Quantitation of IgA in human whole saliva: A comparison of three immunoassays

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Concentration of IgA was measured in saliva samples from 23 healthy adults by solid phase radioimmunoassay (RIA), rocket immunoelectrophoresis (IEF) and single gradial immunodiffusion (SRID). In preliminary experiments it was found that all three methods gave too low values of salivary IgA unless the samples were reduced with dithiothreitol.

After such reduction the mean concentrations of IgA in whole saliva were 100 mg/l (log mean 2.01 ± 0.22) measured with RIA, 90 mg/l (log mean 1.95 ± 0.18) measured with IEF and 120 mg/l (log mean 2.06 ± 0.19) measured with SRID.

Key-words: Solid phase radio-immunoassay; immunoelectrophoresis; single radial immunodiffusion

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Several investigators have studied the concentration of IgA in human whole saliva using different methods (Table 1). The results have varied a great deal, although all methods employed are based on immunoassays. Since salivary IgA is mainly dimeric, consisting of two monomeric IgA units and two additional polypeptide chains, J-chain and secretory component, errors are obtained in IgA values if serum IgA, which is mainly monomeric, is used as a standard (9). When serum IgA has been used as a standard, the underestimation caused by the discrepancy between molecular size of the standard and the salivary IgA has been corrected in most studies by multiplying the obtained values by the factor of 3.25 introduced by Brandtzaeg (9).

In order to obviate the errors that arise from possible differences in molecular size both the standard and the samples can be treated with reducing agents as has been done in some studies with varying success (6, 15, 17, 18, 20, 30).

The purpose of this study is to compare three different methods, namely a solid phase radioimmunoassay (RIA), rocket immunoelectrophoresis (IEF), and single radial immunodiffusion (SRID) in estimating the concentration of human salivary IgA. The effect of reduction on the values obtained with the methods was also studied.

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MATERIALS AND METHODS

15 persons, 30–50 years, were included in the preliminary experiments, and 23 persons of the same age group in the final experiments. They were all healthy and had good oral health. Whole saliva samples and serum samples were collected for IgA quantitation.

Saliva and serum samples

1 ml of unstimulated whole saliva samples were collected and immediately centrifugated at 10.000 g for 15 min at 4°C. The supernatant fluids were divided into portions, frozen and stored at -20°C for less than one month. Samples (6 ml) of the venous blood were obtained from the 15 persons participating in the preliminary experiments. IgA of the saliva samples was measured with RIA, IEF and SRID and of the serum samples with SRID.

Reduction of IgA

The samples and the human IgA standard (186 mg IgA/100 ml, 100 IU/ml, Behringwerke AG) were reduced just before the assays with dithiothreitol (DTT) (Sigma Chemical Co.). The final concentration of DTT was 0.02 M or 0.1 M. The incubation time was 30 min at room temperature (18). For this study the effect of reduction was tested on a whole saliva pool with sucrose gradient centrifugation. The IgA of unreduced salivary IgA forms three frac-

Table 1. *Concentration of IgA in whole saliva obtained by different authors*

Author	Stimulation of saliva	Volume of sample or duration of collection	Method	Standard for IgA	IgA (mg/l) range	Mean
Salmon (1969)	?	?	SSPRIA[a]	serum IgA		45
Brandtzaeg (1970)	–	10 min	SRID	parotid IgA	142–293	194
Sims (1972)	?	20 ml	SRID[b]	serum IgA	4.2–50.2	18.6*
Ben-Aryeh (1976)	–	10 min	SRID	serum IgA	20–190	85
Mach (1976)	–	?	SRID	serum IgA	40–190	77
Arnold (1977)	–	?	SRID	colostrol IgA	74– 287	140
Everhart (1977)	–	10 min	SRID	serum IgA	0– 452	145*
Ben-Aryeh (1978)	–	10 min	SRID	serum IgA	25–130	79,6
Cole (1978)	+	10 min	IEF[c]	colostrol IgA		65
Demetriou (1978)	–	?	SRID	serum IgA		103
Virella (1978)	–	?	Nephelom[d]	serum IgA	22–150	65
Hyyppä (1980)	+	10 ml	SRID	serum IgA		77*

[a]) SSPRIA: Sandwich solid phase radioimmunoassay
[b]) SRID: Single radial immunodiffusion
[c]) IEF: Immunoelectrophoresis
[d]) Nephelom: Laser nephelometry
* Figures are converted from serum IgA standard values by multiplying the obtained values by 3.

tions in sucrose gradient centrifugation; two of them, representing 77 % of the total, sedimented with a constant higher than 7S, and only 19 % sedimented with the same constant as the serum IgA. After the reduction with 0.02 M DTT only one peak was detected. It presented more than 95 % of the total and sedimented with the same constant as the serum IgA.

