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A THERMOANALYTICAL APPROACH TO THE STUDY OF THE TISSUTAL WATER OF MOUSE SALIVARY GLANDS

ROBERTA CURINI * and STEFANO MATERAZZI

Department of Chemical Sciences, University of Camerino, Via S.Agostino 1, 62032 Camerino MC (Italy)

GIOVANNA MENGHI

Department of Cellular Biology, University of Camerino, Via A.Moro, 62032 Camerino MC (Italy)

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ABSTRACT

The salivary glands participate to a different extent when secreting the different components of saliva. The cellular water plays a very important role in this process, but studies concerned with water analysis and characterization in these organ tissues have not been reported in the literature.

The different kinds of water, their percentages and their stabilities were determined by thermal analysis of mouse salivary glands: submandibular, sublingual and parotid. The water was released from the different types of gland in different temperature ranges; the most stable being the submandibular gland, followed by the parotid gland, and then the sublingual gland. The total water per cent was approximately the same (about 72.5%) in the submandibular and sublingual glands even if bound with different energies to the matrix, but a remarkable difference was found between the water per cent corresponding to the first process of the submandibular and sublingual glands. The parotid glands showed a lower per cent (66.6%) for total water.

Finally, the thermogravimetric curves were found using the same analysis, to obtain the ashes content of the glands.

INTRODUCTION

The salivary glands and the kidneys play the most important role in the excretory process and the reabsorption of water. In particular, salivary glands are formed by adenomeres and ductal segments, and the secretory and ductal cells vary widely among different glands for a single species and among different species for corresponding glands [1].

* Author to whom correspondence should be addressed.

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The localization and characterization of glycoconjugate macromolecules by methods in situ and extra situ have allowed further differentiation among the salivary glands and identification of the various chemical species present in these organs [2–15].

More recently, the immunochemistry has been tested by monoclonal antibodies against saliva, salivary gland antigens and biologically active peptides, indicating that this procedure will be useful for detecting these molecules and relating their functions to positive sites [16–19].

The salivary glands participate to a different extent to the secretion of the different components of saliva. The cellular water plays a very important role in this process, but studies concerned with water content and characterization in these organ tissues have not been reported in the literature.

Studies on cell water so far have led to the conclusion that different types of water exist in cells, variously described as bound, hydration, vicinal and bulk water. The traditional, and at the moment the majority, view claims that most, 95% or more, is identical with bulk water, which acts as the space-filling medium. However, as pointed out by Clegg et al. [20], these conclusions are mainly based on interpretation of results from dielectric-relaxation and NMR techniques, which monitor the average rotational motion of single molecules and do not probe long-term collective processes of solvent interaction that underlie long-range effects.

The water interactions in the biological systems are functions of the hydrogen bonds, the van der Waals forces, the London forces etc., and as Watterson [21] suggests the water collectively takes part in mutual interactions on a macromolecular scale, leading to the formation of clusters that do not just flicker on and off, appearing and disappearing at random, but travel as a wave through the medium [22]. These clusters are entities held together by cooperative intermolecular bonds, such as hydrogen bonds, which are very strong in water. The clusters can then act as independent systems and to participate in processes occurring in liquid media [23].

The water in a biological system is bound to the matrix by different energies and so, when the system is heated, the water could be lost in different successive steps when the activation energy necessary to obtain the release of each type of water is reached.

Analysis of the water in animal tissues is commonly carried out by heating the samples at 95 °C until reaching a constant weight (about 48 h) and calculating the water content by difference [24]. By using this method it is impossible to recognize if different types of water are present in the tissues and in what percentages they occur. This choice of the drying temperature is completely empirical and it is possible that the strongest bound water is not released at this temperature because its activation energy has not been reached.

This research has been undertaken with the aim to characterize and quantify, by thermoanalytical techniques, the different types of water in sublingual, submandibular and parotid glands.

EXPERIMENTAL

Instrumentation

Differential scanning calorimeters (Perkin–Elmer DSC-2B) and thermobalances TGS-2 equipped with a data station were used. Atmospheric air was used at a flow rate of 50–100 ml min^{-1} and heating rates ranged between 2.5 and 10 °C min^{-1}.

