Biochimica et Biophysica Acta 861 (1986) 483–488
Elsevier

BBA 70346

BBA Report

Hydrogen ion-coupled transport of D-glucose by phlorizin-sensitive sugar carrier in intestinal brush-border membranes

Takeshi Hoshi, Noriko Takuwa, Masako Abe and Akihiro Tajima

Department of Physiology, Faculty of Medicine, University of Tokyo, Tokyo 113 (Japan)

(Received 2 June, 1986)

Key words: H^+ cotransport; Glucose transport; Sugar carrier; Phlorizin sensitivity; Brush-border membrane; (Rabbit, Toad intestine)

In rabbit intestinal brush-border membrane vesicles, Na^+-independent D-glucose uptake in the presence of an inside-negative transmembrane potential was found to be stimulated by an imposed pH gradient. Na^+-independent, pH-dependent and phlorizin-sensitive D-glucose-evoked potentials could be recorded from isolated toad intestine. The obtained data suggest that phlorizin-sensitive D-glucose carriers of intestinal brush-border membrane can interact with H^+ when Na^+ is absent.

Transport of D-glucose across the intestinal and renal brush-border membranes is known to be absolutely dependent on the presence of Na^+ in the outer medium [1,2]. In the presence of Na^+, D-glucose transport is stimulated by an increase in inside-negative transmembrane potential [3,4] and the stimulation is explained in terms of an increase in the driving force of Na^+, i.e. the Na^+ electrochemical potential difference across the membranes. However, it has been shown by Hilden and Sacktor [5] that, even in the complete absence of Na^+, D-glucose transport across the renal brush-border membrane vesicles is stimulated by an inside-negative transmembrane potential. They also showed that the stimulated D-glucose transport was phlorizin-sensitive. The nature of such Na^+-independent, voltage-accelerated and phlorizin-sensitive transport of D-glucose is unknown at the present time, although some voltage-dependent changes in conformation and/or recruitment of the Na^+/D-glucose cotransporters have been speculated by these authors and others [5,6].

Correspondence: Dr. T. Hoshi, Department of Physiology, Faculty of Medicine, University of Tokyo, Tokyo 113, Japan.

The present study has been designed to examine a possibility of involvement of H^+ in the Na^+-independent, voltage-dependent transport of D-glucose across the intestinal brush-border membrane. A sugar carrier which can utilize either Na^+ or H^+ as a coupling cation has been demonstrated in bacterial (*Escherichia coli*) membranes [7], and recent analysis of amino acid sequences of the same carriers from the mutants revealed that the ability to use either H^+ or Na^+ or Na^+ only was dependent on the difference in amino acid species (serine or proline) at a certain portion of the peptide chain of the carrier molecule [8]. Accordingly, it does not seem unreasonable to suppose that the Na^+/D-glucose cotransporters of vertebrate intestinal and renal brush-border membranes can utilize H^+ under Na^+-free conditions, and that H^+ is involved in this Na^+-independent voltage-accelerated transport of D-glucose. The results obtained in this study strongly support that D-glucose is cotransported with H^+ across the intestinal brush-border membrane when the external medium is completely Na^+-free.

Two different types of experiments were carried out in the present study. One was to measure

D-glucose uptake by the brush-border membrane vesicles in the absence of Na^+ but in the presence of an inside-negative transmembrane potential and to see the effect of a pH gradient across the vesicular membranes. The other was to investigate the properties of D-glucose-induced changes in the transmural potential (the D-glucose-evoked potential) of isolated everted intestine in Na^+-free and low-pH media. Our particular interest in the latter type of experiments was to see whether pH-dependent, phlorizin-sensitive and saturable D-glucose-evoked potentials could be recorded in the complete absence of Na^+.

