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Characterization of transferrin receptor-dependent Ga_C–Tf–Fe_N transport in human leukemic HL60 cells

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Abstract

Background: Understanding the uptake of Ga_C–Tf–Fe_N by cells will provide key insights into studies on transferrin-mediated drug delivery.
Methods: The mechanism of Ga_C–Tf–Fe_N transporting into and out of HL60 cells has been investigated by comparing transports between Ga_C–Tf–Fe_N and apoTf by means of ^{125}I-labeled transferrin.
Results: An association constant for Ga_C–Tf–Fe_N was 2 times that for apoTf. Ga_C–Tf–Fe_N and apoTf of cell surface-bound displayed similar kinetics during the uptake, but the release rates of internalized Ga_C–Tf–Fe_N and apoTf from cells were different which showed characteristic disparate. The release continued to occur during the incubation of Ga_C–Tf–Fe_N in the presence of nonradioactive apoTf. Neither NaN_3 nor NH_4Cl could completely block internalization of Ga_C–Tf–Fe_N, but they prevented the release of Ga_C–Tf–Fe_N from the cells. Excess cold unlabeled apoTf could overcome the block in the release due to NH_4Cl but not NaN_3. The binding and internalization of Ga_C–Tf–Fe_N could be competitively inhibited by nonradioactive apoTf. It implies that both bind to the same receptor on the membrane and the localization of Ga_C–Tf–Fe_N resembles that of apoTf inside cells. Pretreated cells with pronase abolished the binding of Ga_C–Tf–Fe_N significantly.
Conclusion: On the basis of these findings, we proposed the "transferrin receptor" for the mechanism of Ga_C–Tf–Fe_N transport by HL60 cells.

Keywords: Ga_C–Tf–Fe_N; apoTf; HL60 cells; ^{125}I-labeled transferrin; TfR-mediated

1. Introduction

In vivo studies using ^{67}Ga find that all gallium in blood is present in plasma (with traces in leukocytes) and is tightly bound to transferrin [1,2]. Gallium compounds have been used extensively both in the diagnosis and the treatment of human cancers [3]. Ga^{3+} is probably delivered to tumor cells through the serum protein transferrin [4–6], which closely parallels that of iron [6–8]. The uptake of iron and gallium by cells occurs by TfR-mediated endocytosis of Tf–Fe or Tf–Ga. Inside the cell, the TfR–Tf complex translocates to an acidic endosome where iron/gallium dissociates from Tf and trafficks out of the endosome. The receptor–apoTf (metal-free) complex then recycles back to the cell surface where apoTf is released to the exterior [9–11]. The accumulated evidence suggests that the mechanisms involved in iron and gallium transports to target sites by TfR may be of fundamental importance. Under physiologic conditions in vivo approximately one third of plasma Tf is Fe-saturated, leaving the remainder of the protein free to bind other metals [12]. Thus, the Ga_C–Tf–Fe_N can be formed, but the mechanism involved in cycling of Ga_C–Tf–Fe_N by different cell types still remains unclear.

We studied the uptake of iron/gallium from Ga_C–Tf–Fe_N using the human leukemia cell line HL60, which was established in 1977 from a patient with acute myeloid

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leukaemia. The cells largely resemble promyelocytes but can be induced to differentiate terminally in vitro [13]. The feature has made the HL60 cell line an attractive model for such studies [6,8].

In the current study, the mechanism of cycling of Ga_C–Tf–Fe_N by HL60 cells was investigated by comparing transports between Ga_C–Tf–Fe_N and apoTf by means of ^{125}I-labeled Tf. In addition, in order to better understand the mechanism, we have studied the effects of various factors that influence cycling of Ga_C–Tf–Fe_N on the HL60 cells.

2. Materials and methods

2.1. Materials

Human serum apotransferrin (apoTf) was from Sigma. $Na^{125}I$ (13,539 MBq/ml) was purchased from NEN. HL60 cells were from the Second Hospital of Shanxi Medical University. RPMI 1640, penicillin, streptomycin and fetal bovine serum were from Gibco BRL. Pronase was from Beijing–Xinjing Science, Biology and Technology Co. Chloramine-T, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (Hepes), $Fe(NH_4)_2(SO_4)_2$, $Na_2S_2O_5$, sodium azide (NaN_3), and ammonium chloride (NH_4Cl) were analytical grade.

