



# A role of salivary carbonic anhydrase VI in dental plaque

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## Summary

**Objective:** Carbonic anhydrase (CA) VI is a unique secreted isozyme of CA, which catalyzes the reversible reaction  $\text{CO}_2 + \text{H}_2\text{O} \leftrightarrow \text{H}^+ + \text{HCO}_3^-$ . CA VI has been thought to provide a greater buffering capacity to fluids into which it is secreted. This study was performed to confirm this in saliva.

**Design:** Nine healthy subjects participated in the study. The pH of the dental plaque from each subject was monitored after a mouth rinse with 10% sucrose with or without  $10^{-5}$  M acetazolamide, a specific inhibitor of CA. Also CA was examined in plaque by enzyme histochemistry, immunohistochemistry and Western blot analysis.

**Results:** Though sucrose and sucrose plus inhibitor yielded Stephan curves with a similar temporal pattern, the pH values of the latter were significantly lower than those of the former. Plaque exhibited CA activity by enzyme histochemistry. Immunohistochemistry and Western analysis demonstrated that the activity was due to CA VI but not to CA I or CA II.

**Conclusions:** The results indicate that CAVI in saliva penetrates plaque and facilitates acid neutralization by salivary bicarbonate. Therefore, CA VI may be considered an anti-caries protein in saliva.

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## Introduction

Carbonic anhydrase (CA; EC.4.2.1.1), a zinc metalloenzyme, catalyzes the reversible hydration of car-

bon dioxide in a reaction  $\text{CO}_2 + \text{H}_2\text{O} \leftrightarrow \text{H}^+ + \text{HCO}_3^-$ . Among 12 active mammalian isozymes, CAVI is unique in that it is secreted by exocrine glands.<sup>1,2</sup> CAVI was originally described as a salivary constituent produced by the serous acinar cells.<sup>3–8</sup> The isozyme is also secreted by lacrimal, mammary, nasal and tracheobronchial glands.<sup>1,2,9–11</sup> By catalyzing the above reaction, CAVI is thought to provide a greater buffer-

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ing capacity to the secretions, however, the evidence for this is still lacking.

The buffering of saliva is best reflected by neutralization of the acid in dental plaque. The augmentation of the buffering capacity would affect the dental caries process. In fact, saliva expressed higher CA activity in caries-free children than in children with active caries.<sup>12</sup> More recently, salivary CA VI concentration was shown to correlate negatively with the numbers of decayed, missing and filled teeth (DMFT index) especially in individuals with poor oral hygiene.<sup>13</sup> CA VI in saliva has been shown to be adsorbed on to the tooth surface and remain enzymically active.<sup>14</sup>

The aim of the present study was to provide direct evidence of CA VI implication in salivary buffering. To this end, the pH of dental plaque was measured after rinsing with either a sucrose solution or a sucrose solution containing a CA inhibitor, and CA VI was demonstrated in the plaque by histochemistry and Western analysis.

## Materials and methods

The study was approved by the Intramural Ethics Committee of Osaka University Graduate School of

Dentistry and informed consent was obtained from each subject.

## Subjects

The subjects were nine healthy adults (seven males and two females) aged between 21 and 36 years. None of the subjects was on medication and none had any teeth with active caries. The mean DMFT index was 5.7 (0–17) (Table 1). They all had a healthy gingiva without redness, swelling or bleeding. The subjects refrained from all oral hygiene procedures for 48 h to allow plaque to accumulate on their teeth. The subjects did not consume any food or drink for at least 2 h prior to presenting themselves in our laboratory for sampling between 9 and 11 a.m.

## Plaque pH

Plaque pH was measured by a method similar to that described by Higham and Edgar.<sup>15</sup> Plaque samples (about 0.5 mg) were obtained with a sterile curette from all accessible buccal surfaces of premolars and molars. The plaque was suspended in 20  $\mu$ L of distilled water in a plastic micro-titer dish. The pH was read with a glass combination electrode (Model

**Table 1** pH and pH difference ( $\Delta$ pH) of dental plaque after a 2 min mouthrinse of 10% sucrose with or without  $10^{-5}$  M acetazolamide.