The brush-border membrane vesicles were prepared from rabbit intestine by the Ca^{2+}-precipitation method of Kessler et al. [9]. To increase the purity, homogenization and Ca^{2+}-precipitation were repeated twice as described in a previous paper [10]. Average enrichment factors for alkaline phosphatase and sucrase of the finally obtained vesicle preparations were 15 and 18, respectively. The membrane vesicles were stored in liquid nitrogen, according to Stevens et al. [11], until use. Before the use, aliquots of the stored vesicles were thawed, and equilibrated with 50 mM K_2SO_4 or 100 mM KCl at pH 7.5 overnight under ice-cold condition or 4–5 h at room temperature. The measurement of D-glucose uptake was carried out in the following way: the K^+-loaded vesicular preparations were diluted 10-fold with a K^+-free incubation medium containing 1 mM D-glucose, 0.1 μCi D-[U-^{14}C]glucose, 200 mM mannitol, 10 μM valinomycin and 10 mM Hepes/Tris (pH 7.5) or Hepes/Mes (pH 5.5), and incubated for various time intervals at 25°C. For L-glucose uptake studies, L-[1-^{3}H]glucose was used. Both radioactive compounds were purchased from New England Nuclear (Boston). The rapid-filtration method was employed for determination of uptake values per mg protein as described elsewhere [10,12].

The observations of the D-glucose-evoked potential were made in isolated everted intestines of toads (*Bufo vulgaris*). The intestine of this animal species was found to be extremely tolerant to Na^+-free conditions. Quite stable and reproducible D-glucose-evoked potentials could be recorded in vitro in Na^+-free low-pH media for more than 6 h. The method of recording the transmural potential was described elsewhere [13]. Briefly, the everted intestine, 2–3 cm long, was fixed over a fenestrated polyethylene tube, and the potential difference across the intestinal wall was led out by means of thin polyethylene bridges filled with 2% agar in 1 M KCl. The bridges were connected to calomel electrodes. Only uppermost part of the small intestine was used since phlorizin-sensitive electrogenic sugar carriers are mainly located in that part [13]. The spontaneous transmural potential and its changes induced by D-glucose added to the mucosal side were recorded on a

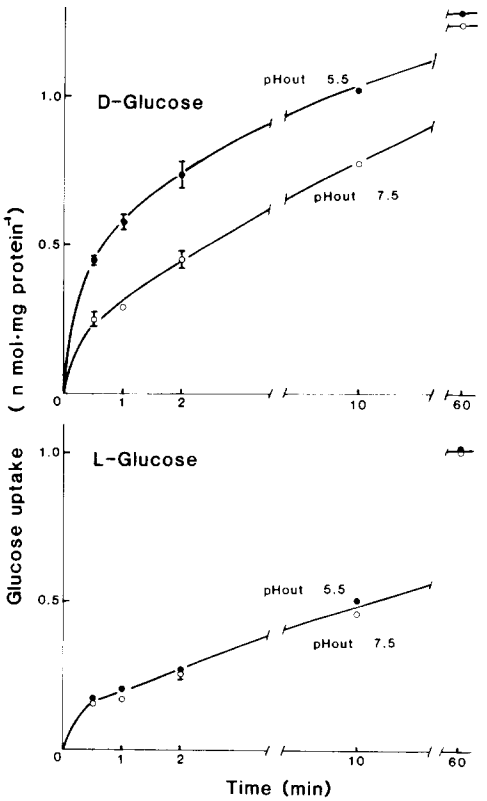


Fig. 1. Effect of lowering external pH (pH_{out}) on Na^+-independent uptake of D-glucose and L-glucose by rabbit intestinal brush-border membrane vesicles. Upper panel shows the results of experiments with D-glucose, and lower panel with L-glucose. Membrane vesicles were prepared at pH 7.5, and all uptake measurements were performed in the presence of valinomycin-induced inside-negative K^+ diffusion potential. Closed circles indicate the condition of pH_{out} 5.5 (the presence of a pH gradient), and open circles the conditions of pH_{out} 7.5 (the absence of a pH gradient). All values are means of four different measurements except for the 10 and 60 min values, where single measurements were performed. Vertical lines indicate the standard error of the mean

