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Radiation-inactivation analysis of the oligomeric structure of the renal sodium/D-glucose symporter

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Abstract

The radiation-inactivation size (RIS) of the rat renal brush-border membrane sodium/D-glucose cotransporter was estimated from the loss of transport activity in irradiated membrane vesicles. The RIS depended on the electrochemical conditions present when measuring transport activity. A RIS of 294 ± 40 kDa was obtained when transport was measured in the presence of a sodium electrochemical gradient. Under sodium equilibrium conditions, the RIS was 84 ± 25 kDa in the presence of a glucose gradient, and 92 ± 20 kDa in its absence. In the absence of a sodium gradient, but in the presence of an electrical potential gradient, the RIS increased to 225 ± 49 kDa. The 294 kDa result supports earlier suggestions that the Na^+ gradient-dependent glucose transport activity is mediated by a tetramer. Individual monomers appear, however, to carry out glucose transport under equilibrium exchange conditions or when a glucose gradient serves as the only driving force. The electrical potential gradient-driven glucose transport RIS appears to involve three functional subunits. © 1997 Elsevier Science B.V.

Keywords: Radiation-inactivation; Glucose cotransporter; SGLT1; RS1; (Kidney)

1. Introduction

The high-affinity Na^+/D-glucose cotransporter from the brush-border membranes of the small intestine and the kidney proximal tubule is among the most extensively studied transport proteins [1–3]. A protein of 73 kDa, designated SGLT1, which mediates electrogenic Na^+ gradient-dependent D-glucose transport, was originally cloned from rabbit small intestine [4]. Highly homologous Na^+-dependent glucose cotransporters have subsequently been cloned from human ileum [5], the LLC-PK porcine kidney epithelial cell line [6] and rat renal cortex [7]. The amino acid sequences of SGLT1 proteins from these different species share 86 to 87% identity [7], but, within a given species, identical SGLT1 cotransporters are expressed in the intestine and kidney [8,9]. SGLT1 also shares strong homology with SGLT2, which mediates low-affinity high-capacity Na^+-dependent glucose transport in the kidney [10,11], with mammalian Na^+-dependent cotransporters for nucleosides (SNST1) [12], *myo*-inositol (SMIT1) [13] and

Abbreviations: HEPES, 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid; RIS, radiation-inactivation size

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neutral amino acids (SAAT1) [14], and with a rabbit kidney protein of still unidentified function (rkST1) [15].

Information concerning the structure-function relationship of the glucose cotransporter was obtained from measurements of its in situ functional size with the radiation-inactivation technique [16–19]. The radiation-inactivation size (RIS) estimated for the Na^+/glucose cotransporter in renal [20,21] and intestinal [22,23] brush-border membrane vesicles ranges from 288 to 434 kDa. A similar value (343 kDa) was also reported for the rabbit renal cotransporter reconstituted in liposomes [24]. Based on the fact that the RIS calculated from the loss of Na^+-dependent glucose transport activity in irradiated rabbit intestinal brush-border membrane vesicles (290 kDa) corresponds to four times the molecular weight of SGLT1, Stevens et al. [22] concluded that the cotransporter functions in situ as a homotetramer. Recently, however, a 67-kDa protein (RS1), which alters the kinetic properties and voltage dependence of SGLT1, was cloned from porcine kidney cortex [25]. Because this protein, which shares no homology with SGLT1, but is present in the cells of the small intestine and kidney, probably associates with SGLT1 and possibly with other homologous cotransporters in the membrane, the glucose cotransporter apparently consists of an oligomer composed of both SGLT1 and RS1 subunits [3].

In the present study, the functional size of the Na^+/glucose cotransporter was estimated from the radiation-induced loss of glucose transport activity, measured under four different electrochemical gradient conditions, in rat renal brush-border membrane vesicles.

