Physicochemical Characteristics of the Glycosaminoglycan–Lysosomal Enzyme Interaction *in vitro*

A MODEL OF CONTROL OF LEUCOCYTIC LYSOSOMAL ACTIVITY

By JOSÉ LUIS AVILA and JACINTO CONVIT

Instituto Nacional de Dermatología, Apartado 4043, Caracas 101, Venezuela

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1. The activities of 30 different lysosomal enzymes were determined *in vitro* in the presence of the sulphated glycosaminoglycans, heparin and chondroitin sulphate, all the enzymes being measured on a density-gradient-purified lysosomal fraction. 2. Each enzyme was studied as a function of the pH of the incubation medium. In general the presence of sulphated glycosaminoglycans induced a strong pH-dependent inhibition of lysosomal enzymes at pH values lower than 5.0, with full activity at higher pH values. However, in the particular case of lysozyme and phospholipase A₂ the heparin-induced inhibition was maintained in the pH range 4.0–7.0. 3. For certain enzymes, such as acid β -glycerophosphatase, α -galactosidase, acid lipase, lysozyme and phospholipase A₂, the pH-dependent behaviour obtained in the presence of heparin was quite different to that obtained with chondroitin sulphate, suggesting the existence of physicochemical characteristic factors playing a role in the intermolecular interaction for each of the sulphated glycosaminoglycans studied. 4. Except in the particular case of peroxidase activity, in all other lysosomal enzymes measured the glycosaminoglycan–enzyme complex formation was a temperature- and time-independent phenomenon. 5. The effects of the ionic strength and pH on this intermolecular interaction reinforce the concept of an electrostatic reversible interaction between anionic groups of the glycosaminoglycans and cationic groups on the enzyme molecule. 6. As leucocytic primary lysosomes have a very acid intragranular pH and large amounts of chondroitin sulphate, we propose that this glycosaminoglycan might act as molecular regulator of leucocytic lysosomal activity, by inhibiting lysosomal enzymes when the intragranular pH is below the pI of lysosomal enzymes. This fact, plus the intravacuolar pH changes described during the phagocytic process, might explain the unresponsiveness of lysosomal enzymes against each other existing in primary lysosomes as well as its full activation at pH values occurring in secondary lysosomes during the phagocytic process.

Polymorphonuclear-leucocyte lysosomes contain more than 20 acid hydrolases (Avila & Convit, 1975), several cationic proteins (Zeya & Spitznagel, 1963) and large amounts of chondroitin 4-sulphate (Olsson & Gardell, 1967; Taniguchi *et al.*, 1974; Murata, 1974).

In a previous paper (Avila & Convit, 1975) we have suggested that these three different lysosomal components interact with each other at the low pH existing inside leucocytic primary lysosomes, and that in the case of acid hydrolases, their interaction with chondroitin 4-sulphate at intragranular pH values below their pI would represent a mechanism of control of lysosomal digestive activity.

However, in that paper the pH-dependence studies of this intermolecular interaction were limited to just eight lysosomal enzymes and no study was made of the effect of time and temperature on the glycosamino-

glycan–lysosomal enzyme complex formation; consequently the present paper has enlarged the pH-dependence studies up to 30 lysosomal activities and has also carefully studied some physicochemical parameters affecting this molecular interaction. Evidence is presented for the fact that all of the 30 lysosomal enzymes studied were inhibited *in vitro* to a different degree by sulphated glycosaminoglycans in a time- and temperature-independent (except for peroxidase) and pH-dependent manner. Evidence was also obtained for the fact that there are strong differences in the physicochemical characteristics of the interaction existing between different sulphated glycosaminoglycans with regard to the same protein.

The above results give additional support to the concept of the chondroitin sulphate–lysosomal enzyme interaction as a regulatory mechanism at the molecular level of leucocytic lysosomal activity.