RESULTS

Submandibular, sublingual and parotid glands of sexually mature male mice (outbred, Swiss strain "Morini") were studied.

Since it was impossible to analyse each tissue immediately after dissection, each sample was quenched in liquid nitrogen immediately after collection and then stored at −80 °C in single polyethylene holders. To check if this treatment could induce any modification in the water arrangement or in the water content, some samples of each studied tissue were divided to give two homogeneous sub-samples immediately after collection. One sample was immediately analysed by thermogravimetry (TG) and by differential scanning calorimetry (DSC), while the second was stored as previously described and analysed after 2 weeks (the time corresponding to the maximum storage of the tissue samples). The results obtained were exactly the same.

The thermal behaviour of the right and left submandibular, sublingual and parotid glands of some animals were studied in parallel. The results were very similar with a negligible number of exceptions. Thus, since the glands were very small and could be placed entirely in the thermobalance sample holder, the TG study was carried out by analysing one whole gland and using the other one for other studies. This way the possible anatomical differences among samples obtained by dividing the same gland are completely avoided and the TG curves are truly representative of the thermal behaviour of the total water present in each gland. Twenty glands of each type were analysed to give a really representative series of results.

Figure 1 shows the TG and the derivative thermogravimetric (DTG) curves of the submandibular, sublingual and parotid glands. The submandibular gland (Fig. 1, curve a) releases water first through a small process between 25 and 97 °C, followed by a second asymmetric process between 97 and 185 °C, and finally a third small process between 185 and 204 °C characteristic of this gland. The dry sample then decomposes through two smooth steps corresponding to the decomposition of the proteins and the fats to give the ashes. Figure 2 illustrates the thermal water release of some glands of the series, which accounts for the constancy and the repeatability of the process.

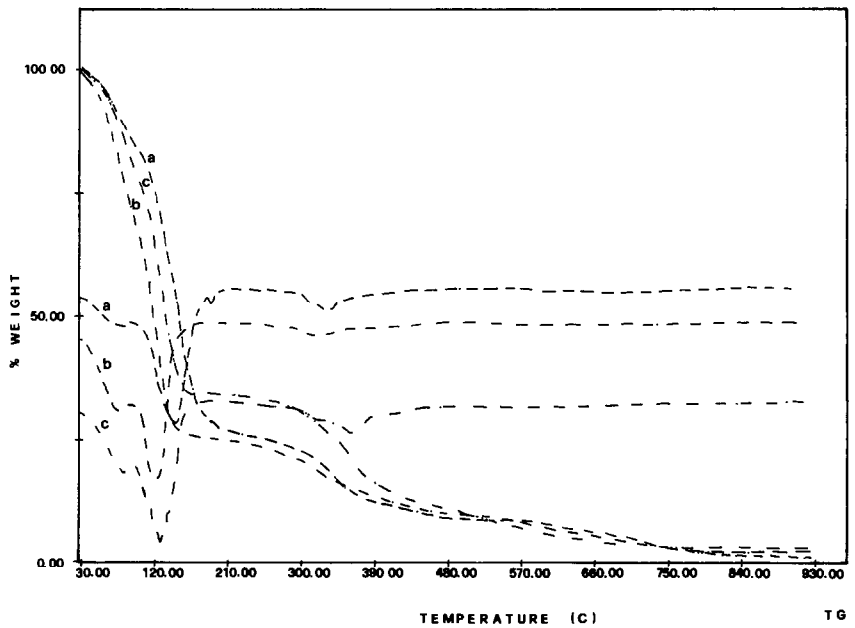

Fig. 1. TG and DTG curves of (a) submandibular, (b) sublingual and (c) parotid glands. Heating rate = 10 °C min^{-1}. Atmosphere: air at a flow rate of 100 ml min^{-1}.

