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Immunochemical Assay of Saliva Parotin A

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Saliva parotin A (SPA) (a protein of molecular weight of 16000 purified from human saliva[2]) decreases serum calcium and protein concentrations and increases the number of circulating leucocytes in rabbits. When it is repeatedly injected in the animals, it produces an antibody[3] which does not crossreact with parotin, S-parotin, α-uroparotin nor with β-uroparotin, although all these parotins induce similar biological responses such as those mentioned above.

In the experiments to be reported below, we devised a highly sensitive and specific assay method for SPA by means of its reaction with its antibody. This assay method was rationalized by demonstrating the correlation between the immunochemical and biological activities of SPA preparations.

Experimental

Saliva Parotin A——Proteins were extracted from human saliva with acetic acid and SPA was purified to homogeneity according to the method described by Ito and Okabe.[4] Seven other preparations of SPA which were partially purified from different batches of human saliva were employed.

Anti-SPA Serum——Two rabbits (male, weighing 3 kg each) were immunized by injecting a mixture of pure SPA (1.5 mg in 0.3 ml of 0.9% NaCl) and Freund's complete adjuvant (Difco) (1.5 ml); two-thirds of the mixture injected subcutaneously and remaining one-third intraperitoneally. Injections were repeated once a week three more times, and then SPA alone (1.5 ml in 1 ml of 0.9% NaCl) was injected intraperitoneally. The booster shot was given once more 10 days later. Seven days after the second booster injection, blood was collected from the animals by heart puncture and serum was separated by centrifugation. One ml of this serum was strong enough to precipitate 300 μg of the purified SPA.

Determination of Leucocytes——Male albino rabbits weighing 2.5 to 3.0 kg were used for the assay of the leucocyte increasing activity of SPA preparations. The animals were housed in a room of a constant temperature (25°) and fed on a commercial diet (Funabashi Farm Co.). The SPA preparation was dissolved in 0.9% NaCl and 0.5 ml aliquot was injected intravenously in the animals. Blood (0.1 ml each time) was collected every 2 or 3 hr from the vein of ear of the animals. The number of leucocytes stained by Türk's solution was counted under a microscope with use of Thoma's hemocytometer. The leucocyte number increased by 80 to 100% in 24 hr after the injection of 5 μg of the purified SPA preparation, although there was a transient decrease by about 50% during the first 2 or 3 hr following the jnjection. The elevated leucocyte number persisted for several hr and gradually returned to normal in 2 days.

Determination of Ultraviolet Light Absorption——The protein solutions were taken in quarz cells of 1 cm light path. Light absorption of the solution was recorded against 0.1N NaOH on a spectrophotometer.

Results

Determination of SPA by Anti-SPA Serum

The SPA preparations were dissolved in 0.9% NaCl solution and a series of dilutions were made. To each 3 ml aliquot of these solutions, one ml of the anti-SPA serum was added. After one-hour incubation at 37°, the formed precipitate was collected by centrifugation.

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3) M. Shinoda, Y. Takagi, and B. Tamaoki, *Yakugaku Zasshi*, **86**, 306 (1966).
4) Y. Ito and S. Okabe, *Endocrinol. Japan.*, **6**, 166 (1959).

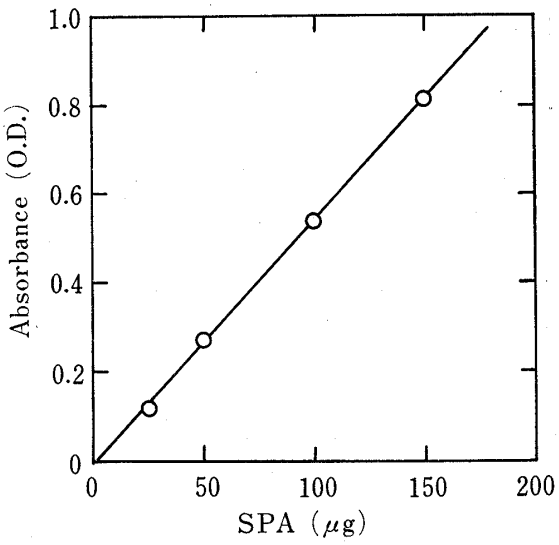

Fig. 1. Relationship between the Amount of Saliva Parotin A added and the Amount of the Precipitate obtained by Its Reaction with the Precipitin

The ordinate represents the light absorbance of the precipitate which was dissolved in 0.1N NaOH (4 ml each).

The precipitate was washed with 3 ml of 0.9% NaCl solution. Washing was repeated twice more. The precipitate was then dissolved in 4 ml each of 0.1N NaOH, and the light absorbance of the solution was determined at 240 mμ. The amount of SPA was calibrated against the values obtained with use of the purified SPA preparation. As it is shown in Fig. 1, the light absorbance of the solutions was linearly related with the amount of SPA added. It will be seen that SPA in an amount as small as 20 to 150 μg could be determined by this method. The magnitude of errors was within 5%, when the determinations were repeated with the same samples.