Experimental

Materials

Substrates for enzyme determinations were the same as used previously (Avila & Convit, 1975). *N*-t-Butoxycarbonyl-L-alanine *p*-nitrophenyl ester, β -methylumbelliferone, heparin (grade I, lot 52C-2160), chondroitin sulphate (grade III, from whale cartilage, 92% chondroitin 4-sulphate and 8% chondroitin 6-sulphate, lot 22C-2100) and deoxyribonucleic acid (type I) were from Sigma Chemical Co., St. Louis, MO, U.S.A. *p*-Nitrophenyl *N*-acetyl-2-acetamido-2-deoxy- α -D-glucopyranoside, 4-methylumbelliferyl elaidate and 4-methylumbelliferyl glycosides were from Koch-Light Laboratories, Colnbrook, Bucks., U.K. Phenyl α -L-iduronide was synthesized by the method of Friedman & Weissmann (1972). Sphingomyelin was prepared as described by De Rooij *et al.* (1975). Heparan sulphate was from Seikagachu Kogyo Co., Tokyo, Japan.

Preparation of leucocyte lysosomal fractions

Polymorphonuclear leucocytes were from freshly drawn human peripheral blood as described previously (Avila & Convit, 1973a). The lysosomal fractions used in these experiments were prepared essentially as described by Avila & Convit (1975).

Enzyme assays

The methods used for enzyme assays were those previously used by Avila & Convit (1975), except for arylsulphatase A, which was assayed with 1 mM-methylumbelliferyl sulphate in a final volume of 0.2 ml for 5 h at 37°C. The reaction was stopped with 2 ml of 100 mM-glycine buffer, pH 10.7. The methylumbelliferone released was determined fluorometrically exactly as described for β -galactosidase (Avila & Convit, 1973b).

Elastase activity was assayed by the method of Visser & Blout (1972), sphingomyelinase by the method of De Rooij *et al.* (1975), α -L-iduronidase and *N*-acetyl- α -glucosaminidase by the method of Liem & Hooghwinkel (1975), α -arabinase by the method of Van Hoof & Hers (1968), β -mannosidase as previously described for α -mannosidase (Avila & Convit, 1973d) and deoxyribonuclease II as described by de Duve *et al.* (1955).

Suitable enzyme and substrate blank assays were performed.

Standard conditions for the study of the influence of sulphated glycosaminoglycan on enzyme activities

In general enzyme assays were done in the pH range 3.6–5.8 by using, under standard conditions, 50 mM-sodium acetate/acetic acid buffer, except for certain enzymes (elastase, ribonuclease, lysozyme and acid lipase), which were assayed with 50 mM-sodium

citrate/sodium phosphate buffer in the pH range 4.4–7.4.

The assay mixture included in certain cases (cathepsin B, cathepsin C, arylsulphatase A, ribonuclease and deoxyribonuclease II) the lowest concentration of activator necessary to obtain adequate enzymic activity; this is because high ionic strength partially blocks the glycosaminoglycan-induced inhibition of leucocytic lysosomal enzymes (Avila & Convit, 1975).

Incubation times were the longest that ensured a linear relationship of time versus activity for each individual enzyme, so as to use the minimum lysosomal protein concentration. However, the glycosaminoglycan/lysosomal protein ratio (w/w) was maintained in all cases at about 2, as this concentration seems sufficient to obtain a strong inhibition of the several lysosomal enzymes studied, as reported previously (Avila & Convit, 1975).

In all experiments, enzyme assays were initiated by adding the substrate to the incubation mixture, which had been preincubated for at least 10 min at 37°C with the enzyme and with the respective glycosaminoglycan under study (test tubes) or with water (control tubes) in the pH range 3.6–7.4, so as to compare, under the same experimental conditions, the enzymic activity obtained in control tubes with that determined in the presence of sulphated glycosaminoglycans.

Isolation of leucocytic glycosaminoglycans

A human polymorphonuclear-leucocyte pellet, obtained as described previously (Avila & Convit, 1973a), was rinsed with acetone and defatted in a diethyl ether/acetone (3:1, v/v) for 4 h at 37°C. The leucocyte preparation was then treated by the method of Taniguchi *et al.* (1974) for the isolation of glycosaminoglycans. Electrophoretic analysis showed two spots: the major spot similar in electrophoretic mobility to chondroitin 4-sulphate, (96% of the total sulphated glycosaminoglycans), the minor spot identical in mobility with heparan sulphate. Similar results have been reported by Taniguchi *et al.* (1974) and by Murata (1974).