Apparatus: We used the HH6003 γradioimmunoassay analyzer, Chinese Nuclear Instrument Corporation, a XW-80A cyclonic mixer, Shanghai Fine-Scientific Co., Ltd., Eppendorf centrifuger, Eppendorf AG-2331, Hamburg, Germany, an HP8453 UV-Vis spectrophotometer, Hewlett Packard Corp., and a Nuaire CO_2-incubator.

2.2. Methods

2.2.1. Ga^{3+} stock solutions

Gallium chloride stock solutions were prepared by carefully weighing pieces of pure gallium metal in a 10 ml beaker, adding 0.5 ml of concentrated HCl, covering it, and gently heating it until all the metal dissolved. More HCl was added periodically as needed to maintain vigorous hydrogen evolution at the gallium surface. This solution was then diluted to 25 ml with a final pH of 0.9. The gallium (Ⅲ) concentration was calculated from the initial weight of the metal in a definite volume and was confirmed by atomic absorption spectroscopy.

2.2.2. Radiolabelling of apotransferrin

Purified apoTf was labeled with ^{125}I by the method of Chloramine-T [14]. The solution of apoTf (4 ml, 3.7 mg/ml) and $Na^{125}I$ (8 μl, 13,539 MBq/ml) were mixed in 0.01 mol/l Hepes, pH 7.4. Chloramine-T (25 μl, 4 mg/ml) was added to the mixed solution. After reacting for five min, $Na_2S_2O_5$ (25 μl, 8 mg/ml) was added to the solution and kept the solution reacting for 1 min. The solution was passed through a 1 × 30 cm Sephadex G-50 column at 1.5 ml per min. By monitoring the radioactivity of iodine-125, eluent fractions containing apoTf were collected. The final concentration of ^{125}I–apoTf was determined from the absorbance at 278 nm using an extinction coefficient of 93,000 cm^{-1} l/mol.

2.2.3. Preparation of Ga_C–Tf–Fe_N [15,16]

One equivalent of ferrous ammonium sulfate was added to a ^{125}I–apoTf solution in 0.01 mol/l Hepes, pH 7.4. This kept the solution reacting for 30 min in the air and at room temperature. Then 0.8 equivalent of Ga^{3+} solution was added to the Tf–Fe_N solution and incubated for another 30 min.

2.2.4. Cells and binding assays

HL60 cells were cultured in RPMI 1640 medium with 10 mmol/l Hepes, 100 mmol/l glutamine, and 10% fetal bovine serum (heat inactivated) in 5% CO_2/95% air atmosphere at 37 °C. Cell viability, as judged by the exclusion of 0.5% trypan blue, was always >99%. Cells harvested were washed twice with RPMI containing 1% serum by centrifugation at 1000 rpm for 10 min in a 50 ml tube at room temperature and subsequently suspended to a final concentration of about 10^7 cells/ml with RPMI containing 1% serum.

The cells were incubated in the presence of ^{125}I–apoTf or Ga_C–Tf–Fe_N, and 0.426 ml mixture were added to 1.5 ml Eppendorf centrifuge tube at 0 °C for 15 min. This was centrifuged for 0.5 min at 6000 rpm, and the pellet was resuspended and washed twice with physiological buffer containing 140 mmol/l NaCl, 5 mmol/l KCl, 1 mmol/l $MgCl_2$, 5.5 mmol/l glucose and 10 mmol/l Hepes (pH 7.4) at 0 °C. Cell-associated counts (CPM) were measured with HH6003γradioimmunoassay analyzer. Specific binding was determined as previously described and maximal Tf binding was calculated according to the method of Scatchard [14].

2.2.5. Internalization and release of ^{125}I–apoTf and Ga_C–Tf–Fe_N

The kinetics of internalization and release of both tracers was examined by using a modification of a previously described method [9,14]. For the both internalization experiments, HL60 cells (~10^6 cell/ml) of the same amount of 0.40 or 0.36 ml and Eppendorf centrifuge tubes containing 25 μl Ga_C–Tf–Fe_N (final concentration 1.87 nmol/l) or 12 μl ^{125}I–apoTf (final concentration 0.61 nmol/l) were put into the ice–water bath, respectively. After 10 min, each of the Eppendorf centrifuge tubes was warmed to 37 °C in a water bath to start endocytosis up to required time and then was immediately placed into the ice–water bath to terminate the reaction. Cell-associated counts (CPM_1) were determined by washing the cells twice with physiological buffer at 0 °C. The cells were resuspended with 0.2 ml of physiological buffer. Then 0.2 ml of 0.25 mol/l acetic acid/0.5 mol/l NaCl (pH 2.3) was added into the tube. After 5 s, 0.2 ml of 1 mol/l sodium acetate was added, which returned the pH to 6.0 [9,14]. The cells were centrifuged again. The

