EFFECT OF pH ON THE FORMATION OF DIFFUSIBLE CARBON DIOXIDE FROM HUMAN SUBMAXILLARY SALIVA

Shibdas Biswas

Department of Oral Biology, The University of Manitoba, Winnipeg, Canada. R3E OW3

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SUMMARY

The effect of pH on the diffusible carbon dioxide from saliva was examined. The pH of saliva was maintained by the addition of a suitable buffering system consisting of either sodium acetate or tris buffer. Actual diffusible carbon dioxide was determined by Conway's diffusion technique and the values were compared with another set of values obtained by calculation, employing the Henderson-Hasselbalch equation. The result indicated that the diffusible carbon dioxide formation was not only dependent on the relative composition of carbonic acid and its salts (HCO_3^- and $CO_3^=$) at a given pH, but it was also dependent on the relative rate of hydrolysis of HCO_3^- and $CO_3^=$ respectively, which are strictly controlled by the available H^+-ions.

INTRODUCTION

While examining properties of human submaxillary saliva as a lyophilic colloid in relation to protein aggregation (1-3), it was observed that the aggregation varies as a function of pH. Since the pH of saliva is largely affected by the relative composition of H_2CO_3, HCO_3^-, $CO_3^=$ and dissolved CO_2, these considerations prompted a re-investigation of the state of diffusible carbon dioxide in saliva.

Consequently, experiments were carried out to determine the diffusible CO_2 in saliva when the saliva was adjusted to a constant pH varying between 3.5 amd 8.7. Carbon dioxide bound to amino groups as carbamino CO_2 was not considered because it was present in saliva in trace amounts as shown previously by Grøn (4). Evaluation of the data showed that diffusible CO_2 formation was the function of the pH as well as the rate of hydrolysis of HCO_3^- and $CO_3^=$, respectively.

METHOD

Stimulated submaxillary saliva was collected by cannulation. The time of cannulation, flow rate (1.25 ml ± 0.08/min/gland) and the subjects were the same each time to minimize day-to-day variation in electrolyte and protein concentrations (5).

An aliquot of 250 µl samples was immediately transferred into a series of serum bottles of 10 ml of volume and then each bottle was tightly closed with a serum stopper. A custom-made Teflon cup, containing 70 µl of 1.0N NaOH was suspended with a stainless-steel wire from the stopper. To each serum bottle, 1 ml of buffer sufficient to maintain a constant pH, was injected through the serum stopper and then samples were incubated at 25°C for varying lengths of time. As gaseous carbon dioxide diffused out of the mixture, it was trapped in sodium hydroxide solution (7).

One duplicate sample, corresponding to each pH (ranging from 3.5 to 8.7 with an approximate difference of 0.5 pH unit) was removed from among the series at varying intervals of time and then the CO_2 content of each cup was analyzed by a back-titration using 0.05 N HCl. The back-titration was performed by using a micro-autoburette (type ABU 1a, Radiometer, Copenhagen).

The amount of carbon dioxide evolved, was determined by a standard curve, which was prepared from known amounts of $NaHCO_3$. The procedure adopted to trap CO_2 from bicarbonate was the same as that described above.

Total CO_2 content (6) (TCO_2 = CO_2 (dissolved) + H_2CO_3 + HCO_3^- + $CO_3^=$) in a separate sample was also analyzed in the same manner. In this case 500 µl 0.1 N HCl was injected to displace gaseous CO_2. At a high pH, some of the HCO_3^- and $CO_3^=$ remained in solution (as indicated by the value of diffusible CO_2 which could not approach the value of TCO_2), and this remaining HCO_3^- and $CO_3^=$ was determined as CO_2 by the same technique following the addition of 500 µl of 0.1 N HCl, sufficient to change the pH of sample from alkaline to a pH as low as 3.0.

Assuming the value of TCO_2 equals approximately 38 mM (actually ob-

tained at pH 3.4) relative compositions of H_2CO_3, and HCO_3^- formed at varying pH, were calculated by the following Henderson-Hasselbalch equation:

$$-\log [H^+] = pk_1 + \log_{10} \frac{[HCO_3^-]}{[H_2CO_3]} \text{ or } \log_{10} \frac{[HCO_3^-]}{[CO_2]} \quad \ldots\ldots\ldots (1)$$

and the experimental and theoretical values were compared.

At a given pH the amount of diffusible CO_2 will measure the relative proportion of H_2CO_3 ($H_2CO_3 \rightleftharpoons H_2O + CO_2$) and the dissolved CO_2. The formation of H_2CO_3 will depend on the rate of hydrolysis of HCO_3^- (see following equation 2).