Results and Discussion

pH-dependent inhibition of lysosomal enzymes by sulphated glycosaminoglycans

Figs. 1 and 2 compare the pH-activity curves of 17 different lysosomal glycosidases and 13 other different lysosomal enzymes respectively in control conditions as well as in the presence of two different sulphated glycosaminoglycans, namely chondroitin sulphate and heparin.

Optimal pH values in control conditions (absence of sulphated glycosaminoglycans) were in the pH range 3.6–3.9 for α -mannosidase, cathepsin C, α -galactosidase, α -L-iduronidase and β -glucuronidase,

Fig. 1. Influence of a fixed incubation-mixture pH on the glycosaminoglycan-lysosomal glycosidase interaction

Acid glycosidase activities were determined as described in the Experimental section. Incubations were in 50 mM-sodium acetate or 50 mM-citrate/phosphate buffer, according to the enzyme, at fixed pH values in the presence of a glycosaminoglycan/lysosomal protein ratio of about 2. Results are expressed as percentage of the control activity obtained for each particular pH in the presence of exogenous glycosaminoglycans regarding the maximal activity obtained at a given pH in control conditions. ●, Control; ○, chondroitin sulphate; ▲, heparin. Values are means of eight different experiments in the case of control experiments, and of four different experiments in the case of each glycosaminoglycan studied. Variation range for each individual pH value was in all cases 5–8%. (a) α -Mannosidase (EC 3.2.1.24); (b) α -arabinase (EC 3.2.1.-); (c) *N*-acetyl- β -galactosaminidase (EC 3.2.1.53); (d) α -fucosidase (EC 3.2.1.51); (e) β -glucuronidase (EC 3.2.1.31); (f) *N*-acetyl- β -glucosaminidase (EC 3.2.1.30); (g) *N*-acetyl- α -glucosaminidase (EC 3.2.1.50); (h) α -galactosidase (EC 3.2.1.22); (i) α -glucosidase (EC 3.2.1.20); (j) β -glucosidase (EC 3.2.1.21); (k) β -fucosidase (EC 3.2.1.38); (l) β -mannosidase (EC 3.2.1.25); (m) β -galactosidase (EC 3.2.1.23); (n) lysozyme (EC 3.2.1.17); (o) α -1-iduronidase (EC 3.2.1.-); (p) sialidase (EC 3.2.1.18); (q) β -xylosidase (EC 3.2.1.37).

between pH 4.0 and 4.3 for sialidase and cathepsin D and between pH 4.4 and 4.7 for acid β -glycerolphosphatase, α -arabinase, acid lipase, *N*-acetyl- α -glucosaminidase, β -mannosidase and β -galactosidase. *N*-Acetyl- β -galactosaminidase, β -xylosidase and β -fucosidase showed optimal activities between pH 4.8

and 5.1; optimal pH values were in the range 5.2–5.5 for *N*-acetyl- β -glucosaminidase, peroxidase, α -fucosidase, arylsulphatase A, phospholipase A₂, acid deoxyribonuclease, cathepsin A and sphingomyelinase, and finally in the pH range 5.6–6.0 for cathepsin B, α -glucosidase and β -glucosidase. Lysozyme, ribo-

Fig. 2. Influence of a fixed incubation-mixture pH on the glycosaminoglycan-lysosomal enzyme interaction