Correlation between the Immunochemical and Biological Assays

Table I shows that the SPA content of several crude SPA preparations determined by the above-described method is reasonably

Table I. Correlation between Immunochemical and Biological Activities of Crude Saliva Parotin A Preparations

Preparation	Immunochemical activity[a] (O.D./mg)	Leucocyte increasing activity	
		Dose of SPA (μg/kg)	Increasing rate[b] (%)
1	5.5	10	102.6± 4.7
		5	116.5±27.4
		2.5	44.5±18.4
2	3.1	18	147.6±28.9
		9	152.5±46.1
		4.5	30.9±13.0
3	2.1	26	105.3±36.2
		13	33.1±15.5
		6.5	50.4±27.4
4	1.7	32	139.3±42.9
		16	118.1±25.6
		8	33.6±15.7
5	0.46	60	108.6± 8.4
		30	79.6±40.3
		15	36.1±29.0
6	0.37	76	157.0±54.8
		38	84.3±11.5
		19	0.2± 4.4
7	0.14	200	207.1±68.7
		100	80.51±30.9
		50	25.2± 6.3
8	0.01	400	33.2± 6.8
		200	48.7± 9.8
		100	5.0±16.5

a) One mg aliquots of various preparations were treated with the precipitin, and the formed precipitate was determined by the optical density at 240 mμ after it was dissolved in 0.1N NaOH (4 ml each).

b) Each figure is the mean ± standard error of the determinations in 3 rabbits at 24 hr after SPA intravenous injection.

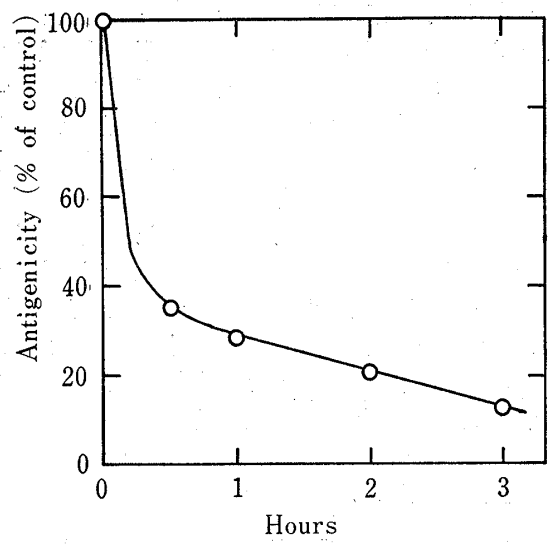

Fig. 2. Decrease of the Immunochemical Potency of Saliva Parotin A by the Treatment with 0.1N NaOH

The ordinate represents the amount of the precipitate produced by the antigen-antibody reaction in the term of % of controls (untreated Saliva parotin A).

correlated with the result of the assay of the leucocyte increasing activity of these preparations.

In a separate experiment, the purified SPA preparation was dissolved in 0.1N NaOH in a concentration of 0.1 mg/ml and incubated at 37°. Portions of the SPA solution were removed from the incubation and brought to pH 7.0 by titration with 0.1N HCl. Fig. 2 shows that the amount of immunochemically reactive SPA was rapidly decreased by the treatment with the strong base. The decrease of the leucocyte increasing activity of SPA was more rapid; half an hour was long enough to accomplish complete loss of the activity. The result suggests that the biological activity of the protein is more labile than its antigenicity.

Discussions

Because SPA is a simple protein, there is no chemical method of determination which is specific for it. Biological as assays based on its action upon serum calcium or upon the number of leucocytes in rabbits are by no means simple. On the other hand, the immunochemical method reported above is quite useful not only because of simplicity of the procedure but also because of high reproducibility of the result. Immunochemical methods similar to that reported above have been successfully applied by Mimura[5] for the determination of parotin and S-parotin. These workers used Folin-Ciocalteau's phenol reagent for the measurement of the mass of the precipitate produced by the antigen-antibody reaction. The method reported in the present paper is based upon ultraviolet light absorbance for the quantitative determination of the precipitate. Light absorbance at 240 mμ was employed rather than the characteristic 285 mμ peak, because the light absorption spectrum of the solution had a slight shoulder around 240 mμ and the absorbance at 240 mμ was more than 3 times as high as that at 285 mμ.

In summary, the immunochemical method reported in the present paper is sensitive enough for the determination of SPA in amounts of 20 to 200 μg. The SPA content of several crude SPA preparations was determined by the above-described method. The magnitude of the leucocyte increasing activity of these preparations was in good agreement with the result of the immunochemical assay.

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5) T. Mimura, *Yakugaku Zasshi*, **82**, 442 (1962).