above acid washing was repeated. Cell-internalized counts (CPM_2) were performed. For the both release studies [9], cells ($\sim 2\times10^6$) were loaded with ^{125}I–apoTf or Ga_C–Tf–Fe_N (final concentration 0.61 nmol/l) for 5 min at 0 °C, then removed to 37 °C for 30 min. After the 37 °C incubation, the cells were cooled to 0 °C, washed twice with buffer, and cell-associated counts were measured (CPM_3). They were then resuspended in RPMI 1640 medium at 37 °C at the initial cell density. Cell were maintained for 0, 5,10, 25, 45, and 60 min at 37 °C, respectively. The samples were cooled to 0 °C at the required time and the pellets were washed twice with buffer and counted for radioactivity (CPM_4).

2.2.6. Effect of unlabeled apoTf and selective inhibitors on the release of Ga_C–Tf–Fe_N

To determine whether Ga_C–Tf–Fe_N release from HL60 cells is affected by external apoTf, the cells were incubated with Ga_C–Tf–Fe_N (final concentration 1.87 nmol/l) at 37 °C for 30 min. The cells were then chilled and washed thoroughly so as to be free of unbound ligand. Next, the unlabeled apoTf containing various concentrations were added and the mixture were resuspended in RPMI 1640 at 37 °C for 0, 2, 5, 10, 20, and 45 min, respectively. When the time was up, the samples were placed on the ice–water bath and the free ligands were washed and counted.

The release of Ga_C–Tf–Fe_N from the cells was tested for sodium azide (final concentration 1%) [9]. Cells were incubated with Ga_C–Tf–Fe_N (final concentration 1.87 nmol/l) at 37 °C for 20 min. Then NaN_3 was added into the mixture for another 10 min. At this time the mixture was chilled and washed off unbound ligand. The cells were divided into two aliquots, one of which received NaN_3 and 100 μg/ml of cold apoTf and the other received NaN_3 alone. They were then resuspended in media at 37 °C. At the required time the samples were cooled to 0 °C and the process of washing for binding assays was repeated. Control cells (no NaN_3) were likewise rewarmed in the presence or absence of excess unlabeled apoTf.

Since ammonium chloride presumably acts as a lipid-soluble weak base to neutralize acidic cellular compartments, it was used to monitor the effect on the release of Ga_C–Tf–Fe_N from the cells. First, the cells were incubated with 21 mmol/l NH_4Cl at 37 °C for 15 min, then they were chilled. Second, Ga_C–Tf–Fe_N was added to the mixture for 30 min at 37 °C and at this time the cells were cooled and washed to remove unbound Ga_C–Tf–Fe_N. Finally, the cells were again put into 37 °C, either in the absence or presence of 100 μg/ml of unlabeled apoTf. Control cells (no NH_4Cl) were likewise rewarmed in the presence or absence of excess unlabeled apoTf.

2.2.7. Influence of unlabeled apoTf and pronase on Ga_C–Tf–Fe_N uptake by HL60 cells

In the experiments, the cells (1.7×10^6/ml) were incubated with Ga_C–Tf–Fe_N in the presence of unlabeled apoTf of various amounts (0, 0.34, 1.2, 3.5, 10.51, and 31.48/10^{-7}mol/l) to find out whether apoTf has a competitive inhibiting effect on Ga_C–Tf–Fe_N transport into the cells. After 5 min at 0 °C, each of the samples was warmed to 37 °C for 15 min. Then they were immediately placed into the ice–water bath to terminate the reaction.

In certain experiments, the cells (1.14×10^6/ml) were incubated with various amounts of pronase (0, 0.0078, 0.016, 0.031, 0.062, 0.25, 0.50 and 1.0 g/l) for 40 min in RPMI 1640 at 37 °C to disable the function of TfR on the cell surface. The cells were centrifuged for 0.5 min at 6000 rpm and liquid above was discarded. The pellet was resuspended and then incubated with Ga_C–Tf–Fe_N in RPMI 1640 at 0 °C for 20 min. Cells without pretreatment of pronase were treated as the control. Each value of CPM represents the mean of triplicate determinations.

