THE INSENSITIVITY OF THE ERYTHROCYTE GLUCOSE TRANSPORT SYSTEM TO BOROHYDRIDE REDUCTION

David R. Evans, Blaine C. White, and Ray K. Brown

Department of Biochemistry, Wayne State University
School of Medicine, Detroit, Michigan

Received July 18, 1967

The recent report of Langdon and Sloan (1967) on the incorporation of glucose-^{14}C into stromal protein by the reduction of human erythrocytes with sodium borohydride prompts us to report some experiments on the facilitated diffusion of glucose in such cells. Prior to the above report we had considered the possibility that the transport of glucose across the erythrocyte membrane involved the formation of an imine and had attempted unsuccessfully to inhibit the transport process by treating erythrocytes with borohydride in the presence of glucose. This paper describes the results of a kinetic study of glucose transport in cells reduced according to the procedure devised by Langdon and Sloan. A more detailed report concerning glucose transport in cells reduced under a variety of conditions will be presented at a later date.

METHODS AND MATERIALS

Human erythrocytes in ACD were donated by the Southern Michigan Blood Center. The cells were washed 3-4 times with isotonic buffer (0.01 M potassium phosphate and 0.145 M sodium chloride, pH 7.4) before use.

The cells were reduced by the method of Langdon and Sloan (1967). The concentration of glucose and the reduction times are indicated. The only modification of their procedure was that in the reduction of the cells which were to be used in the transport experiments, the reaction was stopped by the addition of 10 ml of ice-cold isotonic buffer, and no acetic acid was added.

The preparation of the stromal protein for the ^{14}C incorporation studies was as described by Langdon and Sloan. A Baird Atomic thin window gas flow counter was used to count all protein samples. The counting efficiency was 12.6% and was independent of protein concentration over the range used.

Glucose transport was determined by a modification of the method developed by Sen and Widdas (1962). A suspension consisting of .300 ml of packed erythrocytes and 10 ml of 80 mM glucose in isotonic buffer was incubated with constant stirring at 37^{0} for 30-60 minutes. The cells were isolated by centrifugation, and a 25% suspension of these cells in 80 mM glucose in isotonic buffer was prepared. The exit of glucose from these preloaded cells was determined indirectly by monitoring with time the change in cellular volume which occurs as glucose is released into a hypotonic environment. Initially there is a rapid expansion of the cell as water is taken up. This process can be considered instantaneous, however, relative to the gradual return to the equilibrium volume which occurs as glucose is transported out of the cell.

A Zeiss spectrophotometer (PMQ II) was equipped with a specially designed jacketed cell in which 4.75 ml of isotonic buffer or any one of a series of glucose solutions (2, 4, 6, 8, 10, or 12 mM) in isotonic buffer was maintained at 37^{0} with constant stirring. Two micropipets connected in series were used to inject an aliquot of the 25% suspension of preloaded erythrocytes. The first pipet contained 0.100 ml of the suspension. The second pipet contained 0.250 ml of isotonic buffer (or isotonic buffer containing glucose) which served to rinse the first and facilitated rapid mixing. The percent transmittance at 700 mμ was recorded on a Sargent SRL recorder which was equipped with a bucking

potential for nulling the initial value. The recorder was started as the suspension was injected. The total change in percent transmittance was about 15%, and the recorder scale was expanded and nulled so that full scale corresponded to the range between 45 and 65%.

The resulting curve represented volume changes which accompanied the loss of glucose from the cell. The linear portion of the curve is extrapolated to what corresponds to the equilibrium position. This represents the exit time (t) which would be required to reach equilibrium if the cells had continued to transport glucose at the initial maximum rate. Sen and Widdas (1962) have shown that a plot of t versus the concentration of glucose in the external medium (G_o) is linear and that the extrapolated value of t at $G_o = 0$ is inversely proportional to V_{max} for exit into a glucose free medium. The intercept at $t = 0$ corresponds to the half saturation constant, which can be related to the dissociation constant for the glucose-carrier complex (Levine and Stein, 1966). The concentration of glucose in the external medium increases by 1.2 mM due to the addition of 0.075 ml of 80 mM glucose in which the cells were suspended. There is also an increase of 0.4 mM (mean change in glucose concentration: 0.2 mM) due to the loss of glucose from the cell as a result of transport. Consequently the exit times were plotted against the effective glucose concentration calculated taking these factors into consideration ($G_{o\ effective} = G_o + 1.4$ mM).