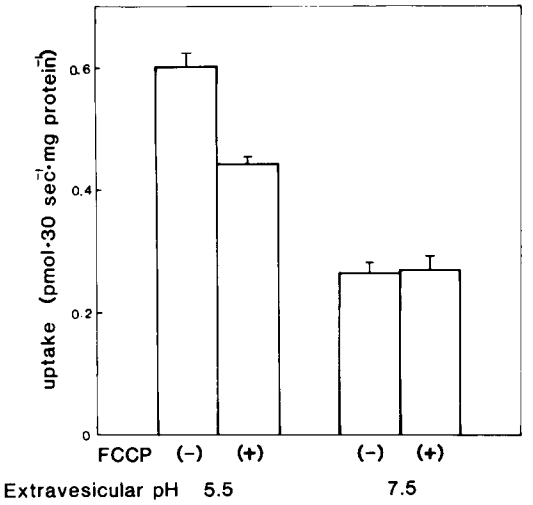


Fig. 2. The effect of FCCP on Na^+-independent uptake of D-glucose in the presence and absence of a pH gradient. K^+-loaded intestinal brush-border membrane vesicles were used. All data are means (±S.E.) of four experiments.

high sensitivity DC recorder (National, VP-6541A).

The time-courses of the vesicular uptake of D-glucose and L-glucose in the absence of Na^+ were shown in Fig. 1. All data were obtained from the K^+-loaded membrane vesicles and in the presence of an inside-negative transmembrane potential (valinomycin-induced K^+ diffusion potential). As previously shown by Hilden and Sacktor [5], Na^+-independent D-glucose uptake in the presence of an inside-negative transmembrane potential was significantly greater than L-glucose uptake during at least initial 10–15 min even in the absence of a pH gradient. When the vesicular uptake was examined at a lowered extravesicular pH ($pH_o = 5.5$), the utpake of D-glucose was further accelerated. Such an effect of the lowered pH_o was not seen for L-glucose. As all the vesicles used had been prepared at pH 7.5, a pH gradient was expected to be imposed across the membrane when the extravesicular pH was lowered. Accordingly, it is considered that a proton gradient is responsible for the acceleration of D-glucose uptake in the low pH medium. To test this possibility, the effect of FCCP (carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone), a protonophore, was examined. The protonophore did not affect the uptake of D-glucose in the absence of a pH gradient ($pH_o = pH_i = 7.5$). In contrast, FCCP