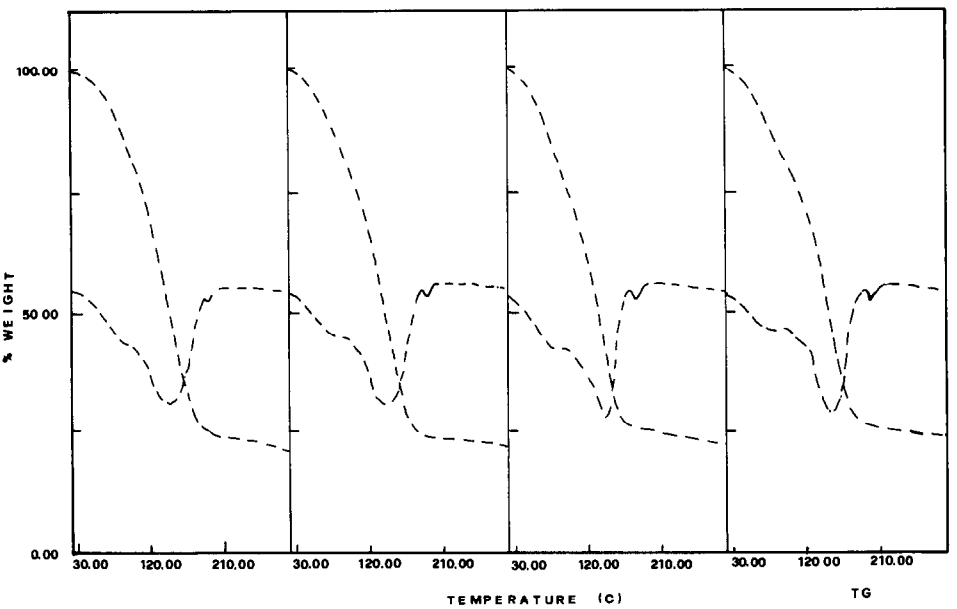

Fig. 2. Water release processes of some submandibular glands. Heating rate = 10 °C min^{-1}. Atmosphere: air at a flow rate of 100 ml min^{-1}.

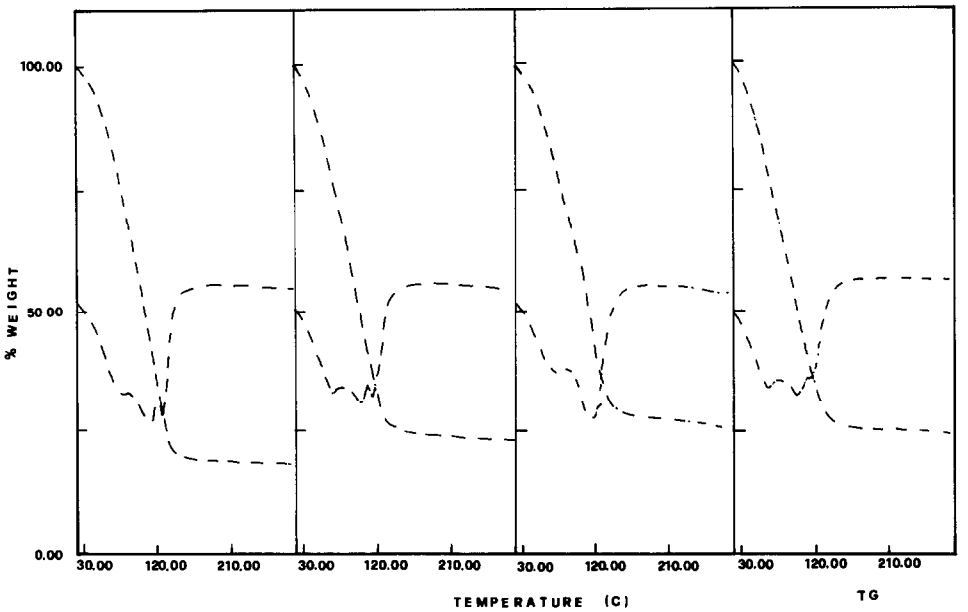

Fig. 3. Water release processes of some sublingual glands. Heating rate = 10 °C min^{-1}. Atmosphere: air at a flow rate of 100 ml min^{-1}.

The sublingual glands (Fig. 1, curve b) lose water in two overlapping processes, the first one between 25 and 91 °C and the second, more pronounced process is between 91 and 167 °C. The dry sample decomposition shows behaviour very similar to that of the dry submandibular gland, giving the ashes at about 900 °C.

