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G. A. Maksimova, et al

Foreign Technology Division
Wright-Patterson Air Force Base, Ohio

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By: G. A. Maksimova and V. F. Runova

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Block	Italic	Transliteration	Block	Italic	Transliteration
А а	*А а*	A, a	Р р	*Р р*	R, r
Б б	*Б б*	B, b	С с	*С с*	S, s
В в	*В в*	V, v	Т т	*Т т*	T, t
Г г	*Г г*	G, g	У у	*У у*	U, u
Д д	*Д д*	D, d	Ф ф	*Ф ф*	F, f
Е е	*Е е*	Ye, ye; E, e*	Х х	*Х х*	Kh, kh
Ж ж	*Ж ж*	Zh, zh	Ц ц	*Ц ц*	Ts, ts
З з	*З з*	Z, z	Ч ч	*Ч ч*	Ch, ch
И и	*И и*	I, i	Ш ш	*Ш ш*	Sh, sh
Й й	*Й й*	Y, y	Щ щ	*Щ щ*	Shch, shch
К к	*К к*	K, k	Ъ ъ	*Ъ ъ*	"
Л л	*Л л*	L, l	Ы ы	*Ы ы*	Y, y
М м	*М м*	M, m	Ь ь	*Ь ь*	'
Н н	*Н н*	N, n	Э э	*Э э*	E, e
О о	*О о*	O, o	Ю ю	*Ю ю*	Yu, yu
П п	*П п*	P, p	Я я	*Я я*	Ya, ya

*ye initially, after vowels, and after ъ, ь; e elsewhere. When written as ë in Russian, transliterate as yë or ë. The use of diacritical marks is preferred, but such marks may be omitted when expediency dictates.

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GRAPHICS DISCLAIMER

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RUSSIAN AND ENGLISH TRIGONOMETRIC FUNCTIONS

Russian	English
sin	sin
cos	cos
tg	tan
ctg	cot
sec	sec
cosec	csc
sh	sinh
ch	cosh
th	tanh
cth	coth
sch	sech
csch	csch
arc sin	$\sin^{-1}$
arc cos	$\cos^{-1}$
arc tg	$\tan^{-1}$
arc ctg	$\cot^{-1}$
arc sec	$\sec^{-1}$
arc cosec	$\csc^{-1}$
arc sh	$\sinh^{-1}$
arc ch	$\cosh^{-1}$
arc th	$\tanh^{-1}$
arc cth	$\coth^{-1}$
arc sch	sech^{-1}
arc csch	csch^{-1}
rot	curl
lg	log

THE EFFECT OF FORMALDEHYDE ON THE ACTIVITY OF ANTHRAX

G. A. Maksimova and V. F. Runova

State Control Institute of Medical Biological Preparations imeni Tarasevicha

(Submitted 27/I, 1970)

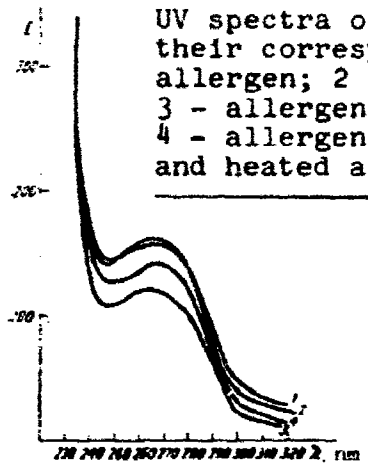
The anthrax allergen, isolated by the previously-described procedure (Runova and Ulanov, 1966), is a protein-polysaccharide-nucleic complex which contains up to 80-86% of protein. The carrier of the specific activity is the protein component of the preparation (Runova and Rudnev, 1968). For the purpose of explanation of the chemical determinant groups of anthrax allergen it was iodinated under varied conditions, which led to the significant loss of activity along with full blocking of phenol groups (Maksimova and Runova, 1969). In connection with this the assumption was made about the significant role of tyrosine in the biological activity of anthrax allergen. Earlier Runova and Rudneva (1968) noted a lowering in the specific activity of preparation following formalin treatment. Some researchers (Zaretskaya, 1959; Musiyko, 1957; Gavrilenkova and Runova, 1967) connected the lowering in the activity of allergen following formalin treatment only with the blocking of amino groups, assuming that the reagent acts selectively on them. However, it is necessary to keep in

mind that the action of formaldehyde on the protein molecule depends on the conditions of the reaction - the duration of the interaction, temperature and pH of the medium (Putmam, 1956). The short-time action of reagent on protein at room temperature and pH 7.0-8.0 is more specific in relation to the amino group. In this case there is compensation of formaldehyde with the amino or imino group and the formation of methylamines. However, during the isolation of the modified product the reaction flows in the opposite direction. The prolonged treatment of protein at 25 or 37° and pH 3.0-9.0 leads to the irreversible or partially reversible reaction with the formation of cross methylene bridges between the amino group, on the one hand, and other groups, in particular phenol and indole, on the other hand.

