and bind firmly with histones, for precipitating and washing the histones with acetone did not separate the sarcolysin from them, for the absorption spectrum of the solution obtained after dialysis of the histone was changed, giving a sharp rise in E at 260 nm (Fig. 2). Under these conditions hydrolyzed sarcolysin also was bound with the histones, but to a lesser

degree. Both sarcolysin and hydrolyzed sarcolysin were found to a greater degree with the S-histone than with the R-histone. The difference between the two histones in the case of hydrolyzed sarcolysin was no less marked.

The use of sarcolysin-H^3 in these experiments also showed that sarcolysin binds with histones and that hydrolyzed sarcolysin binds almost equally with R- and S-histones whereas sarcolysin itself binds to a greater degree with S-histone (Table 2).

Incubation of the cells of the resistant and sensitive lines of the sarcoma for 1 h at 37°C with sarcolysin-H^3, followed by isolation of the histone and determination of its radioactivity, showed that both R- and S-histones incorporate label intensively. Their values were 5080 and 1955 pulses/min/mg, respectively.

The results described above show that sarcolysin binds with the histones of tumor cells, and it does so to a greater degree with the histones of cells sensitive to alkylating agents than with those of cells resistant to them. The ability of histones to bind with alkylating compounds is evidently connected with the resistance or sensitivity of the tumor cells to these compounds.

LITERATURE CITED

1. G. A. Panosyan, Biol. Zh. Armenii, No. 11, 79 (1968).
2. G. A. Panosyan, Biol. Zh. Armenii, No. 10, 32 (1969).
3. G. A. Panosyan, Biol. Zh. Armenii, No. 2, 36 (1970).
4. C. R. Ball and T. A. Connors, Biochem. Pharmacol., 16, 509 (1967).
5. C. R. Ball, T. A. Connors, E. H. Cooper, et al., Neoplasma (Bratislava), 14, 253 (1966).
6. C. R. Ball, T. A. Connors, J. A. Dauble, et al., Internat. J. Cancer, 1, 319 (1966).
7. V. Ujhazy and A. Winkler, Neoplasma (Bratislava), 12, 11 (1964).
8. V. Ujhazy and A. Winkler, Folia Biol. (Prague), 11, 434 (1965).