3. Results and discussion

3.1. Binding of ^{125}I–apoTf and Ga_C–Tf–Fe_N to HL60 cells at 0 °C

The binding of transferrin to its receptor on the surface of HL60 cells at 0 °C is extremely rapid. The process is independent on metabolic energy. A comparative method was used to verify different trait of ^{125}I–apoTf and Ga_C–Tf–Fe_N binding to the receptors, the binding parameters (K_A) of the interaction between HL60 cells and ^{125}I–apoTf or Ga_C–Tf–Fe_N were calculated. An approach used by Zhao CG et al. was followed [14] to determine binding parameters and the number of receptor (n) units per cell at 0 °C. We can get the association constants for ^{125}I–apoTf or Ga_C–Tf–Fe_N, respectively, and the average numbers of receptor on HL60 cell at 0 °C. The values are listed in Table 1.

It has been well determined that the binding of apoTf to TfR is feasible with relatively low affinity in contrast to that of the iron status of Tf using different methods [10,17,18]. In the present study, the Scatchard analysis of the 0 °C binding isotherm revealed a linear curve consistent single type of binding site. Such an analysis led to an estimate of $(4.8\pm0.11)\times10^5$ receptors for per cell. The conformational changes are likely to be of functional importance and play a crucial role in the receptors' recognition. The closing conformation is the more suitable conformation to bind the receptor on the surface of K562 cell [14]. Here, the association constant of Ga_C–Tf–Fe_N binding to the receptor

Table 1
The various parameters[a] for the interaction between HL60 cells and ^{125}I–apoTf or Ga_C–Tf–Fe_N, respectively

Sample	$K_A\times10^8$/l/mol	$n\times10^5$	k_{IR} (min^{-1})	k_{RR} (min^{-1})
^{125}I–apoTf	0.97±0.023	4.8±0.11	0.26±0.068	0.27±0.039
Ga_C–Tf–Fe_N	1.8±0.031		0.28±0.070	0.15±0.052

[a] K_A (l/mol)=the binding parameters; n=the number of receptor (n) units per cell; k_{IR} (min^{-1})=the rate constants for the endocytosis; k_{RR} (min^{-1})=the rate constants for the release.

was about two-fold in contrast to that of ^{125}I–apoTf. This indicates that there is relatively weak affinity when the cells were incubated with ^{125}I–apoTf at 0 °C.

3.2. Internalization of ^{125}I–apoTf and Ga_C–Tf–Fe_N into HL60 cells

The amount of Tf taken up by these cells increases significantly when the incubation is performed at 37 °C. Since the transferrin bound to the surface of cells are released by the quick-twice-acid-wash [9,14], the total transferrin binding to HL60 cells were reflected by CPM_1, and the transferrin entered cells are reflected by CPM_2. Then the endocytosis ratio (IR) can be defined as follows: $IR = CPM_2/CPM_1$. The variations of the endocytosis ratio with time are shown in Fig. 1. Warming the mixture of HL60 cells and ligands induces the endocytosis. From Fig. 1, it can be seen that the fluctuation of the endocytosis ratio follows time. Under the experimental condition, the endocytosis ratio would plateau at about 64% for ^{125}I–apoTf and 81% for Ga_C–Tf–Fe_N in 10 min. Curve fitting by single exponent function reveals the rate constants (k_{IR}) for the endocytosis to be about 0.26 ± 0.068 and 0.28 ± 0.070 min^{-1} for ^{125}I–apoTf and Ga_C–Tf–Fe_N, respectively. The parameters are also listed in Table 1. It was easily drawn that both ^{125}I–apoTf and Ga_C–Tf–Fe_N were internalized at similar rates, but their quantities of internalization depended on transferrin concentration. From the results, it could be inferred that upon both ^{125}I–apoTf and Ga_C–Tf–Fe_N binding to the membrane, both were internalized regardless of the conformational change of the protein. In trophoblast cells that are isolated from term human placenta, both apoTf and Fe_2–Tf (^{125}I-labelled and ^{59}Fe-labelled Tfs) were internalized by receptor-mediated endocytosis at similar rates [19]. It was reported that the Tf was internalized by the reticulocytes regardless of the iron