Subject	Sex/age	DMFT index	0 min		5 min		10 min		15 min		20 min		30 min		60 min			
			pH	$\Delta$ pH	pH	$\Delta$ pH	pH	$\Delta$ pH	pH	$\Delta$ pH	pH	$\Delta$ pH	pH	$\Delta$ pH	pH	$\Delta$ pH		
1	Male/36	3	6.98	-0.92	5.96	-0.92	5.04	-0.92	4.12	-0.92	3.20	-0.92	2.28	-0.92	1.36	-0.92	0.44	-0.92
			6.88	-1.02	5.86	-1.02	4.84	-1.02	3.92	-1.02	3.00	-1.02	2.08	-1.02	1.16	-1.02	0.24	-1.02
2	Male/29	10	6.28	-1.02	5.26	-1.02	4.24	-1.02	3.22	-1.02	2.20	-1.02	1.18	-1.02	0.16	-1.02	-0.86	-1.02
			6.30	-1.12	5.18	-1.12	4.16	-1.12	3.14	-1.12	2.12	-1.12	1.10	-1.12	0.08	-1.12	-0.96	-1.12
3	Male/30	0	6.63	-0.92	5.71	-0.92	4.79	-0.92	3.87	-0.92	2.95	-0.92	2.03	-0.92	1.11	-0.92	0.19	-0.92
			6.54	-1.02	5.52	-1.02	4.60	-1.02	3.68	-1.02	2.74	-1.02	1.80	-1.02	0.88	-1.02	-0.14	-1.02
4	Male/21	1	6.92	-0.92	6.00	-0.92	5.08	-0.92	4.16	-0.92	3.24	-0.92	2.32	-0.92	1.40	-0.92	0.48	-0.92
			6.86	-1.02	5.94	-1.02	5.02	-1.02	4.10	-1.02	3.18	-1.02	2.26	-1.02	1.34	-1.02	0.42	-1.02
5	Male/22	4	6.56	-0.92	5.64	-0.92	4.72	-0.92	3.80	-0.92	2.88	-0.92	1.96	-0.92	1.04	-0.92	0.12	-0.92
			6.65	-1.02	5.63	-1.02	4.71	-1.02	3.79	-1.02	2.87	-1.02	1.95	-1.02	1.03	-1.02	0.11	-1.02
6	Male/27	10	6.67	-0.92	5.75	-0.92	4.83	-0.92	3.91	-0.92	2.99	-0.92	2.07	-0.92	1.15	-0.92	0.23	-0.92
			6.75	-1.02	5.73	-1.02	4.81	-1.02	3.89	-1.02	2.97	-1.02	2.05	-1.02	1.13	-1.02	0.21	-1.02
7	Male/30	6	6.65	-0.92	5.73	-0.92	4.81	-0.92	3.89	-0.92	2.97	-0.92	2.05	-0.92	1.13	-0.92	0.21	-0.92
			6.69	-1.02	5.77	-1.02	4.85	-1.02	3.93	-1.02	3.01	-1.02	2.09	-1.02	1.17	-1.02	0.25	-1.02
8	Female/29	0	6.41	-0.92	5.49	-0.92	4.57	-0.92	3.65	-0.92	2.73	-0.92	1.81	-0.92	0.89	-0.92	-0.03	-0.92
			6.35	-1.02	5.43	-1.02	4.51	-1.02	3.59	-1.02	2.67	-1.02	1.75	-1.02	0.83	-1.02	-0.13	-1.02
9	Female/29	17	6.48	-0.92	5.56	-0.92	4.64	-0.92	3.72	-0.92	2.80	-0.92	1.88	-0.92	0.96	-0.92	0.04	-0.92
			6.52	-1.02	5.50	-1.02	4.58	-1.02	3.66	-1.02	2.74	-1.02	1.82	-1.02	0.90	-1.02	-0.02	-1.02

For each subject, numerals in the upper and lower rows represent pH and  $\Delta$ pH after sucrose without and with acetazolamide, respectively.

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