Thus formaldehyde is not a highly selective reagent on the amino group and therefore with a lowering in the activity of allergen it is necessary to consider its secondary interaction with other groups of the protein molecule. In the present work an attempt is undertaken to reveal the functional groups of protein which are responsible for lowering in the biological activity of anthrax allergen following formalin treatment.

The anthrax allergen was exposed to formaldehyde according to the procedure of Takeya and Mifuchi (1954). The allergen were dissolved in borate buffer at pH 9.1 with the addition of formalin of up to 1.5 and 20% concentration of formaldehyde in the solution. The control sample did not contain formalin. The samples were maintained at 37° for 5 days, dialyzed for 2 days against top water, and for twenty-four hours against distilled water, and then lyophile dried. Taking into consideration that the reaction of formaldehyde with proteins can be reversible (Takeya and Mifuchi, 1954), half of the preparations obtained was dissolved in distilled water, heated at 100° for 30 min, then dialyzed and dried, as is described above. In all samples the relative content of free amino

groups was determined by the ninhydride method (Kebot and Meyer, 1968), the content of tyrosine - according to the method of Folina (Kebot and Meyer, 1968), and the content of tryptophane - according to method of Spies and Chambers (1949).



UV spectra of the absorption of modified preparations and their corresponding control allergens: 1 - control allergen; 2 - control allergen, heated at 100° for 30 min.; 3 - allergen, processed by 5% solution of formaldehyde; 4 - allergen, processed by 5% solution of formaldehyde and heated at 100° for 30 min.

In three preparations, treated with the indicated solutions of formaldehyde, the content of the above-enumerated groups was reduced. In this case the quantity of tyrosine and tryptophane was decreased in them to 30 and 70% respectively and did not depend on the concentration of formaldehyde. The number of amino groups in the preparations treated with formalin was decreased with an increase in the concentration of formaldehyde, comprising correspondingly 43, 37 and 36% of the content of amino groups in the control allergen. After the heating of the formalin-treated preparations the content of tyrosine in them was virtually completely restored (97-100%), the content of tryptophane partially restored, and it comprised 50% in all preparations. The concentration of amino groups also increased somewhat, comprising 65, 53 and 48% respectively for preparations treated with solutions of formaldehyde.

The spectra of absorption in ultraviolet were determined for each preparation with the aid of an SF-4 spectrophotometer. The allergen was dissolved in borate buffer (pH 7.4), the concentration of protein in solution was in this case equal to 100 μg/ml. The figure gives the spectra of absorption of the

preparation, treated with a 5% solution of formaldehyde, and the same preparation after heating, and also their control allergens. The maximum of absorption in all cases was observed at 265-280 nm. The magnitude of absorption in the preparation heated after treatment with a 5% solution of formaldehyde was higher than in the preparation, not subjected to heat treatment. Analogous results are obtained for the preparations treated with 1 and 20% solutions of formaldehyde. It may be concluded that the formalin treatment led to dimunition in the content of cyclic amino acids in the allergen, and the heating of the formalin-treated preparation led to the partial restoration of the total content of tyrosine and tryptophane. Thus spectrophotometric data qualitatively confirmed the results of the chemical analysis of the modification of preparations. For the purpose of the determination of the specific activity of allergen it was dissolved in sterile borate buffer (pH 7.4) up to a concentration of protein of 500 μg/ml (concentration of protein was determined spectrophotometrically by the method of Wadell, 1956). Solutions in a volume of 0.1 ml were introduced into the end of the lateral surface of the body of sensitized guinea pigs weighing 350-400 g 21 days after their immunization with live STI-1 vaccine in doses of 25 million spores. The activity of the modified preparations was evaluated in 24 hours according to the extent of the area of hyperemia and compared with the activity of the control sample. All the preparations were specific, since in nonimmunized guinea pigs a skin reaction was not caused.

The specific activity of the formalin-treated allergens in two parallel experiments turned out to be lowered to an identical measure - it comprised approximately 50% of the activity of the control allergen (Table 1). Lowering in the activity of each preparation in comparison with the control is statistically reliable, since with $T \geq 3$ its probability comprised 99%. Since the activity of all formalin-treated allergens turned out to be equally lowered with a different content of amino groups in them,

Table 1. Change in the activity of anthrax allergen as a result of formalin treatment.