The TG and DTG curves of some sublingual glands are collected in Fig. 3, showing that water is always lost by two overlapping processes, but that the ratio of the areas of the two processes is not constant. On the contrary, the temperature ranges of the two steps, corresponding to the water release, do not change.

The TG and DTG curves of a parotid gland are shown in Fig. 1, curve c. The water is released through a first step (not completely resolved) between 25 and 95 °C, followed by a series of processes completely overlapping in the range 95–182 °C. The dry sample decomposes in two steps giving the ashes at about 900 °C. In Fig. 4 the TG and DTG curves of some parotid glands are shown.

The mean values obtained for the total water present in each type of gland were: submandibular, 72.5 ± 3.0; sublingual, 72.6 ± 5.8; parotid, 66.6 ± 2.6.

The mean values obtained for the water released with respect to the first process in each type of gland were: submandibular, 18.5 ± 1.1; sublingual, 34.1 ± 4.5; parotid, 24.3 ± 1.8.

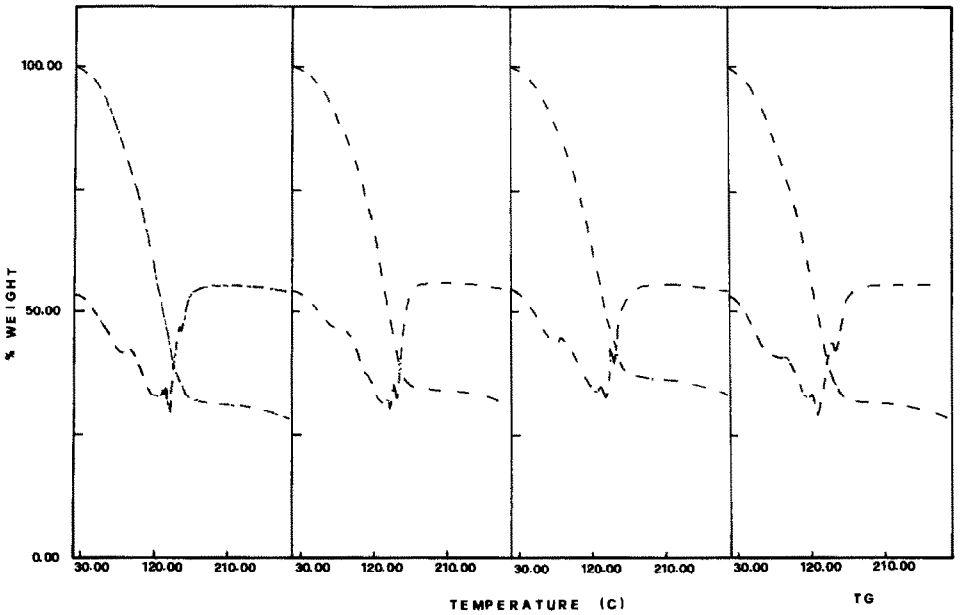

Fig. 4. Water release processes of some parotid glands. Heating rate = 10 °C min^{-1}. Atmosphere: air at a flow rate of 100 ml min^{-1}.

The mean values for the ashes content in each gland obtained by the TG curves were: submandibular, 1.95 ± 0.08; sublingual, 1.08 ± 0.20; parotid, 2.10 ± 0.32.

To recognize the different processes concerning the thermal water release of the three different types of gland, studies were carried out at lower heating rates: 5 °C min^{-1} and 2.5 °C min^{-1}.

Figure 5 shows the TG and DTG curves of the submandibular, sublingual and parotid glands. The submandibular gland (Fig. 5, curve a), shows a three-step water release. The three steps are more separate but still overlap, and the second one appears to be the result of at least two very close processes. The DSC curve (Fig. 6, curve a) shows a first wide asymmetrical peak extending over a temperature range corresponding to the first two DTG peaks, and a second little, sharp peak relating to the third DTG peak. The sublingual gland water (Fig. 5, curve b) is still released in two very overlapping steps, but the second process appears to be a superimposition of two different processes, as confirmed by the DSC curve (Fig. 6, curve b), which after a first wide peak shows a second, two-tipped peak in the same temperature range as the second TG process.

