LACK OF STEREOSPECIFICITY OF GLUCOSE BINDING TO HUMAN ERYTHROCYTE MEMBRANE PROTEIN UPON REDUCTION WITH SODIUM BOROHYDRIDE

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The involvement of an imine intermediate in glucose transport based on the demonstration of D-glucose-^{14}C binding to erythrocyte stroma upon $NaBH_4$ reduction has recently been suggested. To relate this imine intermediate to the transport process, the reaction leading to its formation should possess the same optical specificity characteristic of glucose transport. However, L-glucose incorporation was found to be equal to or greater than that of D-glucose. Furthermore L-glucose inhibited the incorporation of D-glucose. It is therefore concluded that the reaction leading to formation of the imine intermediate is not essential to the transport process.

The formation of a transient imine intermediate involving the aldehyde group of glucose and a lysyl side chain of the membrane protein of human erythrocytes has been postulated by Langdon and Sloan (1) to be an essential reaction in glucose transport. This hypothesis was based on the demonstration of high-affinity binding sites by a kinetic analysis of the incorporation of D-glucose-^{14}C into membrane protein of human erythrocytes resulting from the $NaBH_4$ reduction of the imine linkage. However to identify the glucose incorporation demonstrated by Langdon and Sloan (1) as being associated with glucose transport, it should possess the same optical specificity characteristic of the transport system. This paper provides evidence for the lack of this required stereospecificity.

METHODS AND MATERIALS

Human erythrocyte stroma were prepared from outdated blood by the method of Dodge et al. (2). The experimental conditions for demonstrating glucose-^{14}C

incorporation into stromal protein were as described by Langdon and Sloan (1) with the exception that the delipidated protein was solubilized in 1.0 ml of NCS (Amersham/Searle Corp.). The solubilized protein was counted in 10 ml of a scintillation counting mixture consisting of 4 g of Omnifluor (New England Nuclear Corp.), 80 g of naphthalene, 300 ml of 1,4-dioxane, 300 ml of methyl cellolsolve and toluene to a final volume of 1 liter. The counting efficiency was determined by internal standardization.

D-glucose-U-^{14}C and L-glucose-1-^{14}C were purchased from Amersham/Searle Corp.

RESULTS

In preliminary experiments the amount of D-glucose-U-^{14}C incorporated into stromal protein upon reduction with $NaBH_4$ was found to increase with the age of the $NaBH_4$ solution (stored at 5°) (Table I), reaching a maximum with solutions aged for 12 to 20 days. With freshly prepared $NaBH_4$ solutions, the amount of protein-bound labeled sugar ($P^{14}C$) was similar to that recorded by

TABLE I

Incorporation of D-Glucose-^{14}C into Stromal Protein upon Reduction with $NaBH_4$ as a Function of the Age of the $NaBH_4$ Solution

Age of $NaBH_4$ Solution (Days)	PC^{14}; Nanomoles Glucose/mg Protein
0	3.2
2	6.8
7	9.0
8	14.1
9	17.1
12	19.2
15	18.5
20	19.9

Erythrocyte ghosts were reduced for 7 minutes at 37° in the presence of 50 mM D-glucose-U-^{14}C as described by Langdon and Sloan (1).

Langdon and Sloan (1). The maximum $P^{14}C$ formation obtained with the aged $NaBH_4$ solutions is in agreement with the recent finding of Evans et al. (3). In their experiments, $NaBH_4$ was added to the erythrocyte suspension every 2 minutes over a period of 90 minutes and the pH of the reaction mixture was maintained at 7.4. This continual addition of $NaBH_4$ was necessary to overcome the short half-life of $NaBH_4$ which is 45 seconds at pH 7.4. A possible explanation for our results is the fact that the pH of the $NaBH_4$ solutions increased progressively upon storage from an initial pH of 9.8 to a maximum pH of 11.5 after 12 to 20 days. This higher pH, at which $NaBH_4$ is more stable (4), would increase the effective concentration of $NaBH_4$ added to the stromal suspension by decreasing its rate of acid hydrolysis (3). In all subsequent experiments, only $NaBH_4$ solutions aged for 2 to 3 weeks were used.

Glucose transport in the human erythrocyte is specific for the D-isomer (5). Therefore to relate the glucose incorporation into stromal protein as demonstrated by Langdon and Sloan (1) to the transport process, it is necessary to establish that this incorporation possesses the same stereospecificity. To determine this the $P^{14}C$ formation with D- or L-glucose-^{14}C was measured. At glucose concentrations up to 100 mM, L-glucose incorporation was equal to or

TABLE II

Incorporation of D- or L-Glucose-^{14}C into Stromal Protein upon Reduction with $NaBH_4$ as a Function of the Glucose Concentration

Concentration of D- or L-Glucose (mM)	$P^{14}C$; Nanomoles Glucose/mg Protein	
	D-Glucose	L-Glucose
1.0	0.44	0.57
2.5	1.12	1.44
5.0	2.14	3.09
10.0	5.02	6.29
25.0	13.03	13.31
50.0	26.58	26.70
100.0	38.39	49.60
200.0	36.57	58.63

Erythrocyte ghosts were incubated with varying concentrations of D- or L-glucose-^{14}C for 15 minutes at 37°. All samples contained 1 uC of ^{14}C in a final volume of 1 ml. Treatment with $NaBH_4$ was as described in Table I.