2. Materials and methods

2.1. Membrane preparation

Renal brush-border membrane vesicles were prepared from adult Sprague Dawley male rats (250–300 g) (Charles River, St-Constant, Que., Canada). After decapitation, the kidneys were perfused with 0.85% (w/v) NaCl and brush-border membranes were purified with an $MgCl_2$ precipitation method [26]. The final pellet was washed and resuspended in 14% (w/v) glycerol, 1.4% (w/v) sorbitol, 5 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES)/Tris (pH 7.5), 150 mM NaCl or KCl, with or without 50 μM glucose. To generate electrical potential gradients across the membrane during the transport assays, NaCl and KCl were replaced by 300 mM mannitol or 150 mM sodium gluconate. After incubation at 4°C for 45 min, the membrane vesicles were stored in liquid nitrogen until use. Alkaline phosphatase was assayed as described [27] and protein concentration was measured with the method of Lowry et al. [28]. Membrane preparations were enriched 10–12-fold, over the cortex homogenate, in alkaline phosphatase activity.

2.2. Irradiation procedure

Irradiation was carried out at −78°C in a Gammacell Model 220 ^{60}Co irradiator (Atomic Energy of Canada) at a dose rate of approximately 1.5 Mrad/h as described previously [21,29]. The following equation [17] was used to relate the radiation inactivation size (RIS) to D_{37}, the radiation dose (in Mrad) at which the measured activity has been decreased to 37% of its initial value, and to T, the temperature (in °C):

$$\log \mathrm{RIS} = 5.89 - \log D_{37} - 0.0028T$$

or, for the constant irradiation temperature in these experiments:

$$\mathrm{RIS} = 1.29 \times 10^6 / D_{37}.$$

D_{37} values were obtained from semi-logarithmic plots of transport versus irradiation dose using a least-squares fit.

2.3. Glucose transport

Glucose uptake was measured at 25°C with a rapid filtration technique [30]. The incubation medium contained 14% (w/v) glycerol, 1.4% (w/v) sorbitol, 5 mM HEPES/Tris (pH 7.5), 150 mM NaCl or KCl, and 50 μM [^{14}C]glucose (1 μCi/assay). The reaction was initiated by the addition of 80–120 μg of membrane protein. After 5 s, the reaction was stopped by dilution (1/60) with an ice-cold stop solution composed of 14% (w/v) glycerol, 1.4% (w/v) sorbitol, 5 mM HEPES/Tris (pH 7.5), and 150 mM

KCl. The vesicle suspension was filtered through a 0.45-μm pore size nitrocellulose membrane under vacuum. Filters were washed with 8 ml of ice-cold stop solution and processed for liquid scintillation counting. Non-specific binding to the filters was determined with the same procedure, but omitting membrane vesicles. Na^+-dependent transport was calculated as the difference between the uptake values measured in the presence of Na^+ or K^+.

3. Results

The time course of sodium-dependent glucose transport activity of intact and irradiated rat renal brush-border membrane vesicles was measured under different electrochemical gradient conditions (Fig. 1). As expected, the slowest transport rate (24 pmol/mg protein/5 s) was observed under equilibrium exchange conditions (Fig. 1A). In the presence of a glucose gradient as the only driving force, the initial rate of glucose uptake almost doubled (41 pmol/mg/5 s) (Fig. 1B). It increased further (52 pmol/mg/5 s) when an electrical potential gradient was generated across the membrane, under sodium equilibrium conditions, by diluting vesicles loaded with the sodium salt of a relatively impermeant anion, gluconate, in transport buffer containing sodium chloride (Fig. 1C). Finally, the fastest rate (95 pmol/mg/5 s) was observed when a sodium electro-