Lysosomal enzyme activities were determined as described in the Experimental section. Incubations were carried out in 50 mM-sodium acetate or 50 mM-citrate/phosphate buffer, according to the enzyme, at fixed pH values in the presence of a glycosaminoglycan/lysosomal protein ratio of about 2. Results are expressed as percentage of the control activity obtained for each particular pH in the presence of exogenous glycosaminoglycans regarding the maximal activity obtained at a given pH in control conditions. ●, Control; ○, chondroitin sulphate; ▲, heparin. Values are means of eight different experiments in the case of control experiments, and of four different experiments in the case of each glycosaminoglycan studied. Variation range for each individual pH value was in all cases 5–8%. Peroxidase activity was determined after 30 min of preincubation at 37°C; in the case of elastase activity there was no preincubation period. (a) Ribonuclease (EC 3.1.4.22); (b) deoxyribonuclease II (EC 3.1.4.6); (c) arylsulphatase A (EC 3.1.6.1); (d) acid β -glycerophosphatase (EC 3.1.3.2); (e) cathepsin A (EC 3.4.12.2); (f) cathepsin B (EC 3.4.22.1); (g) cathepsin C (EC 3.4.14.1); (h) cathepsin D (EC 3.4.23.5); (i) peroxidase (EC 1.11.1.7); (j) elastase (EC 3.4.21.-); (k) phospholipase A₂ (EC 3.1.1.4); (l) acid lipase (EC 3.1.1.3); (m) sphingomyelinase (EC 3.1.4.3).

nuclease and elastase activities were optimal at pH 6.2, 6.6 and 7.4 respectively.

Some of these control optimal pH values (as those for β -glucuronidase, α -galactosidase, α -fucosidase, α -mannosidase, cathepsin D and acid β -glycerophosphatase) are very different to those previously reported by us for certain leucocytic lysosomal enzymes (Avila & Convit, 1973a,c,d, 1974; Avila *et al.*, 1973), but compare well with the optimal pH values obtained in these experiments in the presence of heparin; this is because in our previous studies homogenates were always prepared with heparin in the

homogenization medium in order to promote cell rupture.

We may then conclude that, in general, in the presence of sulphated glycosaminoglycans, the optimal pH value of several leucocytic enzymes having a control optimal pH value lower than 5.0 is displaced slightly toward more alkaline pH values (Figs. 1 and 2).

Figs. 1 and 2 show that the bulk of the lysosomal enzymes were strongly inhibited by both sulphated glycosaminoglycans in a pH-dependent manner; thus inhibitions were in most of the cases very strong below

pH4.4, with activity returning to control values as the pH increased, reaching control activity at about pH5.0.

Fig. 1 also shows that, except for α -arabinase and β -fucosidase, the inhibition obtained with a commercial preparation of chondroitin sulphate was always smaller than that obtained with a commercial preparation of heparin when both were used at a glycosaminoglycan/protein ratio of about 2.

On the other hand, highly cationic lysosomal enzymes such as lysozyme (Rindler & Braunsteiner, 1973) and phospholipase A₂ (Weiss *et al.*, 1975) showed a quite different behaviour with regard to the other lysosomal enzymes. Thus they were both strongly inhibited by both sulphated glycosaminoglycans at pH values below pH5.0. However, in the presence of heparin they remained inhibited even at about pH7.0, although in the presence of chondroitin sulphate the inhibition obtained at pH4.0 disappeared totally at about pH5.0, suggesting a quite different interaction between both sulphated glycosaminoglycans and highly cationic enzymes. Further, in the case of α -galactosidase, acid lipase and acid β -glycerophosphatase the behaviour of chondroitin sulphate toward these enzymes was again different from that obtained with heparin, suggesting that apart from the higher degree of sulphation per disaccharide unit existing between both glycosaminoglycans, another physicochemical factor(s) present in the heparin molecule must be playing an important role in the glycosaminoglycan-lysosomal enzyme interaction, thus explaining its stronger differential affinity for certain enzymes. Among the lysosomal acid hydrolases of particular interest were the results obtained for acid β -glycerophosphatase and acid lipase, which, under similar assay conditions, were strongly inhibited by heparin, but only slightly by chondroitin sulphate. Table 1 shows the inhibition obtained leucocytic and commercial preparations of chondroitin sulphate towards acid β -glycerophosphatase. Even by working at a glycosaminoglycan/protein ratio as high as 8, commercial chondroitin sulphate induced a weak inhibition of acid β -glycerophosphatase. That it is true only for commercial preparations of chondroitin sulphate is also shown in Table 1, as when assayed with isolated leucocytic chondroitin sulphate, a strong inhibition of acid β -glycerophosphatase was obtained at a chondroitin sulphate/protein ratio as low as 1. As we have previously emphasized the importance of sulphate groups in the glycosaminoglycan-lysosomal enzymes interaction (Avila & Convit, 1975) the different behaviour between both chondroitin sulphate preparations toward acid β -glycerophosphatase could represent a heterogeneity in their degree of sulphation, and in fact chemical determination of sulphate groups revealed 1.0 and 1.4 sulphate groups per disaccharide residue for commercial and leucocytic chondroitin