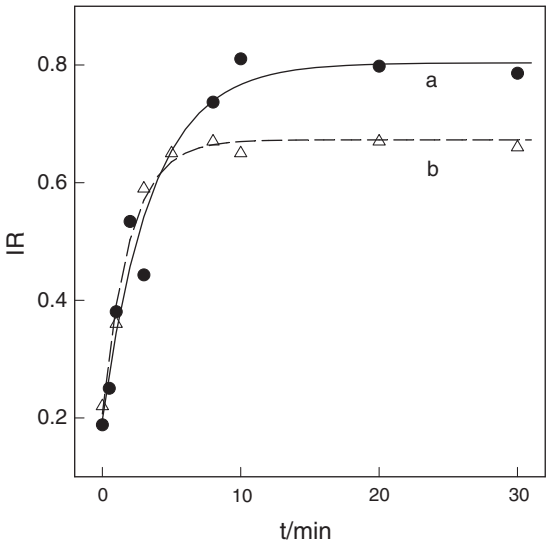

Fig. 1. Change curves of endocytosis ratio with time at 37 °C.

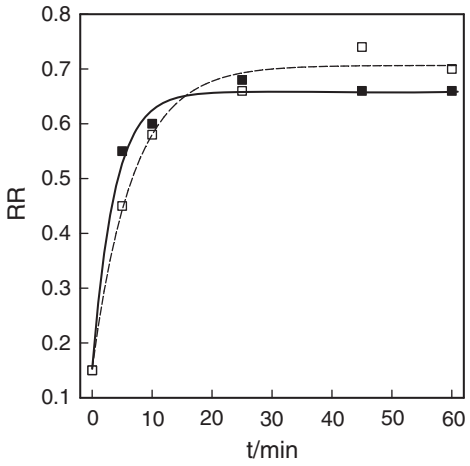

Fig. 2. Change curves of release ratio with time at 37 °C.

content of the protein [20]. All these data support the above possibility.

3.3. The release of cell-bound Tf at 37 °C

We examined the release of cell-bound Tf at 37 °C. The release ratio (*RR*) can be defined as follows: $RR = 1 - CPM_4/CPM_3$. The variations of release ratio with time are shown in Fig. 2. It takes 25 min to plateau at around 65% for ^{125}I–apoTf and Ga_C–Tf–Fe_N. Curve fitting revealed the rate constants (k_{RR}) for the release using a single exponential process. The parameters are listed Table 1 as well. As shown Table 1, after internalization at identical rate both apoTf and Ga_C–Tf–Fe_N were recycled to the cell exterior with Ga_C–Tf–Fe_N having a protracted endocytic cycle. The result implies that metal dissociating from transferrin in acidic endosomes was likely to be the key

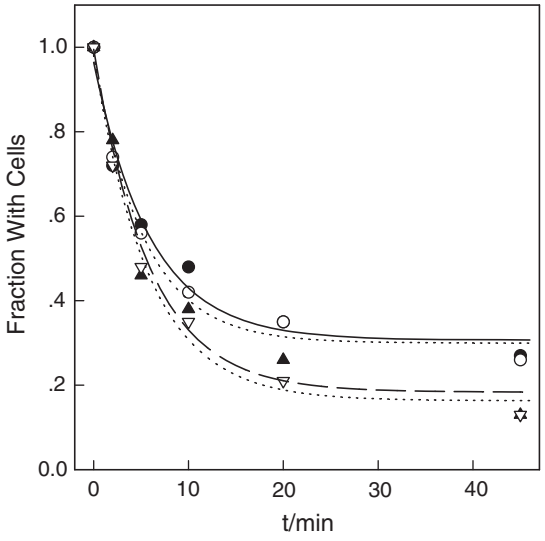

Fig. 3. Effect of unlabled apoTf on the release of Ga_C–Tf–Fe_N from cells at 37 °C. The amount of cold apoTf was none (●), 0.3 μg/ml (○), 30 μg/ml (▲), and 100 μg/ml (▽).