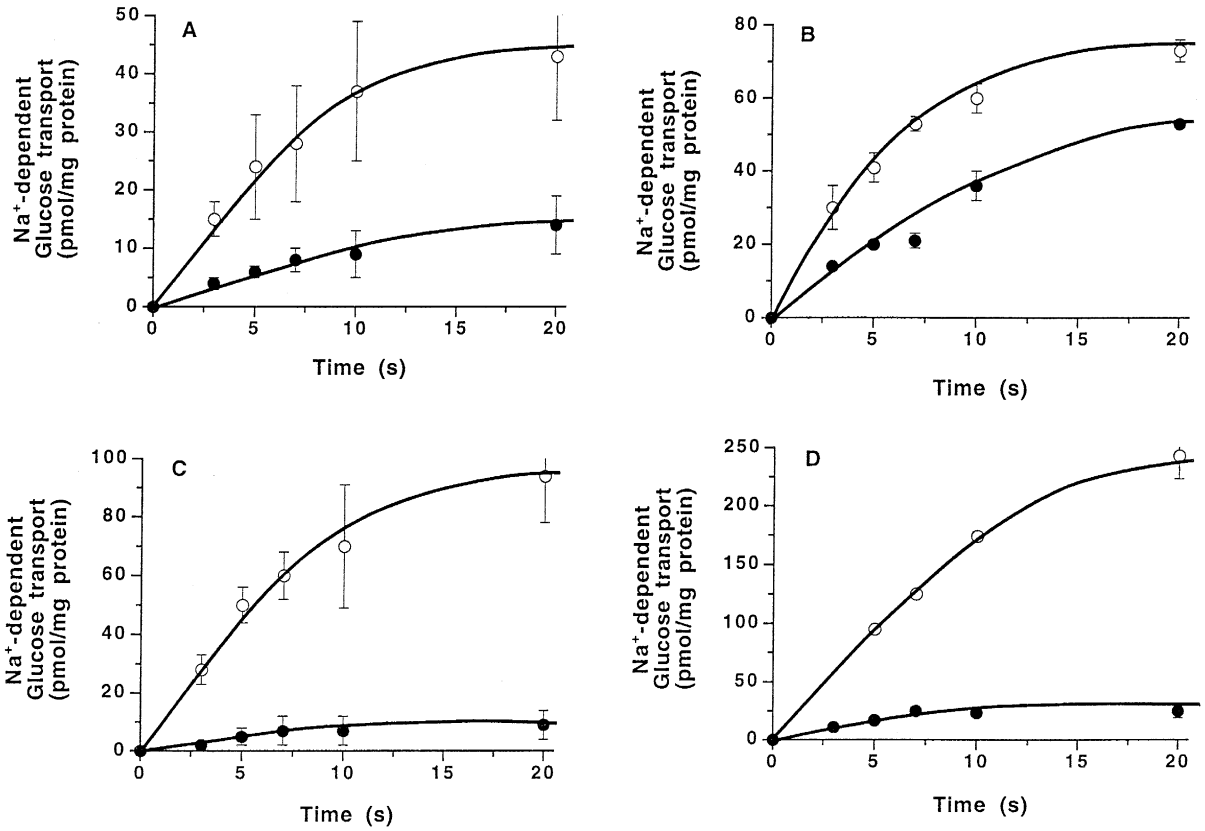

Fig. 1. Effect of irradiation on the time course of glucose uptake under different driving forces. Vesicles were isolated and resuspended in 14% (w/v) glycerol, 1.4% (w/v) sorbitol, 5 mM HEPES/Tris (pH 7.5) and (A) 50 μM glucose and 150 mM NaCl or KCl, (B) 150 mM NaCl or KCl, (C) 150 mM Na^+-gluconate, or (D) 300 mM mannitol before irradiation at 0 (○) or 5 (●) Mrad. Glucose uptake was measured in 14% (w/v) glycerol, 1.4% (w/v) sorbitol, 5 mM HEPES/Tris (pH 7.5), 150 mM NaCl or KCl, and 50 μM [^{14}C]glucose (1 μCi/assay). Na^+-dependent glucose transport was calculated as the difference between the uptake values measured in media containing NaCl or KCl. Each value represents the mean ± S.D. of three experiments, each done in triplicate. Error bars smaller than the symbols are not shown.

