Effect of Detergents and Homogenization on Adenosine Kinase and Adenosine Deaminase of Human Erythrocyte Ghosts[1] (36736)

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Adenosine kinase has been demonstrated in erythrocytes (1, 2), and in studies on the uptake and metabolism of adenosine in human erythrocyte ghosts, it was observed that adenosine deaminase and adenosine kinase were associated with the erythrocyte membrane (3). Therefore, it was of interest to know whether the binding to the membrane reduces the accessibility of the substrate to the enzymes. The present investigation was designed to determine to what extent various disruptive treatments such as detergents and homogenization affect these enzyme activities.

Methods. Red cell ghosts were prepared as described by Schrader, Rubio and Berne (3). Except where otherwise indicated, fresh human blood samples were used and all experiments were conducted on freshly prepared ghosts. These ghost preparations usually contained about 5% hemoglobin.

Adenosine deaminase and adenosine kinase activities were determined in ghost cell suspensions prepared from 12 ml of 50% suspensions of intact red blood cells. The pellet of each ghost preparation was resuspended in 10 ml of isotonic phosphate buffer (310 mOsm, pH 7.4) and 4 m*M* ATP + 4 m*M* $MgSO_4$. Aliquots were withdrawn in separate tubes for detergent treatment (Triton X-100 or deoxycholate) and were incubated in a final concentration of 0.2% detergent for 5 min at 0°. Three milliliter aliquots of these detergent-treated preparations were incubated with 2 m*M* adenosine-U-^{14}C at 25° for 15 min with shaking. The adenosine added to each tube contained 0.125 μCi or 0.022 nmoles of adenosine-U-^{14}C with the balance made up of unlabeled adenosine. Untreated ghost cell suspensions were used as controls. The reaction was stopped by the addition of 5.0 ml of 0.5 *N* perchloric acid. In some experiments ghost cell membranes were disrupted by homogenization in a Polytron homogenizer at a speed of 3000 rpm for 1 min prior to incubation with labeled adenosine.

Adenosine deaminase and adenosine kinase activities were also determined in hemolysates, with and without detergent treatment. The hemolysates represented the supernatant fractions obtained after centrifugation of the lysed erythrocytes at 125,000*g* for 90 min. (Beckman ultracentrifuge Model L3-50). This produced a membrane-free hemolysate. The detergents were added to the hemolysate at a final concentration of 0.2%. Three milliliters of this preparation were assayed for adenosine deaminase and adenosine kinase activities as described above.

In experiments in which the incorporation of adenosine-U-^{14}C into the nucleotide fraction was studied, 0.3 ml of the Triton X-100-treated ghost cells were incubated with different concentrations of adenosine-U-^{14}C at 37° for 5 min. The hemolysates were treated similarly. The reactions were terminated by the addition of 4.0 ml of 0.5 *N* perchloric acid.

The rate of increase of radioactivity in the total nucleotide pool was taken as a measure of adenosine kinase activity and the rate of increase of combined radioactivity in the inosine and hypoxanthine fractions as a measure of adenosine deaminase activity. As shown previously (3), the rate of increase of activity was constant in both cases. The perchloric

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TABLE I. The Effect of Detergents and Homogenization on Adenosine Kinase and Adenosine Deaminase Activities of Human Red Cell Ghosts.[a]

Treatment	Adenosine kinase[b]		Adenosine deaminase[c]	
	Stored	Fresh	Stored	Fresh
Control	10.40	44.75	543.83	632.72
Triton X-100	37.13	95.90	604.25	584.63
Deoxycholate		92.50		562.59
Homogenization		139.69		678.03

[a] Stored = samples more than 4 wk old; Fresh = samples less than 2 days old.

[b] Expressed as nmoles of adenosine incorporated into the nucleotide fraction/ml cells/15 min at 25°.

[c] Expressed as nmoles of adenosine converted to inosine + hypoxanthine/ml cells/15 min at 25°.

acid extracts were centrifuged, supernatants decanted, adjusted to pH 7.0 and then processed for resolution of bases, nucleosides and nucleotides as previously described (4). Finally, these fractions were counted for ^{14}C activity.

Results. Membrane disruption produced either by detergent treatment or homogenization resulted in a significant increase in the adenosine kinase activity of the ghost cells but was without effect on ghost cell adenosine deaminase activity (Table I). The hemolysate enzyme activities were found to be unaffected by the detergent treatments (Table II). Activation of membrane-bound adenosine kinase by Triton X-100, deoxycholate and homogenization resulted in approximately 2-fold increases in enzyme activity in each instance. Blood storage for 4 wk or more caused a reduction in the adenosine kinase activity but did not alter the activity of adenosine deaminase or the activation of the kinase by detergents (Table I).

The results of a typical experiment showing the incorporation of adenosine into the nucleotide fraction of a Triton X-100-treated human ghost cell suspension in fresh and stored blood samples at varying concentrations of adenosine are given in Fig. 1. It is apparent that there is a significant decrease in the adenosine kinase activity of the stored blood samples. Saturation of the adenosine kinase reaction was not observed even at an adenosine concentration of 750 μM.