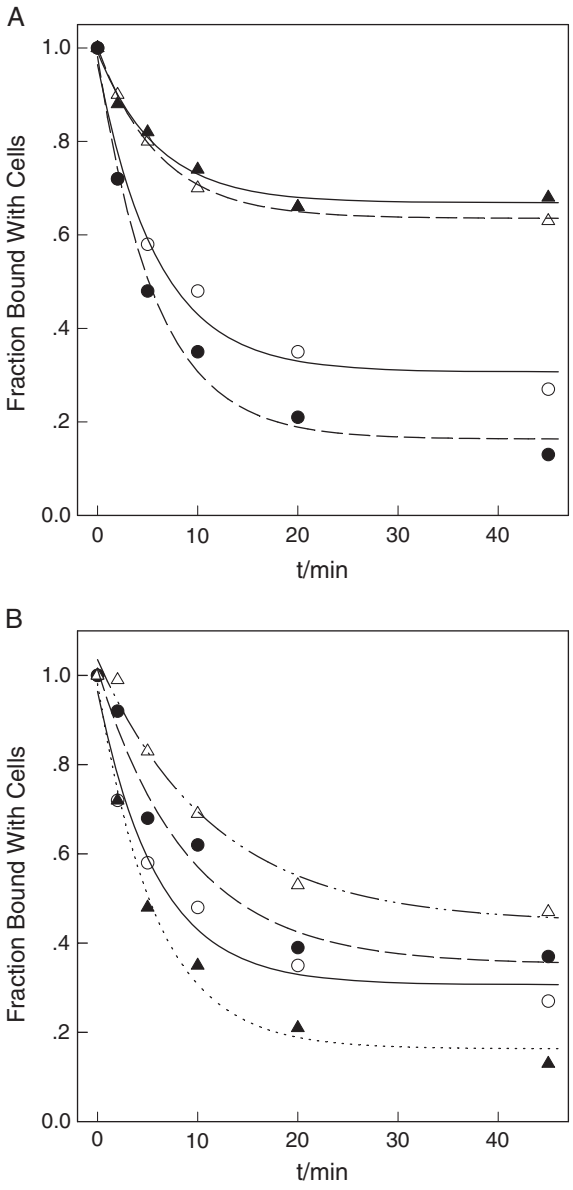

Fig. 4. Effect of drugs on the release of Ga_C–Tf–Fe_N from the cells. (A) Effect of NaN_3 inhibiting the release of Ga_C–Tf–Fe_N by cells at 37 °C, containing no additions (○), 100 μg/ml of cold apoTf (●), 1% NaN_3 (▲), or 1% NaN_3 plus 100 μg/ml of cold apoTf (△). (B) Cells were incubated with 21 mM NH_4Cl at 37 °C for 15 min. Effect on the release of Ga_C–Tf–Fe_N from cells either in the absence (△) or presence (●) of 100 of unlabeled apoTf. Control cells (no NH_4Cl) in the presence (▲) or absence (○) of excess cold apoTf.

step for the circulation. The cycling time from the internalization to release was calculated about $t_{1/2}=5.2\pm0.054$ min for apoTf and $t_{1/2}=7.1\pm0.061$ min for Ga_C–Tf–Fe_N. Subsequently, we found that the addition of increasing amounts of cold unlabeled apoTf led to a progressively greater release of labeled Ga_C–Tf–Fe_N in the medium (Fig. 3). The addition of 0.30 μg/ml apoTf had no effect on the release. When the concentration of apoTf was up to 30 μg/ml, the addition of apoTf led to a progressively greater amount of release for Ga_C–Tf–Fe_N. It elucidated the coupling of receptor occupancy and internalization to the completion of the transferrin cycle. In other words, receptor occupancy would probably work as a driving force for the cycle. If without continued occupancy of the surface receptors, both the release and the cycle would be stopped. Only when transferrin binds to unoccupied surface receptors, the cycle is initiated, leading to the continued release from the "internal" pool [9]. These conclusions are further supported by the results shown in Fig. 3.

3.4. Effect of NaN_3 and NH_4Cl inhibiting the release of Ga_C–Tf–Fe_N by cells

Two types of inhibitors were tested for their effects on the release. The release of Ga_C–Tf–Fe_N from the cells was profoundly affected (Fig. 4). After the cells had taken up Ga_C–Tf–Fe_N at 37 °C, exposure to NaN_3 led to a little release. More strikingly, the addition of a large excess of cold apoTf failed to enhance the release of the Ga_C–Tf–Fe_N (Fig. 4A). NH_4Cl yielded similar results when the