greater than that of D-glucose and in contrast to the D-isomer was not maximal at 100 mM glucose (Table II). Furthermore L-glucose was found to inhibit $P^{14}C$-D-glucose formation (Fig. 1). It is therefore concluded that the reaction

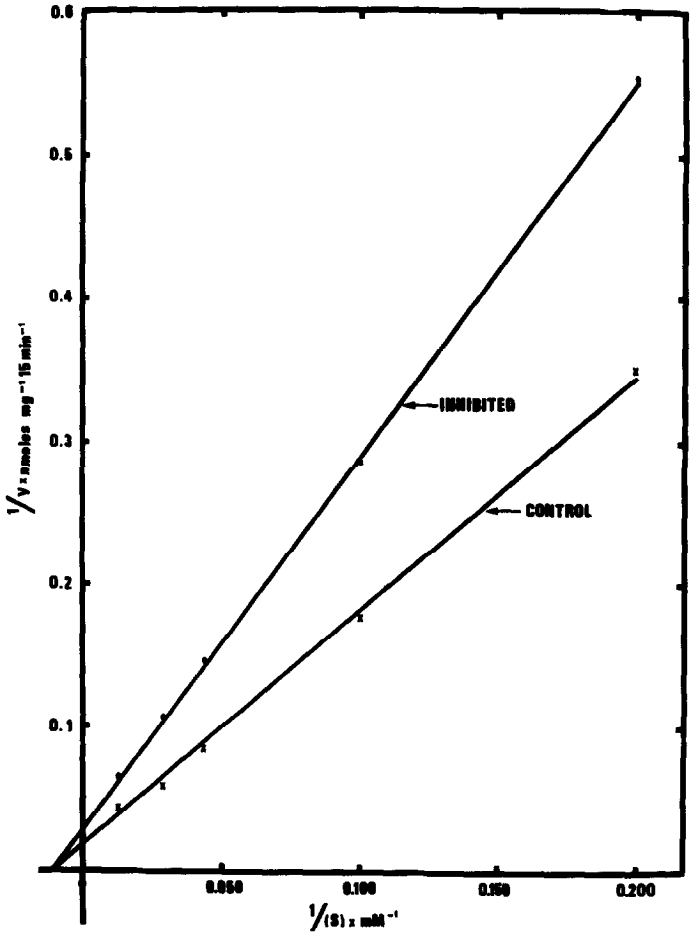

FIGURE 1: Lineweaver-Burk Plot of Incorporation of D-Glucose-U-^{14}C into Stromal Protein in the presence (●) and absence (X) of 50 mM L-Glucose. The lines have been fitted by the method of least squares. The P value for the slope of each line obtained by calculation of the regression coefficients was < 0.05. The coefficient of correlation was significant at the 1 and 5% level for the control and inhibited lines, respectively. Calculation of the Km and Vmax for D-glucose incorporation was not done since the glucose and $NaBH_4$ concentrations do not remain constant during the course of the reaction (3).

involving the formation of an imine linkage between glucose and lysyl residues of stromal protein lacks the stereospecificity characteristic of the glucose transport system. Furthermore at glucose concentrations above 100 mM, sites specific for the binding of L-glucose are evident.

DISCUSSION

The hypothesis that carrier-mediated transport of glucose in erythrocytes involves the formation of an imine intermediate with specific cell membrane proteins has recently been advanced by Langdon and Sloan (1). This hypothesis was based on the demonstration of high-affinity binding sites by a kinetic analysis of the incorporation of D-glucose-^{14}C into erythrocyte membrane protein resulting from the $NaBH_4$ reduction of the imine linkage. Several objections to this hypothesis have recently been raised. Evans et al. (3) have provided evidence, supporting a criticism previously raised by LeFevre (6), that both the glucose and borohydride concentrations do not remain constant during the course of the reaction as had been assumed by Langdon and Sloan (1). Furthermore when extensive amounts of glucose are incorporated into erythrocyte membrane protein as a result of $NaBH_4$ reduction, there is no alteration of the transport kinetics as would be expected if a molecule of glucose had been irreversibly bound to the transport site (3).

Recently, Rose et al. (7) have demonstrated that the glucose transport process does not involve the exchange of the C-1 oxygen of glucose with water and suggested that a carbinolamine rather than an imine intermediate may be involved. However, Evans et al. (3) have demonstrated that the rate of efflux of 1,5-anhydro-D-glucitol from human erythrocytes was decreased by D-glucose. This result was interpreted as evidence against the involvement of a carbonyl group in the glucose transport mechanism of human erythrocytes. But, as pointed out by these authors, this interpretation is open to question since it was not determined if the anhydro sugar is transported by an independent system which nevertheless has a high affinity for D-glucose. The present findings that the binding of glucose to erythrocyte stroma resulting from the $NaBH_4$ reduction

of the imine linkage between glucose and stromal protein lacks the stereospecificity characteristic of the intact glucose transport system, support the interpretation of Evans et al. (3) and argue against the involvement of either an imine or carbinolamine intermediate in glucose transport. It should be pointed out however that the definite exclusion of an imine intermediate in glucose transport based on the presently available data is not possible, since all of the radioactive glucose incorporated upon reduction with $NaBH_4$ may be non-specific and the sites specifically involved in glucose transport are in fact protected from reduction by $NaBH_4$.

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