RESULTS

Intact human erythrocytes were reduced with sodium borohydride in the presence of 50 mM glucose (containing 1 μc of glucose-^{14}C) for 2 minutes at 37^{o} to insure that our reduction procedure was comparable to that of Langdon and Sloan (1967). No sodium borohydride was added to the control, but otherwise these cells were treated identically. As indicated in Table I, the incorporation in our experiments was similar to that obtained by Langdon and Sloan.

TABLE I

Incorporation of Glucose-^{14}C into Stromal Protein

by Reduction of Intact Erythrocytes

	Nanomoles Glucose/mg Protein	
	Experimental	Literature
Control	0	0
$NaBH_4$ Treated	3.7	3.4

Cells which were reduced for 2 minutes at 37° in the presence of 75 mM glucose were used in the kinetic study. Langdon and Sloan found that glucose-^{14}C incorporation into the stroma of cells reduced under these conditions to be 4 nanomoles/mg of protein. A comparison of the results obtained for $NaBH_4$ reduced and untreated cells is presented in Figure I.

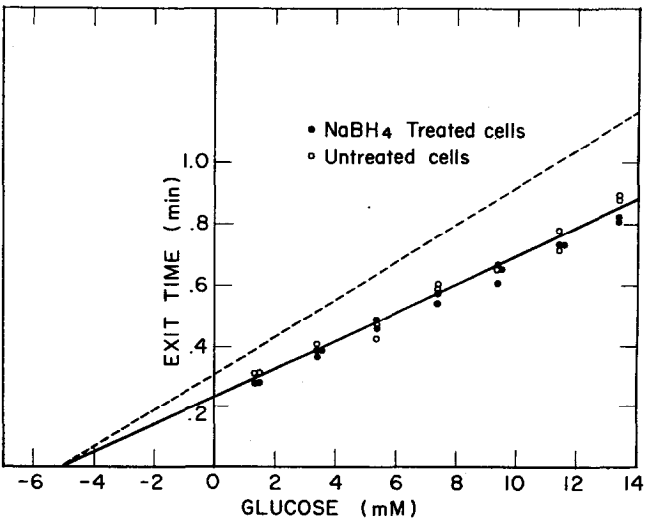

FIGURE I: Kinetics of Glucose Transport in $NaBH_4$ Treated and Untreated Erythrocytes. Plot of exit time versus effective glucose concentration in the external medium. The dashed line represents the theoretical curve for 24% inhibition.

The transport of glucose in reduced cells is indistinguishable from transport in the untreated cells. The half saturation constant is 5.1 mM, and the exit time is .23 minutes. This is in reasonable agreement with the value reported by Sen and Widdas (1962) for normal cells (K_m = 4 mM, t = .18 minutes).

Langdon and Sloan (1967) have devised a mathematical expression which relates the number of sites labeled to the total number of high affinity sites and various other experimental and empirical parameters. Using this equation and the parameters obtained by these authors, we have calculated that 24% of the total number of transport sites should have been labeled under these conditions. Since the exit time is inversely proportional to the maximum velocity which is in turn directly proportional to the number of transport sites, a 32% increase in the exit time would be expected. The corresponding theoretical curve, assuming the half saturation constant remains unaltered, is shown for comparison.

DISCUSSION

It is not unreasonable to expect that reduction of erythrocytes with sodium borohydride in the presence of glucose-^{14}C would result in incorporation of the tracer into the stromal protein. Langdon and Sloan (1967) have shown that significant incorporation occurs with the reduction of albumin, hemoglobin, and insulin. These authors have also presented good evidence that this incorporation into stromal protein involves the reduction of an imine linkage. To relate this to the transport process however, it is necessary to establish that this incorporation occurs at the site on the carrier protein which is specifically involved in the binding of glucose. One criterion for such specificity would be the inhibition of the transport system which would be expected as a result of reduction. It is difficult to imagine that a covalent bond could be formed between glucose and the glucose binding site and not interfere with transport. Our investigation shows that neither the maximum velocity nor the half saturation constant is altered by reduction. This suggests that the incorporation of glucose found by Langdon and Sloan (1967) did not occur at the binding site involved in glucose transport.

ACKNOWLEDGMENTS

A Detroit News Fellowship awarded to David Evans aided this work.

REFERENCES

Langdon, R. G., and Sloan, H. R., Proc. Natl. Acad. Sci., 57, 401 (1967).

Levine, M. and Stein, W. D., Biochem. Biophys. Acta, 127, 179 (1966).

Sen, A. K., and Widdas, W. F., J. Physiol., 160, 392 (1962).