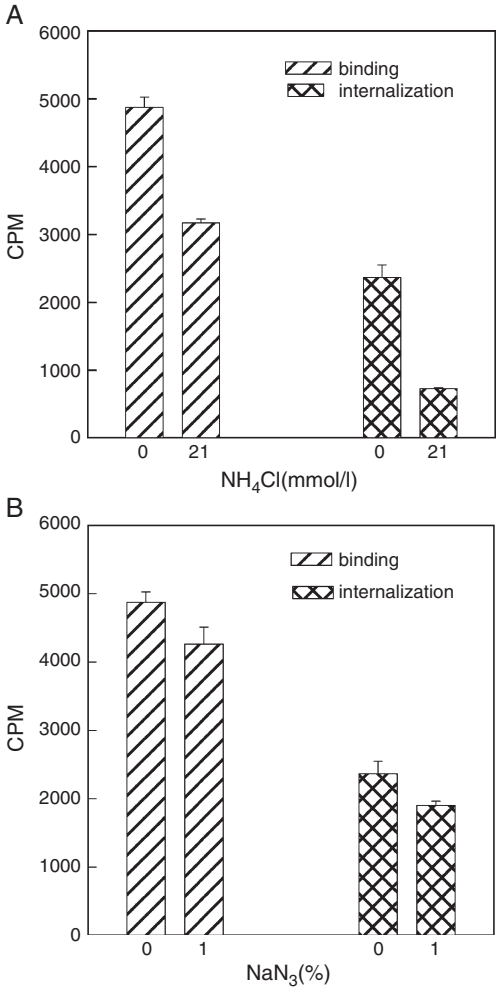

Fig. 5. Effect of NH_4Cl and NaN_3 on the binding and internalization of Ga_C–Tf–Fe_N with cells at 37 °C. (A) NH_4Cl; (B) NaN_3.

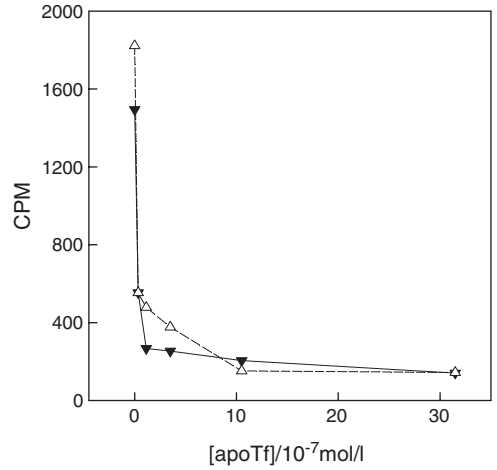

Fig. 6. The binding at 37 °C is specifically inhibited in the presence of unlabeled apoTf. The binding counts of membrane (▼). The binding counts of intracell (△).

release of ligand was monitored. However, in contrast to NaN_3, the addition of excess cold apoTf overcame the NH_4Cl block with the resultant release of the cell-associated ligand, although the rate of release was slowed significantly (Fig. 4B).

It can be predicted under experimental cases where the pH of intracellular compartments is raised when the HL60 cells are treated with NH_4Cl, which raises the pH of acidic compartments including endosomes [9]. The complex of Ga_C–Tf–Fe_N bound to its receptor and was internalized, but the values of CPM on the binding and internalization were evidently decreased compared to that of untreated cells (Fig. 5A). Besides, the release rate and extent of Ga_C–Tf–Fe_N were much slower and fewer than that of untreated cells (Fig. 4B). The findings indicated that Ga_C–Tf–Fe_N uptake by HL60 cells involved initial internalization into acidic receptosomes and NH_4Cl slowed the rate of release (0.086 ± 0.003 min^{-1}). It implied that the detachment of metal and transferrin in acidic endosomes was the rate-limiting step during the release. Moreover, the addition of excess cold apoTf could enhance its release to some extent. The ability to overcome the NH_4Cl block with excess apoTf demonstrates the coupling of receptor occupancy and internalization to the completion of the transferrin cycle.

An ATP-dependent proton pump lowers the pH of the endosome to about 5.5 [21–23]. The acidification of the endosome weakens the association between metal and transferrin. Azide that acts as a metabolic inhibitor can deplete cellular ATP. It could not inhibit the internalization of surface-bound ligand (Fig. 5B), and the values of CPM on the binding and internalization were nearly the same as that of untreated cells. However, it blocked the release of Ga_C–Tf–Fe_N from the cells. Strangely, the addition of excess cold apoTf failed to enhance the release of Ga_C–Tf–Fe_N. The reasons may be as follows: (1) the binding and internalization of Ga_C–Tf–Fe_N to the cells have no use for a metabolic energy; (2) the process of release demands a metabolic energy.