Table 1. *Effect of different concentrations of leucocytic and commercial preparations of chondroitin sulphate on acid β -glycerophosphatase activity*

In these experiments a constant protein concentration (40 μ g) was preincubated for 15 min in 50 mM-sodium acetate buffer, pH4.0, with several different concentrations of exogenous chondroitin sulphate at 37°C. The incubation period (5 h) was initiated by the addition of the substrate to the incubation mixture. Results are expressed as percentage of the activity obtained with the same preparation treated as described, except that chondroitin sulphate was absent. Results are the mean values of four identically treated enzyme preparations.

Chondroitin sulphate/ lysosomal protein ratio	Activity (% of control)	
	Leucocytic	Commercial
0	100	100
1	65	84
2	42	82
3	40	80
4	39	80
6	39	78
8	39	78

sulphate respectively. The sulphation value for our leucocytic chondroitin 4-sulphate preparation is thus higher than that reported by Olsson & Gardell (1967) and Taniguchi *et al.* (1974); it can perhaps be explained by the higher proportion of over-sulphated chondroitin sulphate obtained in our experiments: 30% against 10% reported by Taniguchi *et al.* (1974).

However, we must stress the fact that our previous results do not discard the possibility of the importance of other physicochemical factors in the acid β -glycerophosphatase-glycosaminoglycan interaction.

At this point it is worthwhile to mention the conclusions of Gelman & Blackwell (1974) on the interaction between glycosaminoglycans and cationic polypeptides, which seems to depend on: (a) the length of the polypeptide side chain; (b) the position of the sulphate groups (a stronger interaction occurs where the sulphate is on the C-6 side chain as in heparin than directly on the galactose ring as in chondroitin 4-sulphate); (c) the degree of sulphation; (d) the position of the carboxyl groups; (e) the glycosidic linkages. Regarding point (c), Stone (1972) has reported differences in the helical conformation of heparin and chondroitin sulphate, these depending on the number of sulphate groups per molecule. However, any further interpretation of the glycosaminoglycan-lysosomal enzyme interaction is difficult, as little is known about the chemical structure of lysosomal enzymes.

Table 2. *Influence of the temperature of the incubation medium on the pH-dependent inhibition of leucocytic lysosomal enzymes by sulphated glycosaminoglycans*

These experiments were initiated by the addition of the corresponding substrate to the incubation mixture, which already contained 50 mM-sodium acetate buffer, pH 4.0, leucocytic proteins and the sulphated glycosaminoglycan under study (glycosaminoglycan/leucocytic protein ratio of about 2). Enzyme-activity assays were run simultaneously at 4° and 37°C under otherwise identical experimental conditions. Incubation periods varied between 1 and 18 h according to the enzyme, but were always the same for a given enzyme. The conditions of the enzyme assays were as described in the Experimental section. Values are the percentages of the control activity and are means of three different experiments, each carried out with a distinct leucocytic preparation. Values in parentheses represent the percentage of the control activity when measured at 37°C.

Enzymes	Activity (% of the control)					
	4°C			37°C		
	Control	Chondroitin sulphate	Heparin	Control	Chondroitin sulphate	Heparin
α -Galactosidase	100 (15)	68	30	100	67	28
β -Galactosidase	100 (12)	22	12	100	21	10
<i>N</i> -Acetyl- β -glucosaminidase	100 (19)	40	33	100	43	35
β -Glucuronidase	100 (16)	18	15	100	22	19
α -Fucosidase	100 (11)	30	26	100	31	20
β -Fucosidase	100 (21)	32	49	100	39	51
α -Mannosidase	100 (18)	65	17	100	65	21
Cathepsin C	100 (25)	51	20	100	53	25
β -Glycerophosphatase	100 (21)	82	55	100	86	43
Cathepsin B	100 (16)	59	50	100	55	46
Peroxidase	100 (28)	84	71	100	36	28
Acid lipase	100 (20)	91	25	100	90	18

