INHIBITION OF γ-GLUTAMYLTRANSPEPTIDASE BY TREATMENT OF INTACT LYMPHOCYTES WITH PERIODATE

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1. Introduction

The plasma membrane contains some enzymes whose active sites face the external medium rather than the cytoplasm, and these enzymes have been called 'ectoenzymes' [1,2]. The biological function of an ectoenzyme can thus be explored by treatment of intact cells with impermeant reagents which inactivate the enzyme. Determination of the time course of return of the catalytic activity to steady state after irreversible inhibition has considerable potential for providing information on the biosynthesis and turnover of these specific plasma membrane proteins [3]. However, the lack of suitable irreversible inhibitors has limited the use of these methods for the study of plasma membrane proteins.

γ-Glutamyltranspeptidase (EC 2.3.2.2) is a glycoprotein ectoenzyme which is widely distributed in mammalian tissues and which catalyses the transfer of the γ-glutamyl group of glutathione to certain L-amino acids and peptides [4,5]. In addition to its role in glutathione metabolism a number of functions have been postulated for the enzyme including mercapturate biosynthesis, ammoniagenesis and amino acid transport [5]. It is not yet clear whether the enzyme plays a physiological role in any of these latter processes.

Treatment of intact rat lymph node cells with low concentrations of sodium periodate is shown here to lead to complete inhibition of γ-glutamyltranspeptidase. The enzyme is not reactivated by subsequent reaction with sodium borohydride. The periodate treatment has no effect on 2 other glycoprotein ectoenzymes, 5′-nucleotidase and alkaline phosphodiesterase I, or on alkaline phosphatase, leucine-β-napthylamidase, and *N*-acetyl-β-glucosaminidase.

2. Materials and methods

Adult, male rats of the inbred Lewis strain were obtained from Microbiological Assoc. (Bethesda MD). Hanks' balanced salt solution (HBSS) and phosphate-buffered saline (PBS) were from Grand Island Biological Co. (Grand Island NY). *Vibrio cholerae* neuraminidase (EC 3.2.1.18) was from Calbiochem (San Diego CA). *Dactylium dendroides* galactose oxidase (EC 1.1.3.9), obtained from Worthington Biochemical Corp. (Freehold NJ) was purified before use on Sepharose 6B [6] and assayed as in [7]. Sodium periodate was from Matheson, Coleman and Bell (Norwood OH) and sodium borohydride and all enzyme substrates were from Sigma Chemical Co. (St Louis MO).

Cervical and mesenteric lymph nodes of 3 rats were removed, rinsed in HBSS and forced through a stainless steel screen. The resulting cell suspension was filtered through cotton to remove large debris and the cells were washed twice by centrifugation (375 × *g*, 10 min) and resuspension in HBSS.

For enzyme treatment the cells were resuspended in HBSS at 2×10^7/ml. Neuraminidase and galactose oxidase were added to give final concentrations of 15 and 2.5 units/ml, respectively, and the suspension was incubated at 37°C for 30 min. The cells were then centrifuged and resuspended in HBSS.

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For periodate treatment the cells were resuspended at 2×10^7/ml in PBS and an equal volume of a freshly prepared solution of ice-cold 2.4 mM sodium periodate in PBS was added. The mixture was incubated for 15 min on ice, centrifuged and the cells resuspended in HBSS.

Oxidized cells were reacted with sodium borohydride by resuspending them at 2×10^7/ml in PBS and adding an equal volume of freshly prepared $NaBH_4$ in PBS. The suspension was incubated for 30 min on ice and then centrifuged and the cells washed once in HBSS.

The cells were finally resuspended in 0.15 M KCl, the suspension was divided into portions for the enzyme assays, and they were frozen and thawed once to lyse the cells.

The number of cells in the various suspensions was determined with the model ZBI Coulter counter (Coulter Electronics Inc., Hialeah FL).

γ-Glutamyltranspeptidase [8], alkaline phosphodiesterase I [9], alkaline *p*-nitrophenylphosphatase [9] and *N*-acetyl-β-glucosaminidase [10] were determined by the indicated colorimetric procedures. Leucine-β-naphthylamidase was assayed fluorometrically as in [11]. 5′-Nucleotidase was assayed by colorimetric determination of P_i [12] released from AMP [13]. Under the conditions of the assays all activities displayed linearity with respect to both incubation time and the quantity of lymph node cell lysate. Enzyme activities are expressed in units of μmol/min.

3. Results and discussion

Treatment of intact lymphocytes with neuraminidase and galactose oxidase introduces aldehyde functions at the C-6 position of terminal and penultimate galactose (and *N*-acetylgalactosamine) residues on cell surface glycosylated molecules [14–16]. Low concentrations of periodate generate aldehydes at the exocyclic carbons of terminal sialic acid residues of the same or similar molecules [16,17]. The chemical reagent, as opposed to the enzymatic treatment, probably also covalently reacts with other oxidizable functional groups on cell membrane molecules. Treatments of rat lymph node cells with periodate or with neuraminidase plus galactose oxidase are not cytotoxic and, in fact, if the modified cells are cultured they undergo mitogenesis [16].

