Biophysical Chemistry, 34 (1989) 79–82
Elsevier

BIOCHE 01377

The maximum and minimum water content and cell volume of human erythrocytes in vitro

K. Kageyama [a], Y. Onoyama [b], H. Kogawa [c], E. Goto [d] and K. Tanabe [e]

[a] *Radioisotope Centre and* [b] *Department of Radiology, Osaka City University Medical School,* [c] *Department of School Nurse and* [d] *Department of Health Science, Osaka Kyoiku University and* [e] *Laboratory of Biology, Osaka Institute of Technology, Osaka, Japan*

Received 25 July 1988
Revised manuscript received 18 May 1989
Accepted 19 May 1989

Erythrocyte; Water content; Cell volume; Osmolarity; Gas-liquid chromatography; (Human)

The maximum and minimum water contents of human erythrocytes were measured after exposure to various osmotic pressures. Within a range of osmolarities, at which no haemolysis occurred, the water content reached its maximum, 78.1%, at 180 mosM and its minimum, 54.8%, at 800 mosM. Simultaneously, the mean cell volume increased to 98.5 μm^3 at 180 mosM and decreased to 77.2 μm^3 at 800 mosM.

1. Introduction

It is uncertain as to how much water human erythrocytes can contain and how large the cell volume is under some non-physiological conditions. The values of the maximum and minimum water content and volume of red cells are considered to represent indices of physiological and physicochemical properties of human erythrocytes. Evidence has been presented that the erythrocyte volume can change significantly on altering the osmotic pressure and pH of the suspending medium [1,2]. Thus, in this study, we used buffered solutions with extremely low or high osmotic pressure and pH, conditions under which no haemolysis occurred, to evaluate the maximum and minimum water content and the cell volume of the human erythrocyte. The erythrocyte water content was measured by gas-liquid chromatography and radioisotope analysis [3]. In addition, it was determined whether the changes in water content were reversible to the original level on returning the erythrocytes to the physiological solution.

Correspondence address: K. Kageyama, Radioisotope Centre, Osaka City University Medical School, Osaka, Japan.

2. Materials and methods

To examine the effect of osmotic pressure on the intracellular water content of human red cells, solutions with varying osmotic pressures were prepared using a number of concentrations of NaCl at 25 mM sodium phosphate (pH 7.4). To determine the effect of pH on water content, solutions with varying pH values were made by adjusting the pH using sodium phosphate at constant osmolarity (300 mosM). A control solution (300 mosM at pH 7.4) was also prepared. The osmotic pressure of the phosphate-buffered solutions was determined on an osmometer (Auto STAT omm-6010, Kyoto Daiichi Kagaku, Kyoto). A pH meter (Hitachi-Horiba model F5SS, Kyoto) was used to monitor the pH of the solutions.

Venous blood was collected by venipuncture from healthy male volunteers using a heparinized syringe. The blood was centrifuged at 911 × *g* for

20 min and the plasma was discarded. Approx. 0.5 ml of packed red cells was suspended in 8 ml of each solution prepared as given above, centrifuged at $512 \times g$ for 5 min and the supernatant then discarded. The red cells were washed twice with the same solution. For evaluation of the reversibility of intracellular water content, cells were washed twice with the control solution again. Finally, cells were diluted to make a 50% suspension in the solution.

The water content of red cells was assessed according to a method described previously [3] with a minor modification; namely, isobutanol was used in place of *n*-butanol as an internal standard (IS). Since our preliminary experiments showed that [^{3}H]sucrose is stable at high and low pH values, 2 μCi [^{3}H]sucrose and 100 μg sucrose were added to each sample to determine the intercellular space. The suspension was transferred to Wintrobe tubes and centrifuged at $2050 \times g$ for 30 min. Then, 50 μl of the supernatant was taken with a microliter syringe and injected into a sample bottle containing 1.95 ml methanol and 4% isobutanol (IS) as well as two glass beads to enhance stirring efficiency. The sample bottle was subjected to thorough shaking and allowed to stand. The remaining plasma was then completely removed from the Wintrobe tube. Packed red cells were removed by suction using a capillary pipette and transferred to a barrel of a Pressure-Lok liquid syringe (Precision Sampling, Baton Rouge). 50 μl of the packed red cells were injected into a sample bottle, which contained the solvent mixture mentioned above. The sample bottle was shaken well and centrifuged at $446 \times g$ for 5 min to sediment insoluble materials that were derived from red cell components such as hemoglobin after denaturation in the solvent solution. Three specimens were prepared for testing reproducibility. Standard samples of known water content were prepared for determination of the calibration curve. 2 μl of the supernatant taken from each of the sample bottles were analyzed on a gas chromatograph (Shimadzu model GC-4BPT, Japan) connected to a thermal conductivity detector. The glass column (2 m × 3 mm inner diameter) was filled with 15% poly(ethylene glycol) (PEG 6000) on Shimalite F (fluoresin solid support, 40–60 mesh). The column temperature was maintained at 120° C and the helium flow rate at 60 ml/min. The ratio of the response of water to that of isobutanol (IS) was recorded by printing via an integrator (Shimadzu model I-1A) connected to a gas chromatograph. Intercellular space was determined as follows: 50 μl of the remaining solvent mixture in the sample bottle containing supernatant or packed red cells were placed on glass microfibre paper (Whatman, U.K.) in a vial. Following evaporation of the solvent, the scintillation mixture was added and the radioactivity of the sample was measured in a liquid scintillation counter (Packard Instruments, Downers Grove). The water content in separated red cells was calculated from the following equation:

$$\frac{W - S_2/S_1 \times 50}{50 - S_2/S_1 \times 50} \times 100\ (\%)$$

where W denotes the water content (μl) in the packed red cells, 50 the amount of a blood sample added into a sample bottle, S_1 and S_2 the respective [^{3}H]sucrose radioactivity (cpm) in the supernatant and packed red cells containing trapped fluid, and $S_2/S_1 \times 50$ and $50 - S_2/S_1 \times 50$ the volume of trapped water and red cells, respectively. The water content of red cells was evaluated using the average value from triplicate determinations of the samples obtained from the same subject.