RESULTS AND DISCUSSION

The mean of five experiments carried out on different days is reported below. From Fig. 1, it is evident that at pH 3.5 the diffusible CO_2 **equals** TCO_2. As the pH was progressively increased the diffusible CO_2 gradually decreased perhaps because of decreased H_2CO_3 concentration - the concentration of which was responsible for the formation of diffusible CO_2 at a given pH according to the following equation: $H_2CO_3 \rightleftharpoons H_2O + CO_2$. The concentration of HCO_3^-, however, in these conditions gradually increased and this was theoretically consistent with the values obtained by the Henderson-Hasselbalch equation. As the period of incubation was increased, the value of diffusible CO_2

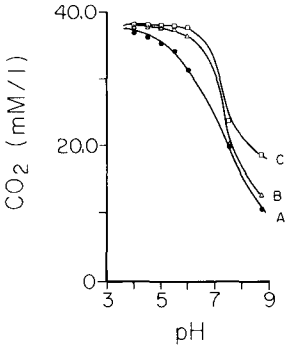


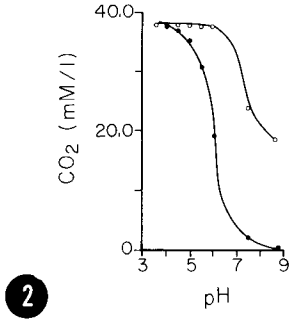
Fig.1. Effect of pH on actual diffusible carbon dioxide. From pH 3.5 to 6.0 sodium acetate/acetic acid buffer (0.2mM) was used.From pH 7.4 and above,tris buffer (0.1 mM) was used. Diffusible time was varied:
(A) —●— =12 hr; (B) —Δ— =30 hr and (C) —□— =96 hr

nearly approached the TCO_2 (see Fig. 1, curve C). It is evident, therefore, that the diffusible CO_2 equals TCO_2 only at an acidic pH below 6.0,whereas at pH 7.0 and above, the diffusible CO_2 although increased even at the end of 96 hr incubation from the previous values, could not approach close to the value of TCO_2. Theoretically, between pH 6.0 - 7.0, the diffusible CO_2 should eventually equal TCO_2 on the basis of the following reactions (6):

$$HCO_3^- + H_2O \rightleftharpoons H_2CO_3 + OH^- \quad \ldots\ldots\ldots\ldots\ldots (2)$$

$$OH^- + HCO_3^- \rightleftharpoons CO_3^= + H_2O \quad \ldots\ldots\ldots\ldots\ldots (3)$$

It appears that these reactions determine the rate of HCO_3^- hydrolysis, and the formation of H_2CO_3 from HCO_3^-. Both the reactions are pH dependent. In acidic pH the equilibrium is shifted toward the right-hand side of the equation (2) under the principal of mass action and thus the diffusible CO_2 in acidic pH equals TCO_2. On the other hand, when the pH is alkaline, the reaction is slowed down leaving HCO_3^- and $CO_3^=$ in solution and therefore at a high pH the diffusible CO_2 could not reach the value of TCO_2 as shown in Fig. 2.



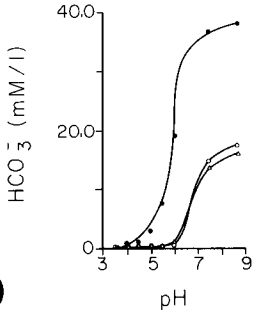


Fig.2. Effect of pH on diffusible carbon dioxide.
— ● — =Theoretical H_2CO_3 equivalent to CO_2,calculated by the Henderson-Hasselbalch Equation,when the total buffer was 38 mM (TCO_2) and pK_1=6.1
— O — =Actual diffusible CO_2, analyzed by Conway's diffusion technique. Reproduction from Fig.1C).

Fig.3. Effect of pH on HCO_3^-.(At an acidic pH, the formation of $CO_3^=$ is negligible,whereas in alkaline pH the hydrolysis of HCO_3^- is poor, hence $CO_3^=$ is regarded as negligible).
— ● — =Theoretical HCO_3^-,calculated by the equation 1,by subtracting $H_2CO_3$from the total buffer,which was 38 mM, equivalent to TCO_2 value.
— Δ — = by analysis from the remaining HCO_3^- in solution.
— O — = Actual HCO_3^- was obtained by subtracting diffusible CO_2 from TCO_2.

Edsall and Wyman (6) have shown that the formation of diffusible CO_2 from blood plasma or from dilute HCO_3^- solution is dependent on the partial pressure of CO_2 (PCO_2). As PCO_2 is diminished, CO_2 and H_2CO_3 are removed from the solution in vapour phase, which is in equilibrium with PCO_2. The H_2CO_3 will then be formed by the hydrolysis of HCO_3^-, as shown above in equation (2). The PCO_2 in a closed system will gradually be diminished because of CO_2 being trapped by the alkali and PCO_2 will remain in equilibrium with H_2CO_3 and CO_2 in vapour phase as long as H_2CO_3 is present in the system. At an acidic pH the formation of H_2CO_3 was favoured, and therefore diffusible CO_2 gradually equalled TCO_2, whereas at alkaline pH, the reaction was slowed down because of the shift of equilibrium, the diffusible CO_2 was nearly $\frac{1}{2}$ TCO_2.

From Fig. 3 it is evident that in the acidic pH, nearly all HCO_3^- was hydrolyzed, whereas at the alkaline pH, the hydrolysis was poor leaving some of the HCO_3^- in solution (without being degraded) which accounted for the formation of almost equivalent amount of CO_2 when acid was added.

From the present study, it is concluded that differences in diffusible CO_2s which vary with the function of pH and time are not due to the diffusion time factor, but are mainly due to the rate of HCO_3^- hydrolysis, which ultimately affects a number of physical and chemical processes viz., vapour pressure of the dissolved H_2CO_3, and PCO_2 and finally TCO_2.

If the stimulated saliva (pH approximately 7.4) is left open in the air, it will take a long time for the total CO_2 to come off and after some time, there will be hardly any change in pH because the evolution of CO_2 will be negligible.

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