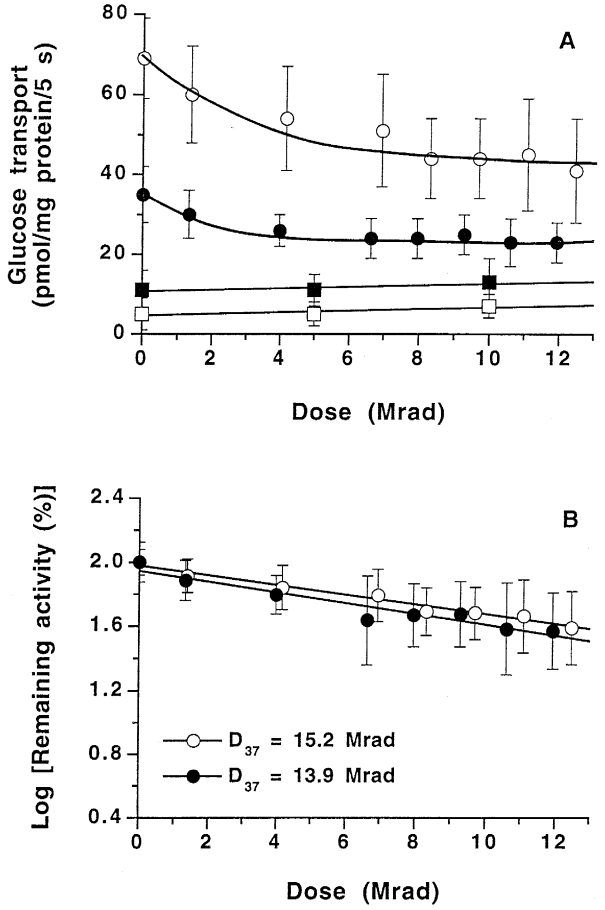

Fig. 2. Radiation-inactivation data for the Na^+-glucose transport activity under sodium equilibrium conditions. (A) Vesicles were isolated and resuspended in 14% (w/v) glycerol, 1.4% (w/v) sorbitol, 5 mM HEPES/Tris (pH 7.5) and 150 mM NaCl (●,○) or KCl (■,□), with (●,■) or without (○,□) 50 μM glucose. The incubation medium was composed of 14% (w/v) glycerol, 1.4% (w/v) sorbitol, 5 mM HEPES/Tris (pH 7.5), 50 μM [^{14}C]glucose (1 μCi/assay) and 150 mM NaCl (○,●) or KCl (□,■). (B) Na^+-dependent glucose transport was calculated as the difference between the uptake values measured in media containing NaCl or KCl and expressed as log of the percentage of the remaining activity. Each value represents the mean ± S.D. of six experiments, each done in quadruplicate.

chemical gradient was established across the membrane (Fig. 1D). Under all conditions tested, irradiation caused a dramatic decrease in transport activity but the initial rate of glucose uptake remained linear for at least 5 s. All subsequent transport measurements were therefore carried out after 5 s of incubation.

Glucose uptake measured under sodium equilibrium conditions, in the presence or absence of a glucose gradient, decreased progressively as a function of the radiation dose (Fig. 2A). In contrast, the Na^+-independent glucose uptake, which is not mediated by a carrier protein, was unaffected at the doses used. A semi-logarithmic plot of these data revealed a simple exponential decay of the activity as a function of the radiation dose (Fig. 2B). Similar radiation inactivation sizes were measured in the presence,

