THE INTERACTION OF ALBUMIN AND CONCANAVALIN A WITH NORMAL AND SICKLE HUMAN ERYTHROCYTES

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Summary

The interaction of human albumin and concanavalin A with normal and sickle human red blood cells previously washed in phosphate buffer at pH = 7.4 was studied by titration calorimetry. The amount of albumin bound to normal cells was (6.8 ± 2.2) x 10^5 molecules/cell. An equilibrium constant of 5 x 10^{10} and an enthalpy change of -(280 ± 90) kcal/mol albumin was determined for albumin interaction with normal cells. The amount of albumin bound to sickle cells was (12.4 ± 1.0) x 10^5 molecules/cell and the enthalpy change for albumin interaction with sickle cells was -(390 ± 140) kcal/mol. Normal cells bound (5.7 ± 2.4) x 10^5 concanavalin A molecules/cell with an enthalpy change of -(840 ± 200) kcal/mol concanavalin. All experiments were conducted at 25°C.

Introduction

Furchgott and Ponder (1) reported that albumin was the anti-sphering agent for maintenance of human red cell shape. These investigators also reported that ∿6 x 10^6 molecules of albumin were adsorbed per red cell (2). This is a quantity sufficient to form a layer ∿50A thick on the red cell surface. More recent *in vitro* studies of red cells by Williams (3), Gul and Smith (4) and Jay (5) indicate that the presence of albumin stabilizes red cells to shear stress (3) probably by stabilizing the red cell membrane (4) and that albumin influences the geometry of the red cell (5). Since previous evidence for the binding of albumin to the red cell mainly involves indirect evidence, we have determined directly the binding of albumin to human red blood cells using a thermometric method. In this communication we report

the amount of albumin adsorbed onto the red cell surface and the ΔH values and equilibrium constants associated with the adsorption process.

Methods

Human blood from twelve hour fasted normal or sickle cell anemic adults was anticoagulated with heparin (0.1 mg/ml), plasma and buffy coat were removed immediately and the red cells washed four times at 4°C with phosphate buffer (pH = 7.4 and 288 mosm). Residual white cells and platelets were removed as previously described (6). The washed cells were then resuspended to a final hematocrit of about 30%. The erythrocyte count was 9×10^9 cells/ml. Blood from patients who were homozygous for sickle cell anemia was supplied to us by Dr. W. C. Mentzer (Department of Pediatrics and Medicine, University of California, S. F.). After preparation the reticulocyte count in the normal cells was <0.1% and the residual white count was $<10^5$ cells/ml. However, in the case of the sickle cell samples, after washing in the same manner as the normal cells, the reticulocyte count obtained was between 5 and 7% with a residual white count $>10^5$ cells/ml. After four washes with phosphate buffer no protein could be detected by the Lowry method (7) in the wash media for any of the blood samples. The albumin used in these studies was Human Albumin Fraction V (Sigma Chemical Co. purity >99% with 0.005% fatty acid). The salt free concanavalin A (Sigma Chemical Co., grade IV) was highly purified from jack beans and substantially free of carbohydrates.

Protein titrant solutions were prepared by dissolving (4 mg/ml) the albumin or the concanavalin in the phosphate buffer. The binding of each protein was studied by continuous calorimetric titration (8) of 0.3 ml of the protein into 2.7 ml of the erythrocyte preparation. A typical titration involved addition of the titrant over a 20 minute time period. Three or four replicate runs were made on each of the six normal and three sickle cell samples studied. Corrections for dilution of the titrant were found to be negligibly small by blank titrations into the phosphate buffer solution. Calculation techniques used in the analysis of the data have been previously described (9-11).

The equilibrium constant for the adsorption of albumin by normal red blood cells was calculated using a procedure described in the literature (10,11).

Results and Discussion

A typical titration curve is shown in Figure 1. The results are given in Table 1. The larger value for the adsorption capacity of albumin with sickle cells compared to normal erythrocytes could be due to the larger concentration of reticulocytes which have a larger surface area (12). The surface area of a typical human red blood cell is 147 μ^2 (4). The red cell will interact with 7×10^5 albumin molecules. The calculated area occupied per adsorbed albumin molecules is 22×10^3 $A°^2$/molecule and the effective radius of the albumin molecules is 83A°. In solution the albumin

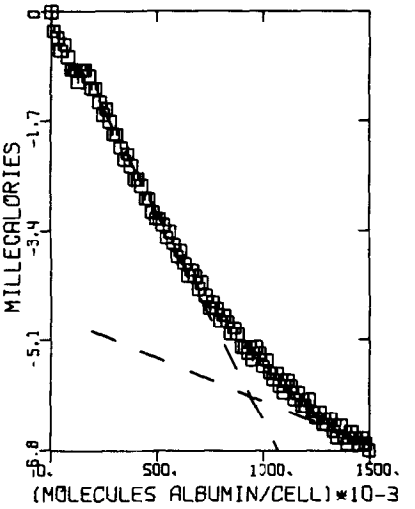

Figure 1. Computer Plot of the Titration of Normal Erythrocytes with Albumin.