In table 1 we show the effects of periodate or neuraminidase plus galactose oxidase treatments on the activity of a number of lymphocyte enzymes. Three of these enzymes, γ-glutamyltranspeptidase, 5′-nucleotidase and alkaline phosphodiesterase I are known to be plasma membrane ectoenzymes on the basis of subcellular fractionation [18,19], cytochem-

Table 1
Effects of neuraminidase and galactose oxidase or periodate treatments on rat lymphocyte enzymes

Enzyme	Activity (mU/10^9 cells)		
	Untreated[a]	NGO[b]	Periodate[c]
γ-Glutamyltranspeptidase	131 ± 25	130 ± 24	4 ± 6
5′-Nucleotidase	431 ± 61	372 ± 65	358 ± 32
Alkaline phosphodiesterase I	1765 ± 16	1584 ± 324	1747 ± 348
Alkaline phosphatase	454 ± 93	393 ± 69	461 ± 152
Leucine-β-naphtylamidase	330 ± 48	341 ± 60	329 ± 43
N-Acetyl-β-glucosaminidase	192 ± 45	162 ± 22	140 ± 21

[a] Represents mean ± SD for 3 samples of control cells from same cell preparation: 1 sample was set aside after preparation, 1 was subjected to a mock neuraminidase + galactose oxidase treatment, and 1 was subjected to a mock periodate treatment

[b] All cells were treated with neuraminidase plus galactose oxidase as in the text. They were then divided into 4 portions and treated with 0, 0.1, 1.0 or 10 mM $NaBH_4$; number represents mean ± SD for these 4 samples

[c] Same as in [b] except that cells were initially treated with periodate as described in text

istry [20,21] and enzyme inactivation [20,22–25] studies. All 3 enzymes are sialoglycoproteins. Alkaline phosphatase and leucine-β-naphthylamidase of rat lymph node cells are partly soluble (2/3rds) and partly associated with the plasma membrane (E. H. H., unpublished). It is not known whether these enzymes are ectoenzymes or glycoproteins. *N*-Acetyl-β-glucosaminidase is an intracellular, lysosomal acid hydrolase that is a glycoprotein [26]. Of the 6 enzymes studied, treatment of intact cells with periodate or with neuraminidase plus galactose oxidase had no effect on their activity with one striking exception: periodate treatment completely inhibited the cell surface γ-glutamyltranspeptidase. This inhibition was not reversed by subsequent treatment with up to 10 mM sodium borohydride, conditions under which all of the periodate generated aldehydes on cell surface sialic acid residues are reduced to alcohols [16]. Our observation that treatment of intact cells with neuraminidase plus galactose oxidase has no effect on the activity of γ-glutamyltranspeptidase agrees with observations [27] that treatment of the purified enzyme with neuraminidase alone does not effect the catalytic activity.

The fact that periodate treatment completely and irreversibly inactivates γ-glutamyltranspeptidase while treatment with neuraminidase plus galactose oxidase has no effect immediately raises the question of the chemical mechanism of periodate inactivation. In the absence of detailed structural analyses it is, of course, impossible to explain our results in chemical terms. However, considering the study [16] on cell surface glycoproteins of rat lymph node cells, we can suggest one possibility. When cells are oxidized with periodate and treated with [^{3}H]borohydride, 4 labeled glycoproteins can be seen on subsequent SDS gel electrophoresis which are not labeled if lymph node cells are oxidized by neuraminidase plus galactose oxidase; these are in addition to ~20 components labeled in common after either method of oxidation. These 'periodate specific' glycoproteins may contain 4-O-acylated sialic acids which are resistant to neuraminidase but susceptible to periodate [28,29]. Two such glycoproteins on lymph node cells have M_r-values of ~28 000 and 30 000 which are near that of the small, catalytic subunit of rat γ-glutamyltranspeptidase [21]; one of these is sensitive to papain. Another lymph node cell glycoprotein that can be labeled after either periodate or neuraminidase plus galactose oxidase treatment has app. $M_r \sim 70\,000$ and is sensitive to treatment with papain, properties of the large subunit of the enzyme [21]. Thus, it is possible that inactivation of γ-glutamyltranspeptidase by periodate is due to the presence of periodate-susceptible but neuraminidase-resistant sialic acids on the catalytic subunit of the enzyme.

Regardless of the mechanism of inactivation of γ-glutamyltranspeptidase by periodate, the phenomenon may offer useful approaches to questions concerning the biosynthesis and function of the enzyme. The fact that periodate specifically inactivates γ-glutamyltranspeptidase but not other ectoenzymes suggests that it could be used to study the turnover of γ-glutamyltranspeptidase, as has been done for other cell surface enzymes using more broadly reactive reagents such as the diazonium salt of sulfanilic acid [24,25]. Finally, by completely inhibiting γ-glutamyltranspeptidase in intact cells, periodate may have advantages over other compounds such as the 6-diazo-5-oxo-L-norleucine which will largely but not completely inhibit the enzyme in intact cells [23]. This would be especially important for experiments on proposed transpeptidase functions where it is often difficult to interpret the results when there is 10–20% residual enzyme activity after inhibition.

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