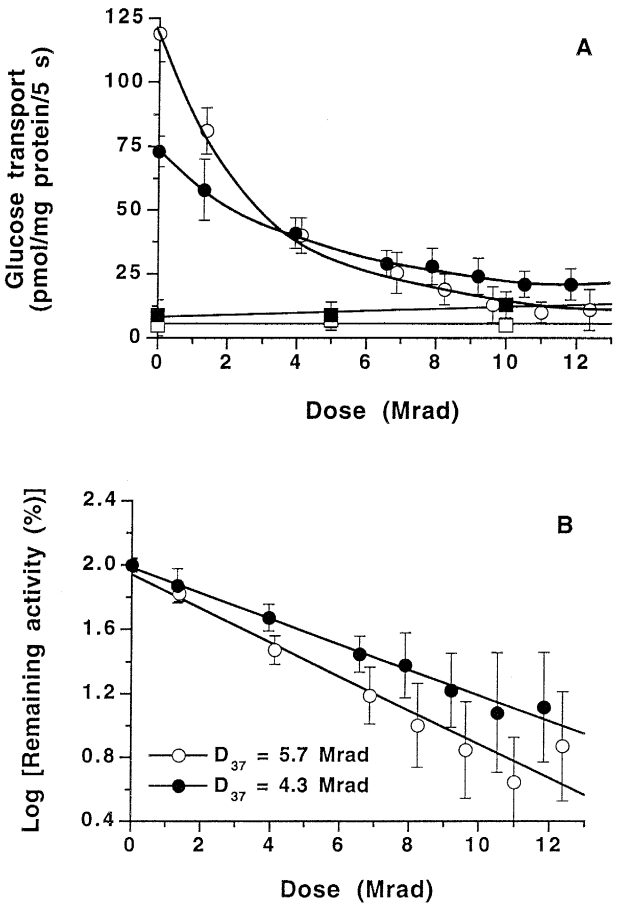

Fig. 3. Radiation-inactivation data for glucose transport activity driven by an electrical potential or a sodium electrochemical gradient. (A) Vesicles were isolated and resuspended 14% (w/v) glycerol, 1.4% (w/v) sorbitol, 5 mM HEPES/Tris (pH 7.5) and 300 mM mannitol (○,□), 150 mM Na^+-gluconate (●), or 150 mM K^+-gluconate (■). The incubation medium contained 14% (w/v) glycerol, 1.4% (w/v) sorbitol, 5 mM HEPES/Tris (pH 7.5), 50 μM [^{14}C]glucose (1 μCi) and 150 mM NaCl (○,●) or KCl (□,■). (B) Na^+-dependent glucose transport was calculated as the difference between the uptake values measured in media containing NaCl or KCl and expressed as log of the percentage of the remaining activity. Each value represents the mean ± S.D. of four experiments, each done in quadruplicate

84 ± 25 kDa, or absence, 92 ± 20 kDa, of a glucose gradient.

The effect of radiation dose on the rate of glucose transport was also measured in the presence of a transmembrane electrical potential generated by chloride diffusion into the vesicles, under a sodium equilibrium or sodium gradient condition. Glucose transport activity decayed faster with increasing radiation dose in the presence of a sodium gradient than in its absence (Fig. 3A). Again, the Na^+-independent glucose uptake was unaffected. The RIS was 294 ± 40 kDa when measured in the presence of a sodium gradient, and 225 ± 49 kDa in its absence (Fig. 3B).

4. Discussion

In the present study, different radiation-inactivation sizes were measured for the sodium-dependent D-glucose cotransporter of the rat renal brush-border membrane depending on the conditions under which transport activity was assayed (Table 1). The size measured in the presence of a transmembrane sodium electrochemical gradient (294 kDa) was in close agreement with those previously estimated for the glucose cotransporter of renal (288 kDa) [21] and intestinal (290 kDa) [22] brush-border membrane vesicles, in the absence of a transmembrane chloride gradient. Because these values are about four times larger than those of SGLT1 (73 kDa) [4] and RS1 (67 kDa) [25] polypeptides, the sodium electrochemical gradient-dependent transport activity is probably mediated by a tetrameric protein, as was suggested earlier [3,21,22]. A similar tetrameric structure thus appears to function, in the presence of a transmembrane sodium gradient, whether or not an electrical potential gradient is imposed across the membrane.