3.5. Effects of unlabeled apoTf and pronase on Ga_C–Tf–Fe_N uptake by HL60 cells

We investigated further the effect of unlabeled apoTf on Ga_C–Tf–Fe_N transport in HL60 cells. The addition of various concentrations of apoTf into the incubation medium from 0 °C for 5 min, to 37 °C for 15 min was found to significantly decrease membrane as well as the values of CPM intracellularly for Ga_C–Tf–Fe_N (Fig. 6). The findings indicated that the addition of apoTf could effectively inhibit Ga_C–Tf–Fe_N binding to membrane and transport into HL60 cells. These results implied the existence of a competitive and special inhibition of apoTf on Ga_C–Tf–Fe_N binding and transport, showing that both bind to the same receptor on the membrane. The result is consistent with the report by Du et al. [17]. Therefore we deduced that both are a receptor-mediated endocytosis in HL60 cells.

Fig. 7 showed that pre-treatment of HL60 cells with 0.03 g/l of pronase leads to a significant decrease in CPM for Ga_C–Tf–Fe_N. The cells pre-treated with different concentrations of pronase suggested that pronase in 0.20 g/l is enough to disable the TfR function on the cell surface. These findings imply a possible role for TfR in Ga_C–Tf–Fe_N transport into the cells.

It has been shown previously that gallium binds to transferrin in the specific Fe^{3+} binding sites with a similar affinity [24]. Transferrin containing gallium binds to its specific receptors on the cell plasma membrane without modification. After gallium enters the cell, it is transferred to cellular ferritin, as is iron itself [25]. Furthermore, within cells, gallium is found mostly as a phosphate salt in lysosomes [25]. This fruitful work explains that in endosomes gallium dissociates from transferrin. Therefore we

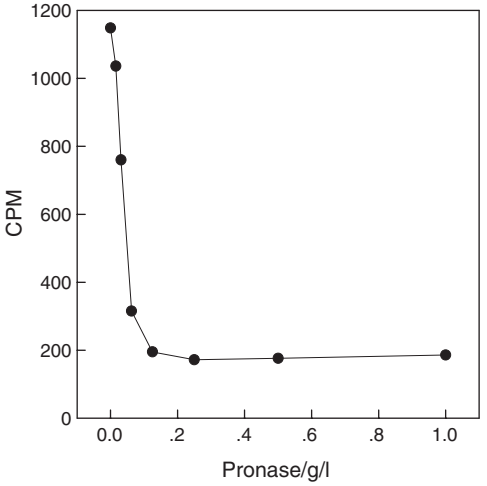

Fig. 7. Effect of pronase treatment on Ga_C–Tf–Fe_N binding by HL60 cells at 0 °C.

inferred that iron/gallium was detached from Ga_C–Tf–Fe_N in acidic endosomes after internalization, and then apoTf was cycled to the cell exterior.

In summary, the results of this research suggest that transferrin receptor plays an important role in the mechanism of Ga_C–Tf–Fe_N passing in and out HL60 cells by comparing Ga_C–Tf–Fe_N and apoTf transports by HL60 cells. Some literatures have reported that certain cells can acquire iron and gallium (as low molecular weight chelates) through a Tf-independent uptake system [26–30], which become apparent when Tf is in short supply or saturated with iron or other metal ions [5,31]. Furthermore, Chitambar CR, et al. suggested that expression of wild-type HFE in cells produces a decrease in ^{67}Ga uptake due to a reduction in available Tf binding sites for ^{67}Ga–Tf on the TfR [32]. Hence the drugs containing Ga may be not completely delivered to the target cells to lead to harm healthy cells. Transferrin has received major attention in the area of drug targeting since the protein is biodegradable, non-toxic, and non-immunogenic [6,33]. More critically, it can achieve site-specific targeting due to the high amounts of its receptor present on the cell surface [34–36]. Therefore our current studies are to specifically deliver Ga_C–Tf–Fe_N to the target cells to control the proliferation of malignant cells. Further work is in progress to define the role of targeting of intracellular processes by gallium. Such information may enhance our understanding of cell metal metabolism and the development of Tf-mediated drug delivery.

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