The TG curve of the parotid gland obtained at 2.5 °C min^{-1} (Fig. 5, curve c) confirms the complexity of the mechanism of water release from the parotid gland, which appears to be a convolution of a number of different processes. The DSC curve (Fig. 6, curve c) shows that after a first, partially isolated peak, the curve becomes a complex representation of the release of

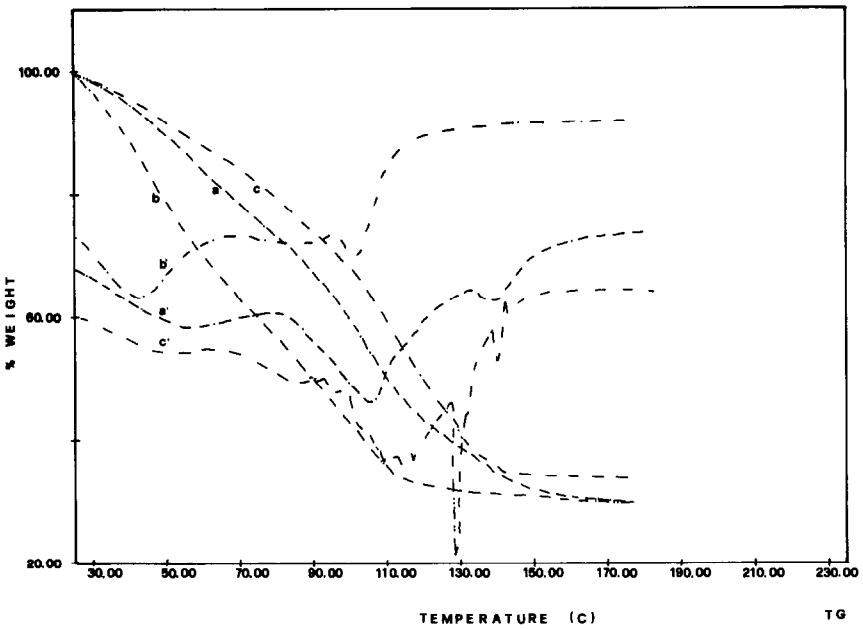

Fig. 5. TG and DTG curves of (a) submandibular, (b) sublingual and (c) parotid glands. Heating rate = 5 °C min^{-1}. Atmosphere: air at a flow rate of 100 ml min^{-1}.

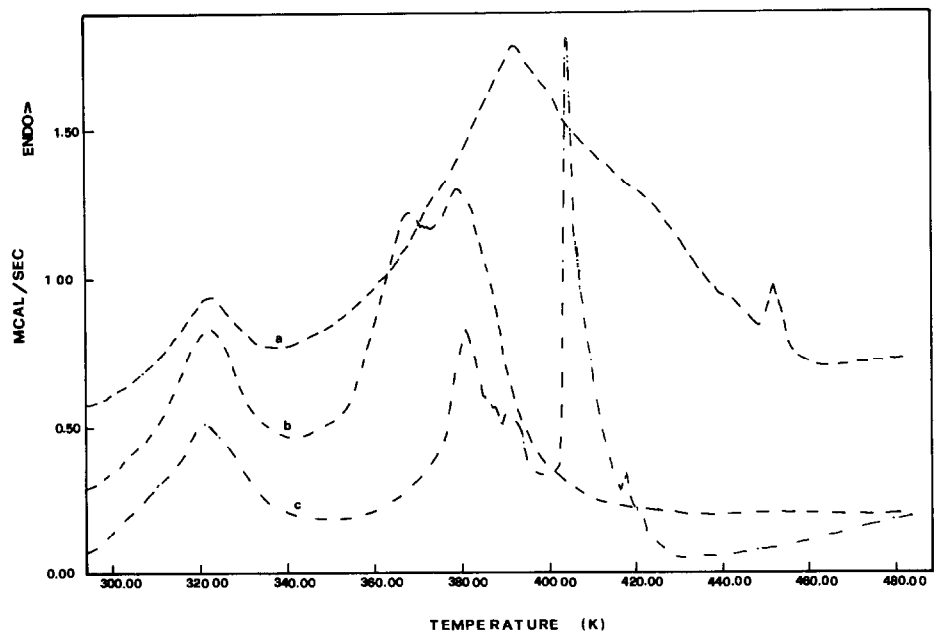

Fig. 6. DSC curve of (a) submandibular, (b) sublingual and (c) parotid glands. Heating rate = 10 °C min^{-1}. Atmosphere: air at a flow rate of 100 ml min^{-1}.