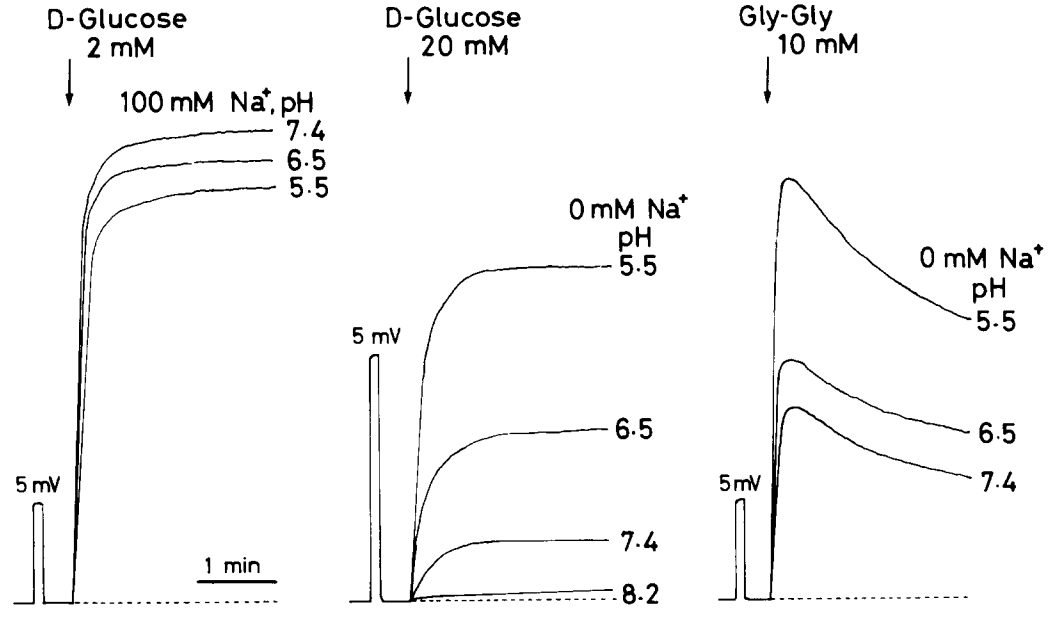


Fig. 3. Effects of pH of the bathing medium on the D-glucose- and glycylglycine-evoked potentials of everted toad intestine. (Left) D-Glucose-evoked potentials in the presence of 100 mM Na^+. (Middle) D-Glucose-evoked potential in the absence of Na^+ (in the mannitol-substituted Ringer's solution). (Right) Glycylglycine-evoked potentials in the absence of Na^+ (the same solution as in middle panel). Each set of PD tracings was obtained from a single typical experiment.

significantly reduced the rate of D-glucose uptake when a pH gradient was present (Fig. 2). The results suggest that the proton gradient is essential for the acceleration by lowering external pH.

In the series of PD-recording experiments, the preparations were preincubated in a Na^+-free solution (the mannitol-substituted Ringer's solution) for a sufficiently long time (2–3 h) in order to wash out Na^+ remaining in the tissue. Even after such a long preincubation, the addition of D-glucose to the mucosal medium evoked a small but definite increase in the transmural potential in the complete absence of Na^+. Interestingly, lowering medium pH to 6.5 and 5.5 caused graded increases in the amplitude of the glucose-evoked potential as shown in Fig. 3. Such pH-dependent increases in amplitude were observed quite reproducibly, and the feature of the graded increase was similar to those seen for dipeptides which are known to be cotransported with H^+ [10,14–16]. In contrast, L-alanine and L-proline never caused such changes in the transmural PD in the Na^+-free medium. This indicates that the observed PD increase caused by D-glucose was not due to the presence of a low concentration of Na^+ in the vicinity of cell surface but due to a H^+-coupled mechanism similar to that in the peptide transport. The amplitude of the D-glucose-evoked potential recorded in the Na^+-free medium was saturable as seen in the Na^+-dependent D-glucose-evoked potentials recorded in the presence of Na^+ [13]. The double-reciprocal plot of the data of the Na^+-independent potential gave a straight line, indicating that the D-glucose-evoked potentials recorded in the Na^+-free medium also conform to simple Michaelis-Menten kinetics as does the Na^+-dependent D-glucose-evoked potential. The mean value of K_t for D-glucose measured in Na^+-free medium of pH 5.5 was 9.6 mM, the value being about 50-times higher than K_t for D-glucose estimated in the presence of 100 mM Na^+ (Table I). The values of ΔPD_{max} were 7.1 mV in the mannitol Ringer's solution, and 11.0 mV in the 100 mM Na^+, SO_4^{2-}-Ringer solution, respectively. The values of the transmural resistance of the everted intestines under such two different medium conditions were 1210 ± 100 and 476 ± 79 $\Omega \cdot cm^2$ (serosal area), respectively. Calculation of the equivalent short circuit current [17] from the values of the evoked potential and the transmural resistance revealed that the maximum short circuit current was not identical between the two conditions. In the Na^+-free medium of pH 5.5, the maximum current was 5.9 $\mu A/cm^2$, while it was 23.1 $\mu A/cm^2$ in the presence of 100 Na^+, thus the former being about one fourth the latter.