Radioimmunoassay (RIA)

A solid phase radioimmunoassay was used (2,10). First the inner surfaces of the 70 x 10 mm polystyrene tubes were coated with a 0.3 ml solution of rabbit anti-human IgA (Behring Institut) in dilutions of 1/15.000 – 1/30.000. Following an overnight incubation of these tubes at 4°C, they were washed twice with 1 ml of 0.9 % saline and filled with saline containing 5 % normal rabbit serum (NRS) and 0.02 % NaN_3. After an overnight incubation at 4°C and two washes with saline the sample dilutions, either unknown or IgA standards, were added in a volume of 0.1 ml. The dilutions were made in phosphate buffered saline containing 0.5 % gelatine and 0.04 % sodium azide. Immediately after this 0.3 ml of labeled IgA-myeloma protein made in 1 % NRS containing approximately 30.000 cpm was added in. Following the incubation overnight at 22°C the tubes were washed twice with saline. Finally the tubes were counted and the inhibition curves were drawn. High control tubes were coated with antibody and samples were replaced by a pure saline-gelatine solution. In the low control tubes the antibody was replaced by saline and no inhibitor was added.

Purification of IgA from myeloma serum

IgA was purified from the IgA-myeloma serum No 12 by the caprylic acid precipitation method (14). The globulins of the IgA myeloma serum were first precipitated by $(NH_4)_2SO_4$, then the precipitate was dissolved and dialyzed and caprylic acid was added to the solution to precipitate impurities which were insoluble in ammonium sulphate. This left IgA and IgG in the solution. The IgA was then absorbed to DEAE cellulose and eluted off with a 0.09 M acetate buffer at pH 5.7. The resulting IgA myeloma protein was relatively free of impurities, and the yields by this method were good. The final product did not contain any IgG as detected by immunoelectrophoresis. The purified IgA preparation was analyzed with a sucrose gradient and it was found that more than 90 % of it was monomeric.

Radioiodination of IgA

Purified 7S IgA myeloma protein No 12 was radiolabeled with ^{125}I (Radiochemical Center, Amersham, England) by the chloramine-T method of Hunter and Greenwood (16).

Rocket immunoelectrophoresis (IEF)

Rocket immunoelectrophoresis was carried out with rabbit antiserum to human α-chain (Behringwerke) in 1 % agarose A 37 (Pharmindustrie, Clichy) (3, 19). Electrophoresis was carried out overnight at 90 mV on 14 x 14 cm plates. Four different dilutions of standards were used on each plate to construct the standard curve. The heights of the rockets obtained were plotted on linear graph paper and compared with those of the standards to obtain the values of the samples.

Single radial immunodiffusion (SRID)

Anti-human IgA LC-Partigen® immunodiffusion plates (Behringwerke AG,

Marburg, W. Germany) were used (23). Diffusion time was 2 days at room temperature. The squared diameters of the precipitation rings were calculated and compared with those of the standards.

The samples were analyzed with and without reduction. All RIA, IEF and SRID measurements were done in triplicate, the means of which were used for statistical analyses.

In order to compare the salivary IgA values obtained by the three methods, the standard moment correlation coefficients between RIA, IEF and SRID were calculated. The statistical analysis was made both for the native and reduced salivary IgA values. The correlation of serum and salivary IgA was tested in the preliminary experiments.

Table 2 *The standard product moment correlation coefficients between the methods tested in this study*

Treatment of saliva		Correlation coefficients
native	RIA- IEF	0.90
	RIA- SRID	0.91
	IEF - SRID	0.84
0.02 M DTT	RIA- IEF	0.67
	RIA- SRID	0.84
	IEF - SRID	0.89
0.1 M DTT	RIA- IEF	0.70
	RIA- SRID	0.81
	IEF - SRID	0.85

RESULTS

In preliminary experiments with 15 unreduced whole saliva samples the mean values obtained for IgA were 53 mg/l (log mean 1.72 ± 0.23) with RIA, 26 mg/l (log mean 1.42 ± 0.23) with IEF, and 36 mg/l (log mean 1.55 ± 0.27) with SRID. When 0.02 M DTT was used the corresponding values were 98 mg/l (log mean 1.99 ± 0.22) with RIA, 57 mg/l (log mean 1.75 ± 0.19) with IEF, and 88 mg/l (log mean 1.94 ± 0.15) with SRID. When 0.1 M DTT was used the mean values were 93 mg/l (log mean 1.97 ± 0.21) with RIA, 72 mg/l (log mean 1.86 ± 0.10) with IEF, and 110 mg/l (log mean 2.04 ± 0.16) with SRID. Geometric means were used, because the normal distribution of salivary IgA was logarithmic.

