Thermochimica Acta, 162 (1990) 445–448
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

Note

MICROCALORIMETRIC STUDY FOR SUCCESSIVE DILUTIONS OF AQUEOUS SOLUTIONS

MICHEL LABADIE

Laboratoire de Biochimie Médicale, Faculté de Médecine, 2 rue du Docteur Marcland, F-87025 Limoges Cedex (France)

JEAN-CLAUDE BOLLINGER

Laboratoire de Chimie Générale et Analytique, Faculté des Sciences, 123 avenue Albert-Thomas, F-87060 Limoges Cedex (France)

(Received 24 October 1989)

ABSTRACT

A batch microcalorimeter was used to measure the mixing enthalpies for pure water with aqueous solutions (10 g l^{-1}) of five biologically interesting substances: bovine hemoglobin, human albumin, polyethylene glycol, D(+)-saccharose and ascorbic acid. The values obtained are low (a few mJ) and become negligible after dilution to 1%, even with "dynamization" of the solution, Therefore, in contrast with some previous claims, the memory effects of water cannot be observed in these systems; this is discussed in terms of the structure of water and of hydrophobic interactions.

INTRODUCTION

In 1988, Benveniste and his colleagues reported [1] the somewhat puzzling biological behaviour of highly diluted aqueous solutions of anti-IgE antiserum against human polymorphonuclear basophils; this work has given a new impulse to the controversy concerning the real efficiency of undermolecular homoeopathic dilutions, and it has been discussed at considerable length in some subsequent articles [2].

It should be recalled that, at dilutions such as 10^{-24}, no solute molecule can be found in the final solution; moreover, Hauton [3] has pointed out the total disappearance of the genuine solvent molecules used in the initial solution at these dilutions. A multi-centre round-robin study recently published [4] does not confirm the behaviour of *Opium* or of *Raphanus* at dilutions of 10^{-30} and 10^{-10}, respectively, against an intestinal transit return in the case of digestive surgical interventions.

Therefore, we have investigated some aqueous solutions of substances of biological interest, in order to ascertain whether any mixing enthalpy can be

0040-6031/90/$03.50

measured after successive dilutions. Our results are discussed in terms of the known structure of aqueous solutions and of hydrophobic interactions.

MATERIALS AND METHODS

Solutions

The solvent was distilled water, redistilled twice in a silica apparatus. The origin of the chemicals used as solutes is given in Table 1. From initial solutions of 10 g l^{-1} (a concentration typical of homoeopathic preparations), the following successive dilutions: 1×10^{-2}, 1×10^{-4},..., 1×10^{-8}, were obtained with "dynamization", according to the homoeopathic pharmacopea by vigorous mixing for 15 s in a blender (see ref. 1).

Microcalorimetry

An LKB "batch" microcalorimeter No. 2107 [5] was used, thermostatted at 25 °C and equipped with two gold twin-cells; the electrical calibration was standardized by comparison with the dilution enthalpy for aqueous solutions of saccharose (exothermic [6]) or urea (endothermic [7]).

The larger compartment of each cell contained 4 ml of water; there was 2 ml of water in the smaller compartment of the reference cell and, in the measuring cell, 2 ml of the solution under study. After sufficient time had elapsed for thermal equilibrium to be reached, the system was mixed by rotation of the calorimeter and the corresponding thermogram was recorded. Then an electrical calibration was performed. In the present case, each solution studied had a dilution enthalpy in a 1 : 3 ratio.

TABLE 1

Mixing enthalpies at 25 °C for aqueous solutions (2 ml at 10 g l^{-1}) with tridistilled water (4 ml)

Chemical substances (origin)	Molar weight (Dalton)	Mixing enthalpy (mJ)
Globin from bovine hemoglobin (Calbiochem, no. 34.598)	68000	+2.5
Human albumin (Calbiochem, no. 126.656)	66000	+2.1
Polyethylene glycol 6000 (Merck, no. 807.491)	5000–7000	−4.5
D(+)-saccharose (Fluka puriss., no. 84100)	342	−1.8
Ascorbic acid (Fluka puriss., no. 95210)	176	+19

RESULTS AND DISCUSSION

In the study of the initial solutions (at 10 g l^{-1} concentration) of the five compounds (with molar masses between 176 and 68 000 Dalton) there were three endothermic and two exothermic values for the enthalpy of mixing with water (see table). Following the first dilution (1×10^{-2}), no thermal effect can be measured at the precision of the background noise, i.e. approximately 0.25 mJ, with or without "dynamization".