TABLE I

KINETIC PARAMETERS OF THE Na^+-DEPENDENT AND Na^+-INDEPENDENT D-GLUCOSE-EVOKED POTENTIALS AND K_i VALUES FOR PHLORIZIN IN THE PRESENCE AND ABSENCE OF Na^+

Data are given as mean ± S.E. of the number of experiments given in parenthesis.

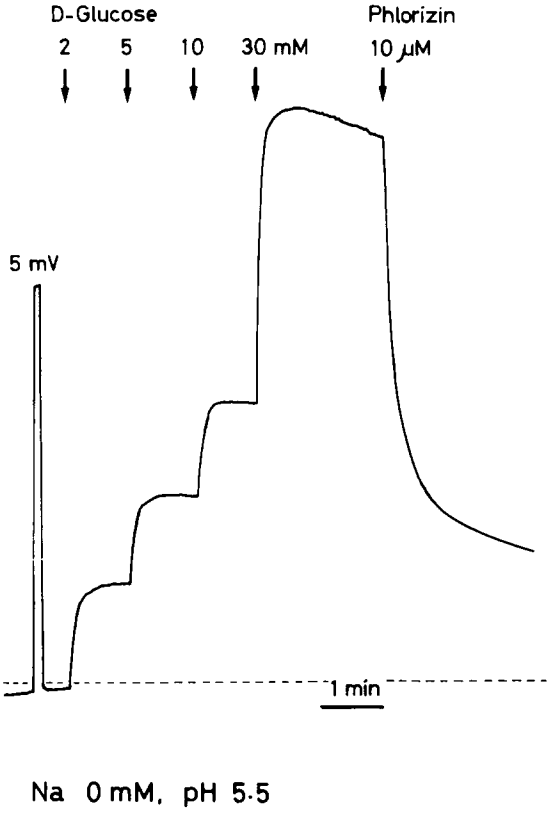
Na^+ concn. (mM)	pH	K_t for D-glucose (mM)	ΔPD_{max} (mV)	K_i for phlorizin (μM)
100 [a]	7.4	0.19 ± 0.02 (8)	11.0 ± 2.3 (8)	0.20 ± 0.03 (4)
0 [b]	5.5	9.6 ± 1.4 (8)	7.1 ± 0.9 (8)	1.27 ± 0.25 (6)

[a] The solution contained 50 mM Na_2SO_4, 104 mM mannitol 1.5 mM $CaSO_4$, 1 mM $MgSO_4$, 1.24 mM K_2HPO_4, 0.42 mM KH_2PO_4.

[b] The solution contained 204 mM mannitol, 1.5 mM $CaSO_4$, 1 mM $MgSO_4$, 1.24 mM K_2HPO_4, 0.42 mM KH_2PO_4. pH was adjusted with Hepes/Mes.

Phlorizin was found to effectively abolish the glucose-evoked potential in the Na^+-free medium. A typical example of the phlorizin-inhibition of the evoked potential is shown in Fig. 4. The inhibition appeared promptly when added to the medium, as seen in the presence of Na^+ [13]. Although the inhibitor constant (K_i) for phlorizin estimated in the Na^+-free medium was 6-times higher than that estimated in 100 mM Na^+ Ringer's solution, the obtained value was still very low, namely the order of μM (Table I). A limited number of experiments showed that the value of K_i for phlorizin was pH-dependent; lowering of medium pH tended to decrease the value of K_i. Even in the absence of Na^+, phlorizin action was found to be purely competitive with D-glucose as shown by the Lineweaver-Burk type plot of the data (Fig. 5).