Concentration of formaldehyde in %	Characteristics of preparations				Residual activity in %
	M	σ	m	r	
0	257	61.7	23		100
	261	73.0	30		
1	131	31.2	11.7	4.9	51
	130	40.3	16.0	4.0	50
5	130	23.0	8.7	5.1	50
	138	42.5	17.0	3.6	53
20	121	37.2	15.0	5.0	47
	124	45.8	19.0	3.9	47

Note. In the upper row - the results of the first, in the lower - the results of the second experiment (in each experiment there were 7 guinea pigs).

it can be assumed that the lowering in biological activity, apparently, is not connected with blocking of the latter. At the same time a correlation was observed between the change in activity and the content of determined cyclic amino acids - the activity and concentration of tyrosine and tryptophane in the formalin-treated preparations was maximally reduced already with the lowest concentration of formaldehyde.

Not in one of the preparations, heated after treatment with formaldehyde, was a statistically proved lowering of allergen activity observed (Table 2). The restoration allergen activity in the heated formalin-treated preparations apparently cannot be connected with an increase in the content of free amino groups, since the concentration of the latter varied from 48 to 65%, in this case the activity of all preparations was restored virtually completely. Besides this, the content of amino groups in the allergen treated with a 20% solution of formaldehyde and warmed thoroughly, and in the allergen treated with 1% solution of formaldehyde (not warmed thoroughly), were comparable (48 and 43% respectively), nevertheless the activity in the first case is equal to the control, but in the second is lowered by 50%. The results obtained make it possible to conclude that the amino groups of anthrax allergen apparently are not essential for its activity. This proposition is confirmed by the results of selective N-acetylation of the anthrax allergen: with acetylation of the latter by acetic anhydride in a half-saturated solution of sodium acetate 80% blocking of amino acids occurs, nevertheless the biological activity of preparation is not changed (T=0.8).

Table 2. The statistical interpretation of the activity of the preparations, heated thoroughly after formalin treatment.

Concentration of formaldehyde (in %)	Characteristics of preparations			
	m	σ	m	T
0	256	34.3	13	—
	261	65.7	27	—
1	222	44.7	17	1.6
5	218	51.8	20	1.6
	197	63.2	26	1.7
20	213	66.8	25	1.5
	202	67.0	27	1.5

The fact that the full restoration of phenol groups is accompanied by the full restoration of activity after the heating of preparations, indicates the dependence of the specific activity of anthrax allergen on the content of tyrosine. Since the activity of the heated preparations is retained in the presence 50% indole groups, apparently the latter are not essential for the biological activity of the allergen.

In conclusion it is necessary to note that the results of this work, which indicate the connection of the specific activity of anthrax allergen with the content of tyrosine, confirm the assumption made previously (Maksimova and Runova, 1969) about the possible participation of tyrosine in the mechanism of the allergic reaction.

Conclusions.

1. The lowering of the specific activity of anthrax allergen following formalin treatment is connected with the action of formaldehyde on the phenol groups.

2. Amino and indole groups are not essential for the biological activity of the preparation.

BIBLIOGRAPHY

Гаврилюкова В. Ю., Рухова В. Ф. Ж. микробиол., 1967, № 5, с. 21.— Максимова Г. А., Рухова В. Ф. Биохимия, 1966, т. 5, с. 1015. — Мусайко В. А. Значение функциональных групп белка бруцелл для его аллергенных свойств. Автореф. дисс. канд. Одесса, 1957. — Рухова В. Ф., Рудакова О. А. Ж. микробиол., 1968, № 7, с. 139. — Оцимае Тэи ит, № 12, с. 90. — Рухова В. Ф., Уланова А. А. В кн.: Материалы межинститутской конференции памяти Л. А. Тарасевича. М., 1966, с. 228. — Кабот Е., Мейер М. В кн.: Экспериментальная иммунохимия. М., 1968, с. 663. — Spies J. R., Chambers D. C., Analyt. Chem., 1949, v. 21, p. 1249. — Путнам Ф. В кн.: Белки (под ред. Г. Нейрат, К. Бэйли). М., 1956, с. 310.— Takeya K., Mifuchi I., Enzymologia, 1954, v. 16, p. 366. — Wadell W. J., J. Lab. clin. Med., 1956, v. 48, p. 311.

The authors studied the effect of 1.5 and 20% formaldehyde solutions at pH 9.1 on the biological activity of the anthrax allergen. The activity of all the modified preparations decreased equally (by approximately 50%) and was independent of the concentration of formaldehyde. Reduction of tyrosin and tryptophane content occurred in analogous way (by 30 and 70%, respectively), at the same time the amount of free amino groups diminished with elevation of formaldehyde concentration. Heating of formalinized preparations at 100° C for 30 minutes led to complete restoration of specific activity in all the preparations, along with complete restoration of the content of phenol groups. The concentration of amino groups and tryptophane in these preparations averaged 50 to 60% of their concentration in the crude allergen.

The results obtained led to a conclusion that the activity of anthrax allergen was associated with the content of phenol groups, whereas amino and indol groups were apparently of no significance for biological activity of the preparation.