Discussion. In the present study, the increase in adenosine kinase activity by detergent treatment or homogenization of red cell ghosts indicates that this enzyme is less accessible to the substrate and/or other cofactors responsible for its optimum activity. However, the lack of activation of adenosine deaminase by these procedures suggests that its active sites are already exposed. A direct effect of the detergents or of mechanical disruption on the enzyme molecule itself is possible. However, such an action is not compatible with the lack of activation of the hemolysate enzyme by these treatments. The existence of two separate adenosine kinases has not been ruled out by these studies.

These results are in agreement with those of Zamudio, Cellino and Canessa-Fischer (5) and with the observations of Duchon and Collier (6) who showed an increase in the activity of various enzymes in red cell ghosts fragmented by dilution with water. These en-

TABLE II. Adenosine Kinase and Adenosine Deaminase Activities in Human Red Cell Hemolysates.

Treatment	Adenosine kinase[a]	Adenosine deaminase[b]
Control	587.0	2436.1
Triton X-100	484.6	2345.8
Deoxycholate	486.1	2557.8

[a] Expressed as nmoles of adenosine incorporated into the nucleotide fraction/ml cells/15 min at 25°.

[b] Expressed as nmoles of adenosine converted to inosine + hypoxanthine/ml cells/15 min at 25°.

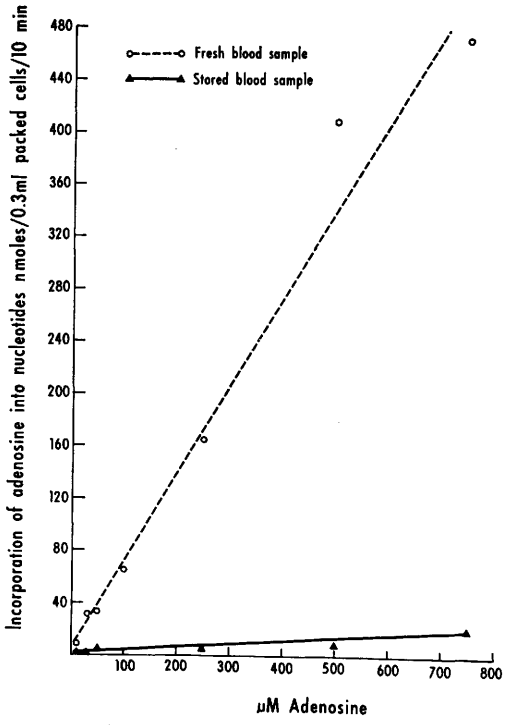

FIG. 1. Effect of substrate concentration on the incorporation of adenosine into red cell ghost nucleotides. Incubated at 37°.

zyme activities were also affected by changes in pH, ionic strength, presence of Ca^{2+} or Mg^{2+} or treatment with Triton X-100.

Schrier (7) has proposed that in the triple layer model of the membrane in which a lipid layer is surrounded by two protein layers, the active sites of the membrane-bound enzyme can be oriented either inwardly or outwardly. On the basis of the Schrier (7) model it would appear that adenosine kinase is oriented in an inward direction and adenosine deaminase in an outward direction.

Failure to saturate adenosine kinase at substrate levels of 750 μM is also consonant with exposure of enzyme sites by the detergent, since in intact ghosts saturation was attained at 10 μM (3). The possibility of a kinetically different enzyme (isoenzyme) in the intact and disrupted ghosts cannot be dismissed.

Our results indicate that storage of blood causes a decrease in erythrocyte adenosine kinase activity, hence a reduction in the ability of the cells to synthesize adenine nucleotides. The latter compounds are important in the preservation of stored blood (8).

Summary. Detergent treatment and homogenization enhance adenosine kinase activity but do not affect adenosine deaminase activity in red cell ghosts. Furthermore, adenosine kinase activity is lost during blood storage whereas adenosine deaminase and detergent activation of adenosine kinase are unaffected. Adenosine kinase was not saturated at substrate concentrations as high as 750 μM whereas saturation occurs at 10 μM in intact ghost cells. Finally, adenosine kinase and deaminase activities are not altered by detergent treatment of hemolysates. These findings suggest that membrane disruption exposes previously inaccessible sites for adenosine kinase activity although the possibility of two separate adenosine kinases has not been ruled out.

1. Miech, R. P., and Santos, J. N., J. Physiol. Chem. Phys. 1, 127 (1969).

2. Miyazaki, H., and Minaki, Y., J. Biochem. 71, 173 (1972).

3. Schrader, J., Rubio, R., and Berne, R. M., Amer. J. Physiol. 223, 159 (1972).

4. Rubio, R., Berne, R. M., and Katori, M., Amer. J. Physiol. 216, 56 (1969).

5. Zamudio, I., Cellino, M., and Canessa-Fischer, M., Arch. Biochem. Biophys. 129, 336 (1969).

6. Duchon, G., and Collier, H. B., J. Membrane Biol. 6, 138 (1971).

7. Schrier, S. L., Amer. J. Physiol. 210, 139 (1966).

8. Bartlett, G. R., *in* "Advances in Experimental Medicine and Biology" (George J. Brewer, ed.), Vol. 6, p. 245. Plenum, New York (1970).

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