The results obtained in this study strongly suggest that D-glucose is cotransported with H^+ when Na^+ is absent in the medium, and that an increase of inside-negative transmembrane potential acts



so as to increase the electrochemical potential gradient of H^+ across the membrane. This is supported by the following facts demonstrated in this study: (a) Na^+-independent voltage-accelerated D-glucose transport is further stimulated by lowering external pH and this effect was inhibited by a protonophore. (b) The properties of the D-glucose-evoked potentials recorded in the Na^+-free medium are quite similar to those of the dipeptide-evoked potentials which are shown to be directly related to the H^+/dipeptide cotransport [18]. (c) The Na^+-independent pH-dependent D-glucose evoked potential exhibits kinetic behaviors quite similar to those of the Na^+-dependent D-glucose evoked potential. Accordingly, our results seem to provide reasonable explanation for Na^+-independent voltage-accelerated transport of D-glucose which was first observed by Hilden and Sacktor [5] in renal brush-border membrane vesicles.

Fig. 4. A typical recording showing the effect of phlorizin on the D-glucose-evoked potential in the absence of Na^+. The mannitol-substituted solution of pH 5.5 was used as the bathing medium.

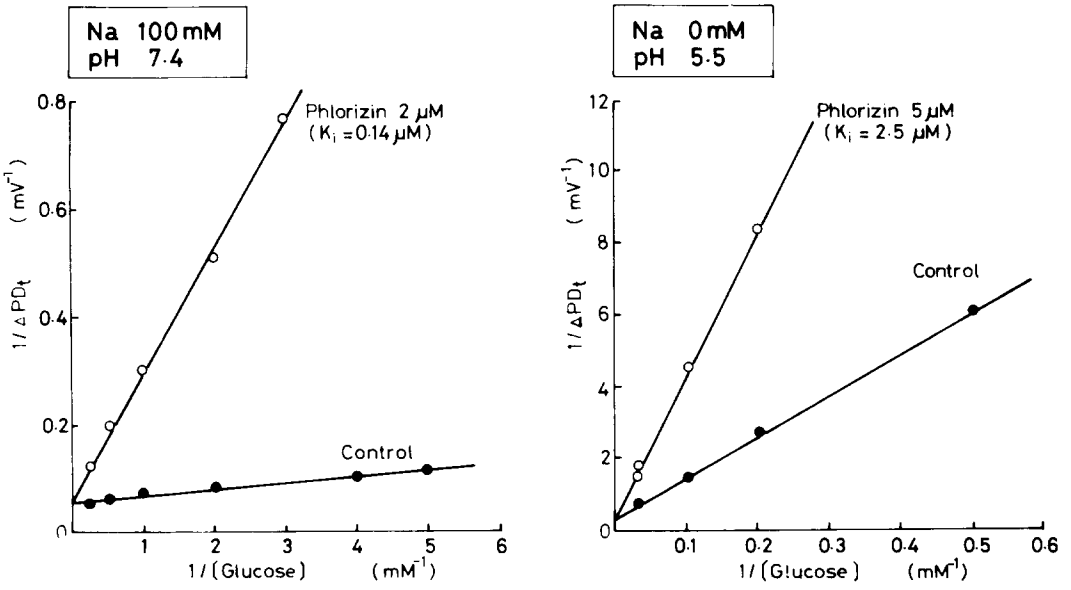


Fig. 5. Inhibition of the D-glucose-evoked potential by phlorizin in the presence (left panel) and absence (right panel) of Na^+. Lineweaver-Burk plot. Medium conditions and concentrations of added phlorizin are shown in the figure. Paired measurements under control conditions (closed circles) and in the presence of phlorizin (open circles) were performed in a single preparation. Typical single experiments.

