Biochimica et Biophysica Acta, 555 (1979) 349—351

BBA Report

BBA 71394

A HIGH AFFINITY SITE FOR SUGAR TRANSPORT AT THE INNER FACE OF THE HUMAN ERYTHROCYTE MEMBRANE?

D.M. FOSTER[a], J.A. JACQUEZ[b], W.R. LIEB[c] and W. D. STEIN[d]

[a]*Laboratory of Theoretical Biology, Bldg. 10, Room 4B-56, National Cancer Institute, National Institutes of Health, Bethesda, MD 20205,* [b]*The University of Michigan Medical School, Ann Arbor, MI 48109 (U.S.A.),* [c]*Department of Biophysics, King's College, London WC2B 5RL (U.K.) and* [d]*Institute of Life Sciences, The Hebrew University, Jerusalem (Israel)*

(Received April 6th, 1979)

Key words: Glucose transport; High affinity site; Parameter fitting; Non-linear fitting; (Erythrocyte, Human)

Summary

A disagreement centering on a method of analysis as to the existence of a high affintiy site for glucose transport at the inner face of the human red cell membrane is resolved by using direct fitting methods to confirm the original parameter estimates.

There has been disagreement as to whether the data of Hankin et al. [1] justify the conclusion that there is a high affinity site for glucose transport at the inside of the human erythrocyte membrane [2, 3]. Foster and Jacquez [2] felt that the method of data analysis used by Hankin et al. [1] could have led to an erroneous estimate of the affinity parameter K_m. Lieb and Stein [3] showed, however, that three other independent data analysis procedures produced estimates of K_m very similar to that obtained by Hankin et al.

The original numerical analysis procedure used by Hankin et al. [1] involved a transformation of their raw data into a linear form, from which K_m was extracted using the method of least squares. This transformation yields a plot in which there is error in both ordinate and abscissa. In such a case, analysis of data of poor quality will result in an underestimate of the true slope and hence of K_m [3, 4]. Since the original [1] and the later [3] estimates of K_m agreed, Lieb and Stein concluded that the data given by Hankin et al. [1] were precise enough to allow a correct estimate of K_m using the linearization procedure [3].

In general, if one does not know the precision of the data, the linearization method can be quite hazardous [2] and it is safer to use direct, non-linear curve fitting procedures. Both Lieb and Stein [3] and now Foster and Jacquez have carried out analyses of the original data using such direct fitting method and find values of K_m close to the value originally reported [1].

The basic equation [1, 2] used in the direct non-linear fitting procedure is

$$\frac{dN}{dt} = \frac{vK}{K + \frac{N(P+S_2)}{P+N}} \quad (1)$$

where:

N = cellular glucose concentration at time t
t = time
P = osmolarity of non-penetrating salts in both the extracellular solution and in the isotonic cells
S_2 = extracellular glucose concentration
v = V_{max}
K = K_m

In the experiments reported by Hankin et al. [1], P and S_2 were assumed to be constant throughout the experiment, and N was determined at 0, 10, 20, 30, 40 and 3600 seconds. Five experiments were reported [3] for a given P and S_2 giving a mean value of N for each sample time.

The advantage in using the direct non-linear fitting procedure is that Eqn. 1 can be used directly, and estimates of v and K obtained to give the best fit to the experimental data. The errors in N can be incorporated directly giving estimates of the errors for v and K. Thus all problems arising from transforming the data (and using the integrated form of Eqn. 1) are alleviated.

There are many non-linear least squares fitting routines available. The one we have used is SAAM (Simulation, Analysis and Modeling) [5].

The results of fitting the data given in Table I of Lieb and Stein [3] using SAAM give $K = 1.18 \pm 0.39$ mM and $v = 68.3 \pm 18.3$ mmol·cell unit^{-1} ·min^{-1} ; these compare with $K = 1.26$ mM and $v = 66$ mmol·cell unit^{-1} ·min^{-1} given by Hankin et al. [1], and also fit within the 95% confidence limits reported from graph 2 in Lieb and Stein [3].

Thus, while the linear transformation procedure employed by Hankin et al. [1] might have led to an erroneous value of K_m [2], it turns out that direct fitting methods confirm the original estimate of K_m.

In conclusion both groups have confirmed the original estimate of K_m given by Hankin et al. and this supports Lieb and Stein's argument for the existence of a high affinity site for sugar transport at the inner face of the human red blood cell membrane. Other independent studies by Ginsburg and Stein [6] and Baker and Naftalin [7] also identify an inner high affinity site.

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