IDENTIFICATION OF STAPHYLOCOCCAL PROTEASES BY ANTITRYPTIC PRECIPITATING SERUM

Yu. S. Krivoshein, V. A. Protsenko,
V. V. Karpitskii, and I. F. Spitsyn

UDC 576.851.252.098.31:
577.152.34.087.4

A protease having common antigens with trypsin was obtained from the supernatant fluid of a culture of staphylococci by the use of a specific antitryptic precipitating serum obtained by intraconjunctival immunization of rabbits with crystalline trypsin. The result was confirmed by the accumulation of trypsin during cultivation of the staphylococci, by inhibition of proteolytic activity by the antitryptic precipitating serum, and by the formation of a protein precipitate during incubation of the supernatant with the antitryptic precipitaing serum.

KEY WORDS: staphylococcal proteases.

Many investigations pointing to the presence of proteolytic enzymes in saprophytes and pathogenic microorganisms have been published [2, 4, 6, 7].

On the assumption that the proteolytic activity of microorganisms may be due to an enzyme of the trypsin type, the writers used an antitryptic precipitating serum (ATS) to identify proteases in various strains of staphylococci.

EXPERIMENTAL METHOD

Altogether 10 strains of staphylococci isolated in the Department of Microbiology, Crimean Medical Institute, were used. Strains Nos. 105, 122, 124, 126, and 135 possessed plasma-coagulating, hemolytic, and lecithinase activity and the ability to form a lemon yellow pigment. Strains Nos. 249, 250, 257, 260, and 261 did not possess these properties. ATS was obtained after intraconjunctival immunization of rabbits with crystalline trypsin (Leningrad Meat Combine) [5]. The ATS titer in the ring-precipitation test was 1:160,000, with a zone of equivalence of 1 ml ATS to 80 μg trypsin. The ATS did not give a precipitation reaction in agar gel with commercial preparations of pepsin, chymotrypsin, and kallikrein. The organisms were grown in nutrient broth (MPB) and medium M-9 at 37°C for 24-120 h. The culture was centrifuged for 15-20 min at 3000 rpm. The supernatant was clarified at 10,000 rpm for 10 min and 1 ml of it was incubated for 30 min at 37°C with 0.5 ml ATS or physiological saline, after which the proteolytic activity was determined from the hydrolysis of BAPNA (N-benzoyl-DL-arginyl-p-nitroanilide) by Elander's method in Shaternikov's modification [1]. As the control the same operations were carried out with MPB and with medium M-9. In the immunochemical investigations 1 ml of supernatant was incubated with 1 ml ATS for 30 min at 37°C and for 24 h at 4°C, after which the quantity of precipitate was determined by Heidelberger's method [3]. The quantity of trypsin in the protein precipitate was calculated by means of a calibration curve in micrograms. A similar investigation was carried out with MPB and medium M-9 as the control.

EXPERIMENTAL RESULTS

Proteolytic activity was demonstrated as hydrolysis of BAPNA to the extent of 0.2-0.5 milliunit (Table 1) in the supernatant of strains Nos. 107, 122, 124, 126, and 135 24 h after cultivation of the staphylococci in MPB. After cultivation in medium M-9 proteolytic activity was discovered only in strains Nos. 107 and 126,

Department of Microbiology and Problem Laboratory and Department of Pathological Physiology, Crimean Medical Institute, Simferopol'. (Presented by Academician of the Academy of Medical Sciences of the USSR N. N. Zhukov-Verezhnikov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 80, No. 9, pp. 59-61, September, 1975. Original article submitted February 5, 1974.

TABLE 1. Characteristics of Proteolytic Activity of Staphylococci

	No. of strains of staphylococci							
	107	122	124	126	135	246, 250,257 260, 261	MPB	Medium M-9
Plasmocoagulase	3 h	2 h	2 h	2 h	1 h	Absent		
Hemolysins	+	+	+	+	+	—		
Lecithinase	+	+	+	+	+	—		
Pigment	Au-reus	Au-reus	Au-reus	Cit-reus	Cit-reus	Albus		
	Growth on MPB							
Activity (milliunits):								
after 24 h	0,4	0,2	0,5	0,3	0,3	0,0	0,0	
» 72 h	0,5	0,6	—	0,4	0,1	—	0,0	
» 120 h	0,6	0,5	0,4	0,2	0,2	—	0,0	
Trypsin (μg/ml):								
after 24 h	7,5	6,6	4,7	4,6	7,1	0,0	0,0	
» 72 h	8,0	8,0	—	7,0	9,0	—	0,0	
» 120 h	11,4	13,3	10,1	11,9	12,3	—	0,0	
Activity (milliunits) after treatment with ATS	0,0	0,0	0,0	0,0	0,0	—	0,0	
	Growth on medium M-9 for 72 h							
Activity (milliunits)	0,2	0,0	—	0,3	0,0	—		0,0
Trypsin (μg/ml)	11,0	12,5	—	10,0	9,0	—		0,0

to the extent of 0.2-0.3 milliunit. With an increase in the period of cultivation in MPB to 120 h the proteolytic activity increased in strains Nos. 107 and 122 and decreased a little in strains Nos. 124, 126, and 135. Incubation of the supernatant with ATS led to complete inhibition of proteolytic activity.

In the quantitative immunoprecipitation test of the supernatant with ATS, a protein precipitate was thrown down. Its content, calculated from the calibration curve, was equivalent to 4.6-7.5 μg trypsin, and during cultivation it increased to 10.1-13.3 μg/ml. A relatively high content of the protein precipitate was observed during cultivation of the staphylococci on medium M-9 (9-12.5 μg/ml). No direct dependence of the proteolytic activity, as reflected in hydrolysis of BAPNA, and the quantity of protein precipitate or, consequently, the quantity of trypsin could be found. In the case of staphylococci Nos. 249, 250, 257, 260, and 261 no proteolytic activity was found and no protein precipitate was formed in the immunochemical test. The increase in BAPNA-splitting activity during cultivation of the staphylococci, the inhibition of this activity by ATS, and the formation of the protein precipitate during incubation of ATS with the supernatant of the staphylococcal culture are all evidence of the existence of common antigenic properties between staphylococcal proteases and trypsin. The supernatant of a broth culture of staphylococci in which the largest quantity of protein precipitate was formed gave a positive precipitation reaction in agar gel with ATS.

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