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molecule has a radius of only ∿40A° (13). It is reasonable, however, to postulate that the albumin molecule is unfolded when bound to the surface of the cell, and the binding results in complete monolayer coverage of the cell.

The enthalpy change associated with the binding of albumin or concanavalin A to erythrocytes results from at least three processes, (a) conformational changes in the protein and/or membrane, (b) solvation changes of the protein and membrane, (c) formation of protein-membrane hydrogen bonds. Previous data (14) suggest enthalpy changes associated with protein structural changes will be endothermic and small compared to the heat effects observed in this study. It is difficult to predict the enthalpy effects due to hydration. Since albumin is easily removed from the cells by washing it is reasonable to assume that it is bound to the cell via the formation of hydrogen bonds. The heat of formation of a hydrogen bond is -3.4 kcal/mol (15). The majority of the measured heat may be due to this process. The heat of adsorption of albumin divided by -3.4/kcal/mol gives ∿80 hydrogen

TABLE 1

Adsorption Capacities, ΔH and K Values for the Interaction at 25°C of Albumin or Concanavalin A with Phosphate Washed Erythrocytes.[a)]

Erythrocytes		Adsorbant	Molecules of Adsorbant/ Cell x 10^{-5}	ΔH (kcal/mol Adsorbant)	K x 10^{-10} (l/mole Adsorbant)
Normal	1	Albumin	3.9 ± 0.1	-430 ± 150	9.8
	2		5.2 ± 0.2	-280 ± 20	2.5
	3		8.3 ± 0.3	-210 ± 60	2.0
	4		9.3 ± 0.3	-210 ± 69	6.3
	5		7.5 ± 0.1	-260	---
		Average	6.8 ± 2.2	-280 ± 90	5.1 ± 3.6
Sickle	1	Albumin	13.5 ± 2.5	-510 ± 110	
	2		12.2 ± 2.5	-420 ± 130	
	3		11.6 ± 0.4	-240 ± 10	
		Average	12.4 ± 1.0	-390 ± 140	
Normal	6	Concanavalin A	5.7 ± 2.4	-840 ± 200	

a) Uncertainties are given as the standard deviation of the mean.

bonds formed in the interaction of albumin with the red cell surface. However, there are many times more groups than this available in albumin for hydrogen bonding, i.e., threonine, serine, tyrosine and imidazole, as well as the amide groups (16). It would not be expected that glutamic and aspartic side chains would be involved in bonding to the red cell surface since the cell surface is negatively charged.

The larger ΔH value for the binding of concanavalin A (as compared to albumin) to erythrocytes may be due to the agglutination which is known to accompany the binding of concanavalin A (17).

The amount of concanavalin A adsorbed at the red cell surface determined in this study is 5.7 x 10^5 molecules/cell. Phillips, Furmanski and Lubin (18) reported 2.2 x 10^6 and Nicolson (19) found ~8 x 10^5 concanavalin A

binding sites/cell. Shore and Shore (17) have reported 1.3 x 10^4 concanavalin A binding sites/cell of sheep erythrocytes. Their work, however, was carried out at protein concentrations which were probably too low to saturate the binding sites. If the equilibrium constant for binding of concanavalin A to erythrocytes is assumed to be the same as that for albumin binding (Table 1), their data can be corrected to give 2 x 10^5 concanavalin A binding sites/cell. Fischer and co-workers (20) have recently reported the binding capacity of normal erythrocyte ghosts for hemoglobin to be 60 μg/mg of ghost protein. Using the values of Dodge et. al., (21) for the protein content of erythrocyte ghosts, we calculate an adsorption capacity of 4 x 10^5 hemoglobin molecules/erythrocyte membrane.

It appears that proteins can be expected to adsorb strongly to erythrocytes to form a monolayer on the membrane surface. Erythrocytes *in vivo* can be assumed to have a monolayer of adsorbed protein.

Acknowledgements

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