It is now well-known that liquid water consists [8] of a three-dimensional network of H_2O molecules connected by hydrogen bonds in a continuous dynamic exchange. This orderly structure and the hydrogen bonds explain some of the "anomalous" properties of water; they also allow compounds to solubilise, to ionize and to become solvated ; these properties can also be analysed in terms of hydrophilic/hydrophobic interactions.

Hydrophobic interactions have been treated in detail [9] by a combination of classical thermodynamics and statistical mechanics. They essentially originate from the solvent itself and can be classified as solute–solvent interactions and solute–solute pair-wise interactions. In the most widely used models for theoretical calculations describing the structure of hydrophobic solutes in aqueous solutions [10], the water molecule is usually represented as a hard sphere. These molecular interactions, explained as distribution functions for the short-range/long-range interactions, constitute the difference between hydrophobic and non-hydrophobic solutes.

This notion of hydrophobic solutes is of particular importance in the case of macromolecular proteins and other biopolymers, especially as far as their conformational changes in solution are concerned; this is also indicated as the lipophilicity concept. However, Ben-Naïm [11] has recently pointed out that intramolecular hydrophilic interactions, i.e. solvent-induced interactions between two functional groups forming a hydrogen bond, are probably more important during biochemical processes.

Pure liquid water consists of a macroscopically connected random network of hydrogen bonds, with frequently occurring strained and broken bonds, that is continually undergoing topological reformation. Totally hydrogen-bonded water molecules are in equilibrium with free OH-groups and free lone-pairs on oxygen atoms

$$(H_2O)_{bulk} \rightleftharpoons (OH)_{free} + (\text{lone pair on O})_{free}$$

The behaviour of a solute, when added to water, depends on its affinity for hydrogen bonds: an acceptor will shift the equilibrium towards disruption of the water hydrogen-bonds (structure-breaking effect) whereas a hydrophobic solute will induce their reinforcement (structure-making effect).

Therefore, the addition of hydrophobic molecules (and especially of macromolecules) will induce important changes in the structure of liquid water. However, it can be observed from the table that the enthalpic effect

(as measured for a $\frac{1}{3}$ dilution) is weak (from -4.5×10^{-3} to $+2.5 \times 10^{-3}$ J) for solutes with no ionic properties (neither ionophores nor ionogenes). The value is greater for ascorbic acid ($pK_2 = 4.17$; $pK_1 = 11.57$) whose dissociation coefficient is increased by dilution (a simple calculation gives a variation from 3.5% to 6.0%).

With the diluted solutions obtained from the initial solutions, the mixing enthalpy as measured in our calorimetric system has the order of magnitude of the background noise, i.e. the mixing enthalpy of water with itself (about 0.25×10^{-3} J).

Thus it can be concluded that even after a 1×10^{-2} dilution, the changes in the water structure are negligible compared to the thermal agitation; therefore there is no "memory" effect in the water structure, even after dissolution of macromolecules (globin, albumin, PEG) or ionogenic compounds (ascorbic acid), in spite of a previous "dynamization" of the solution by vigorous stirring.

REFERENCES

1 E. Davenas, F. Beauvais, J. Amara, M. Oberbaum, B. Robinzon, A. Miadonna, A. Tedeschi, B. Pomeranz, P. Fortner, P. Belon, J. Sainte-Laudy, B. Poitevin and J. Benveniste, Nature, 333 (1988) 816.

2 See, among other papers, the Correspondence sections in volume 334 of Nature, pp. 285–286, 375–376 and 559 (Summer 1988) and also: J. Maddox, J. Randi and W.W. Stewart, Nature 334 (1988) 287; J. Benveniste, Nature, 334 (1988) 291 and Nature, 335 (1988) 759; and J. Maddox, Nature 335 (1988) 760.

3 C. Hauton, Biochimie, 70 (1988) 1849.

4 G.R.E.C.H.O., U. 292 I.N.S.E.R.M., A.R.C. and G.R.E.P.A., Presse Med., 18 (1989) 59.

5 I. Wadsö, Acta Chem. Scand., 22 (1968) 927.

6 F.T. Gucker, H.B. Pickard and R.W. Planck, J. Am. Chem. Soc., 61 (1939) 459.

7 F.T. Gucker and H.B. Pickard, J. Am. Chem. Soc., 62 (1940) 1464.

8 F.H. Stillinger, Science, 209 (1980) 451.

9 A. Ben-Naim, Hydrophobic Interactions, Plenum, New York, 1980.

10 D.Y.C. Chan, D.J. Mitchell, B.W. Ninham and B.A. Pailthorpe, in F. Franks (ed.), Water: A Comprehensive Treatise, Vol. 6, Plenum, New York, 1979, Chap. 5, pp. 239–278.

11 A. Ben-Naim, J. Chem. Phys., 90 (1989) 7412.