The H^+-dependent transport of D-glucose observed in the Na^+-free medium may be mediated by the so-called Na^+/D-glucose cotransporter since the transport is highly sensitive to phlorizin. However, there remains a possibility that the brush-border membranes may have two separate carriers for D-glucose which are different in cation selectivity but equally sensitive to phlorizin. Two distinct Na^+/D-glucose cotransport systems which exhibit different sugar specificity have been reported for hamster intestine [19], and two systems which exhibit different temperature sensitivity have been found in guinea pig small intestine [20]. However, additional findings seem to support, at least at the present time, that the carriers exhibiting Na^+-dependence or H^+-dependence are identical. One of the findings is that the Na^+-independent pH-dependent D-glucose-evoked potentials can be recorded from the same limited portion of toad intestine where Na^+-dependent sugar transport is active and Na^+-dependent D-glucose-evoked potential of high amplitude can be recorded. Another finding is that the D-glucose-evoked potential recorded in the presence of a sufficiently high concentration of Na^+ is not significantly influenced by lowering medium pH from 7.4 to 5.5 (Fig. 3), but at a low concentration of Na^+, lowering pH significantly reduces the amplitude of the evoked potential [13]. This seems to indicate that Na^+ and H^+ may compete for the common cation binding site on the same D-glucose carrier.

This study was supported by Grant-in-Aids from the Ministry of Education, Science and Culture of Japan (No. 60115006 and 60480107).

References

1 Crane, R.K. (1968) in Handbook of Physiology, Sect. 6: Alimentary Canal, Vol. 3 Intestinal Absorption (Code, C.F., ed.), pp. 1323–1351, Am. Physiol. Soc., Washington
2 Schultz, S.G. and Curran, P.F. (1970) Physiol. Rev. 50, 637–718
3 Carter-Su, C. and Kimmich, G.A. (1980) Am. J. Physiol. 238, C73–C80
4 Beck, J.C. and Sacktor, B. (1975) J. Biol. Chem. 250, 8674–8680
5 Hilden, S. and Sacktor, B. (1982) Am. J. Physiol. 242, F340–F345
6 Aronson, P.S. (1984) in Electrogenic Transport: Fundamental Principles and Physiological Implications (Blaustein, M.P. and Lieberman, M., eds.), pp. 49–70, Raven Press, New York
7 Tsuchiya, T. and Wilson, T.H. (1978) Membrane Biochem. 2, 63–79
8 Yazyu, H., Shirota, S., Futai, M. and Tsuchiya, T. (1985) J. Bacteriol. 162, 933–937
9 Kessler, M., Acuto, O., Storelli, C., Murer, H., Muller, M. and Semenza, G. (1978) Biochim. Biophys. Acta 506, 136–154
10 Takuwa, N., Shimada, T., Matsumoto, H., Himukai, M. and Hoshi, T. (1985) Jpn. J. Physiol. 35, 629–642
11 Stevens, B.R., Wright, S.H., Hirayama, B.S., Gunther, R.D., Ross, H.J. Harms, V., Nord, E., Kippen, I. and Wright, E.M. (1982) Membrane Biochem. 4, 271–282
12 Ohsawa, K., Kano, A. and Hoshi, T. (1979) Life Sci. 24, 669–678
13 Hoshi, T. and Komatsu, Y. (1968) Jpn. J. Physiol. 18, 508–519
14 Ganapathy, V. and Leibach, F.H. (1983) J. Biol. Chem. 258, 14189–14192
15 Ganapathy, V., Burchhardt, G. and Leibach, F.H. (1984) J. Biol. Chem. 259, 8954–8959
16 Takuwa, N., Shimada, T., Matsumoto, H. and Hoshi, T. (1985) Biochim. Biophys. Acta 814, 186–190
17 Macknight, A.D.C., Dibona, D.A. and Leaf, A. (1980) Physiol. Rev. 60, 615–715
18 Hoshi, T. (1986) in Ion Gradient-coupled Transport (Alvarado, F. and Van Os, C.H., eds.), pp. 183–191, Elsevier, Amsterdam
19 Honegger, P. and Semenza, G. (1973) Biochim. Biophys. Acta 318, 390–410
20 Brot-Laroche, E., Serrano, M.A., Delhomme, B. and Alvarado, F. (1985) Ann. N.Y. Acad. Sci. 456, 47–50