Table 1
Summary of the radiation-inactivation sizes estimated for the rat renal brush-border membrane Na^+/D-glucose cotransporter under different electrochemical gradient conditions

Na^+ gradient	Potential gradient	Glucose gradient	Glucose function measured	RIS (kDa)
−	−	−	Exchange	92
−	−	+	Uptake	84
−	+	+	Uptake	225
+	+	+	Uptake	294

Radiation-inactivation sizes of 84 and 92 kDa were estimated from the loss of glucose transport activity measured under sodium equilibrium conditions, in the presence or absence of a glucose gradient. It cannot be excluded, on the basis of these radiation-inactivation data, that glucose transport under sodium equilibrium conditions could be mediated by a still unidentified protein, slightly larger than SGLT1, or by a dimer formed by SGLT1 and another polypeptide of about 10–20 kDa. The measured RIS values are not, however, significantly different from the size of the SGLT1 monomer and suggest that such monomers may have the ability to act as sodium-dependent permeases. This interpretation is consistent with the fact that the target size derived from the radiation-induced fragmentation of SGLT1 polypeptides (66 kDa) also corresponds to that of the monomer [22]. Following a radiation hit, the energy absorbed by individual monomers does not therefore appear to spread sufficiently among other subunits of the tetramer to cause their fragmentation. The results of the present study further suggest that the transferred energy is also insufficient to cause denaturation of adjacent monomers. Also consistent with a sodium-dependent transport activity attributable to monomers of the glucose symporter is the earlier demonstration, using covalent fluorescent probes, that glucose and sodium binding sites are present on the same polypeptide [31,32].

The radiation-inactivation size, measured in the absence of a sodium gradient but in the presence of a transmembrane electrical potential, was 225 kDa, a value which suggests the involvement of three subunits. Because of considerable overlap between the transport values measured in the presence or absence of a sodium gradient, especially at the higher radiation doses (Fig. 3), however, further studies will be required to ascertain the existence and composition of this oligomeric species.

Oligomers of the sodium symporter are most probably composed of both SGLT1 and RS1 subunits although the exact proportion of each polypeptide remains to be ascertained [3,25]. In addition to having a strong effect on the kinetics of glucose transport when coexpressed with SGLT1, RS1 is detected by

Western blot analysis, along with SGLT1, in a protein complex of about 300 kDa following electrophoresis in non-denaturing gels [25]. This size is in remarkably good agreement with those estimated with the radiation-inactivation approach. SGLT1 was shown, however, to carry out electrogenic sodium gradient-dependent glucose transport when expressed in frog oocytes [4] in which RS1-homologous mRNAs were not detectable [25]. Possibly a smaller oligomeric assembly could be capable of glucose transport in the absence of RS1 subunits. Furthermore, in the absence of energy transfer between monomers, an oligomer composed of SGLT1 subunits could possibly remain functional in brush-border membrane vesicles following the destruction of RS1 subunits by irradiation. Unfortunately, radiation-inactivation estimates of the molecular size of the glucose cotransporter in oocytes have not, to our knowledge, been reported. The monoexponential decay of glucose transport in irradiated brush-border membrane vesicles (Fig. 2) suggests that such smaller oligomeric structures contribute, at most, very little to the sodium gradient-dependent glucose transport activity. It remains possible however that, because the affinity for glucose is much lower in the absence of RS1 [25], a cotransporter composed of SGLT1 subunits only would not have been detected in the present and previous [21,22] radiation-inactivation studies in which transport was assayed at relatively low substrate concentrations (2–50 μM).

In agreement with the conclusions of previous studies, high-affinity sodium gradient-dependent glucose transport in brush-border membrane vesicles thus probably requires the concerted action of all subunits within a tetrameric protein. Our results strongly suggest, however, that different subunit combinations are involved when glucose transport is carried out in the absence of a sodium gradient.

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