The mean cell volume at low or high osmotic pressures was unmeasurable on a Coulter counter, since the apparatus employs isotonic buffered solution. Hence, the mean cell volume was calculated from the equation,

$$V = V_0\left(\frac{W}{100} - \frac{W_0}{100} + 1\right)$$

where V represents the mean cell volume (μm^3) of test cells being exposed to low or high osmotic pressures and V_0 the value for control cells at 300 mosM that was measured with a Coulter counter (model S-Plus, Coulter Electronics, U.S.A.), and W and W_0 the water contents (%) of test and control cells, respectively.

3. Results and discussion

The changes in water content of red cells suspended at various osmotic pressures are listed in table 1. At osmotic pressures between 180 and 800 mosM, no haemolysis occurred. The water content at 300 mosM (control solution) was 70.05%, nearly equal to that of fresh, unsuspended cells (70.22%). When red cells were suspended at low osmotic pressure (180 mosM), the water content reached the maximum value of 78.1%, equivalent to 111.5% of the control. The water content decreased to 54.78%, equivalent to 78.2% of the control, at high osmotic pressure (800 mosM). These changes in water content were completely reversible, recovering to the normal level on cells being returned to control solution.

The changes in water content at various pH values are shown in table 2. The water content of red cells exposed to the solution at pH 4.5–5.0 was 74.5%, up to 105.7% of the control value at pH 7.4. Elevation to pH 9.0–10.0 resulted in little change in water content which amounted to 98% of the control level. These slight changes in water content did not return to the original level on resuspending the cells in control solution (pH 7.4).

The mean cell volume for control cells was 91.3 μm^3, while for those suspended in solutions of 180 and 800 mosM were 98.5 μm^3 (107.9% of control at 300 mosM) and 77.2 μm^3 (92.1% of control), respectively.

Table 1

Changes in water content of human red cells exposed to hypotonic or hypertonic solutions

(A) Red cells exposed to test solutions; (B) red cells washed with control solutions (300 mosM) after exposure to test solutions. The value for fresh red cells was 70.22 ± 0.68%.

Osmolarity of solution (mosM)	Mean water content (%, v/v) (± S.E.)	
	A	B
180	78.11 ± 0.29	70.54 ± 0.18
200	77.06 ± 0.19	70.35 ± 0.14
300	70.05 ± 0.43	–
700	56.61 ± 0.27	70.13 ± 0.30
800	54.78 ± 0.31	70.56 ± 0.49

Table 2

Changes in water content of human red cells exposed to solutions of low or high pH

(A) Red cells exposed to test solutions; (B) red cells washed with control solutions (pH 7.4) after exposure to test solutions. The value for fresh red cells was 70.74 ± 0.59%.

pH of solution	Mean water content (%, v/v) (± S.E.)	
	A	B
4.5	74.50 ± 0.58	73.33 ± 0.52
5.0	74.47 ± 0.56	72.98 ± 0.46
7.4	70.78 ± 0.33	–
9.0	69.35 ± 0.32	70.54 ± 0.20
10.0	69.01 ± 0.54	69.82 ± 0.12

The present experiments have shown that the water content of human erythrocytes can be substantially changed by alterations in the osmotic pressure and pH of the suspending medium. The procedure of determining water content with [^{3}H]sucrose and gas-liquid chromatography has been shown to be precise and gives accurate values as compared with the hematocrit method, i.e., measuring the difference between wet weight and dry weight of packed erythrocytes [1]. Thus, we believe that the maximum water content is 78.1% and the minimum content 54.8%. Although these values were obtained under non-physiological conditions, they were fully reversible on returning the erythrocytes to physiological conditions (300 mosM).

Thus, variations in water content under normal physiological conditions should be well within the maximum and minimum values obtained in this study. Consistent with this proposed behaviour, we observed previously that the water content of human red cells changed from 69.97 to 73.96% on incubation of whole human blood in vitro at 37 °C for a certain period until haemolysis occurred [4].

In contrast to the variations in water content induced by osmotic pressure, those occurring at both acidic and alkaline pH values were less marked and irreversible. Non-physiologically low or high pH value of the suspension medium presumably damages a mechanism in the erythrocyte membrane by which water is transported across the membrane.

References

1 D. Savitz, V.W. Sidel and A.K. Solomon, J. Gen. Physiol. 48 (1964) 79.
2 J.R. Murphy, J. Lab. Clin. Med. 74 (1969) 319.
3 K. Kageyama, T. Yamashita, T. Kobayashi and E. Kimura, Experientia 37 (1981) 748.
4 K. Kageyama, Nouv. Rev. Fr. Hematol. 21 (1979) 161.