The standard product moment correlation coefficients between RIA, IEF and SRID are presented in Table 2. On the basis of these preliminary experiments the authors of the present paper decided to reduce the samples with 0.02 M DTT for RIA and 0.1 M DTT in the other two assays. The IgA values of the reduced saliva samples are presented in Table 3. The average values of the 23 saliva samples were found to be 100 mg/l (log mean 2.01 ± 0.22) with RIA, 90 mg/l (log mean 1.95 ± 0.18) with IEF and 120 mg/l (log mean 2.06 ± 0.19) with SRID.

The effect of alkylation with 0.035 M or 0.15 M recrystallized iodoacetamide (18, 25) was tested in separate experiments (not reported here). The salivary IgA values obtained were as high as the values obtained with reduction alone (2.0 or 2.6 times the values obtained without reduction). The reducing agent did not seem to interfere with the assays as the reduction of serum IgA with 0.02 or 0.1 M DTT only caused a 1.2 fold increase.

The standard product correlation coefficient between serum and saliva IgA values obtained with SRID was 0.11, which is not statistically significant ($p > 0.1$).

DISCUSSION

The data presented here indicate that the salivary IgA values obtained by the three methods described have a fair concordance. The authors suggest that the reduction should be performed in all three assays since it has a significant effect on the values. As the effect of reduction is different in individual saliva samples we do not propose that the native values should be converted by factors of x2–x3 (based on differences in the mean native and reduced values) depending on the method. This would decrease the accuracy of the individual values.

Concentrations 0.02 M and 0.1 M DTT had the same effect (average 2-fold increase over unreduced values) when the RIA was employed. A 0.1 M concentration had approximately the same effect when the IEF or the SRID test was employed (2.6-fold increase over unreduced values), but a 0.02 M reduction

Table 3. *Concentrations of IgA in whole saliva and serum*

Volunteer No	Saliva IgA (mg/l) SRID a)	Saliva IgA (mg/l) IEF a)	Saliva IgA (mg/l) RIA b)	Serum IgA (g/l) SRID
1	200	150	190	1.4
2	93	52	48	1.8
3	130	69	85	1.1
4	180	83	180	1.4
5	120	72	130	2.3
6	140	85	80	1.2
7	58	65	95	2.1
8	100	73	130	1.4
9	74	72	80	1.4
10	110	64	100	2.3
11	62	59	38	2.9
12	150	76	170	1.9
13	140	77	160	4.3
14	62	57	45	1.4
15	130	68	120	1.6
16	160	160	140	
17	100	120	66	
18	350	260	300	
19	160	150	160	
20	87	84	92	
21	87	120	88	
22	73	95	54	
23	150	150	110	
mean*	120 mg/l (2.06 ± 0.19)	90 mg/l (1.95 ± 0.18)	100 mg/l (2.01 ± 0.22)	1.8 g/l (0.25 ± 0.16)

a) sample was reduced with 0.1 M DTT
b) sample was reduced with 0.02 M DTT

*figures derived from logarithmic calculations.

only increased the values by an average factor of 2.2. or 2.4. We do not know why the 0.02 M DTT was more efficient in the RIA procedure than in the IEF or the SRID procedure. The reduction was performed with 1/10 diluted samples in the RIA procedure and practically undiluted samples in the IEF and SRID procedures. This may have caused the difference. Whatever happened in the reduction of the saliva samples probably had to do either with the degree of polymerization or the presence of the secretory component.

A small fraction of salivary IgA originates directly from the blood (28). Its presence is usually attributed to leakage through the gingival crevice (8, 24, 29) and there is evidence that the leakage is increased via the gingival pockets in patients with periodontal disease (21). Brandtzaeg (7) has also found that most gingival IgA immunocytes are monomer producers. The persons included in this study had good oral health and they did not have periodontal diseases. Still it is possible that varying proportions of the serum IgA contributed to the different reduction effects observed in different samples. Another explanation could be the variable resistance to reduction of salivary IgA molecules (6).

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