TABLE 1

Percentage of the total water lost in isothermal mode at 95 °C and the amount of residual water as a percentage of the total water at 95 °C

Sample	Water lost (%)	Residual water (%)
Submandibular	88 ± 3	12 ± 2
Sublingual	93 ± 5	7 ± 2
Parotid	81 ± 4	19 ± 2

numerous different types of water, with the predominance of a tall sharp peak with a maximum at 406 K corresponding to the DTG peak at 134 °C.

Studies were then carried out working in isothermal mode at 95 °C, the temperature commonly used to dry animal tissues, as referred to in the literature [24]. Five samples for each type of gland were analysed. The results are collected in Table 1.

DISCUSSION

The experimental data show that the thermal behaviour of water is quite different for the three studied glands.

In terms of the complexity of the process and of the different types of water present in each system, it can be seen that the complexity of the signals increases from the sublingual to the submandibular gland and becomes a complex convolution of different superimposed processes in the case of the parotid gland.

Different types of water are thus present in each one of the different studied glands, as shown by the thermoanalytical curves. The process of water release can always be divided into two different portions. A first portion, represented by the first DTG peak, corresponding to a single type of water, and a second portion represented by peaks corresponding to at least two different types of water, as in the case of the sublingual gland and the more complex case of the parotid gland. The first portion can be interpreted as the one corresponding to the extracellular tissue water, while the second portion could correspond to the intracellular water. The intracellular water is then differentiated into bulk water, which has a lower bond energy and so is lost at a lower temperature, and bound water with a higher bond energy.

Moreover, the bound water can show different bond energies as a result of the different possible interactions of the water molecules with the biological matrix. If bond energies of similar magnitude correspond to these interactions, the thermal signal becomes continuous and represents a unique

convolution of different unresolved signals. The more complex the biological system, the more this behaviour is possible because increasing the types of water bond decreases the energy differences between the different bonds, and the thermal signal becomes a continuous function without there being any resolution among the different processes. This could be the case for the sublingual and the submandibular glands that are very differentiated at cellular level. However, the complexity of the signals of the parotid gland could be explained in terms of the low cellular complexity of this tissue.

The water is released from the different types of gland in different temperature ranges, the most stable being the submandibular gland (temperature range 25–204° C), followed by the parotid gland (temperature range 25–182° C) and then the sublingual gland (temperature range 25–167° C).

The total water per cent is almost the same, about 72.5%, in the submandibular and sublingual glands, even if bound with different energies to the matrix, while the parotid gland has a lower value of 66.6%. But by considering the water corresponding to the first process it is possible to see a remarkable difference between the submandibular and sublingual glands. The water of the sublingual gland loses a considerably greater amount of water in the first process, while the parotid gland shows a loss similar to that of the submandibular gland.

When working in isothermal mode at 95° C it is found that none of the three glands loses all its water at this temperature (Table 1), and that the residual water is still a considerable percentage of the water present in the glands. So the analysis of the water carried out at 95° C until a constant weight is reached does not give correct analytical data because the water with the highest bond energy is not released at this temperature.

By looking at the thermoanalytical curves of the glands it can be seen that the decomposition of the dry systems is similar for the sublingual and the submandibular glands. It happens through two partially overlapping steps of about the same magnitude. The dry parotid gland still decomposes in two overlapping steps, but the first one is about twice the height of the second one showing that the decomposition of the parotid tissue is quite different from that of the other two glands.

Finally, by using the thermogravimetric curve it is also possible, by the same analysis, to obtain the ashes content of the glands. The highest ashes results were obtained for the parotid glands (2.10%), followed by the submandibular glands (1.95%), while the sublingual glands showed a very low ashes content (1.08%).

So thermoanalytical techniques, which are very accurate and sensitive, allow us to analyse the water in the mouse salivary glands, distinguishing between the different types of water present. This is also very reliable and allows us to obtain the results in times much shorter than the classical method still used.

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