Influence of the temperature of the incubation medium on the pH-dependent inhibition of leucocytic lysosomal enzymes by sulphated glycosaminoglycans

Table 2 shows that, except for peroxidase, all of the several lysosomal enzymes studied were inhibited as strongly at 4°C as at 37°C; further, the inhibition profiles obtained at 4° and 37°C in the presence of sulphated glycosaminoglycans were absolutely similar for these enzymes.

On the other hand, owing to the very low enzyme activities obtained at 4°C other lysosomal enzyme activities were not measured.

Influence of the type of ion used to increase the ionic strength of the incubation medium on the interaction sulphated glycosaminoglycan-lysosomal enzymes

It can be seen in Fig. 3 that, independently of the type of ion used, any increase of the ionic strength of the incubation medium was able to block the glycosaminoglycan-induced inhibition of lysosomal enzymes, giving further support to the existence of an intermolecular electrostatic interaction between sulphated glycosaminoglycans and lysosomal enzymes.

Influence of the time of contact at pH 4.0 on the sulphated glycosaminoglycan-lysosomal enzymes complex formation

Table 3 shows that the glycosaminoglycan-lyso-

somal enzymes complex formation is a time-independent phenomenon. It seems thus that at least a few minutes at 37°C are enough for the optimal formation of glycosaminoglycan-lysosomal enzymes complexes since a longer period of incubation at 37°C did not further increase the degree of inhibition already obtained. On the other hand, Tables 2 and 3 show that, of the several lysosomal enzymes studied, only peroxidase showed a temperature- and time-dependent inhibition in the presence of sulphated glycosaminoglycans. Thus it can be seen that the degree of inhibition obtained for peroxidase was absolutely dependent on the temperature and time in which the glycosaminoglycan/lysosomal proteins interaction took place, thus explaining our previous failure (Avila & Convit, 1975) to show any effect of sulphated glycosaminoglycans on peroxidase activity, since this enzyme was previously determined for 3 min at 25°C. However, that this is not a rule is shown in the case of lysozyme and elastase activities, which were both also inhibited at 25°C (after only 3 min of contact with sulphated glycosaminoglycans), although we must stress the fact that the former enzyme is a highly cationic protein and thus perhaps has stronger affinity for anionic glycosaminoglycans than peroxidase or elastase.

Biological significance of this molecular interaction

Regarding the bulk of the lysosomal enzymes, the

Fig. 3. Blocking of the glycosaminoglycan-lysosomal enzyme interaction by changes in the ionic strength of the incubation medium

A highly purified lysosomal fraction was preincubated for 30 min at 37°C in 50 mM-sodium acetate buffer, pH 4.0, with the glycosaminoglycan under study at a glycosaminoglycan/lysosomal protein ratio of about 2. The incubation period was initiated by adding a given volume of a mixture containing enough substrate and the substance under study in order to obtain the optimal substrate concentration described for each enzyme in the Experimental section and a concentration range between 0 and 300 mM for the substance under study. Values are means of three different experiments. Results are expressed as percentage of the activity obtained in the presence of exactly the same concentration of the substance under study, but in the absence of exogenous glycosaminoglycans. (a) β -Galactosidase; (b) β -glucuronidase; (c) α -mannosidase; (d) *N*-acetyl- β -glucosaminidase; (e) α -fucosidase; (f) cathepsin C. Experiments (a), (c) and (e) were in the presence of heparin, and experiments (b), (d) and (f) in the presence of chondroitin sulphate. ●, KCl; ○, NaNO₂; △, KI; □, potassium gluconate; ▲, NaClO₄; ■, KBr; ▼, NaNO₃; ▽, NaF.

Table 3. Influence of the time of contact at pH 4.0 and 37°C on the heparin-lysosomal enzyme interaction

These experiments were initiated by adding the enzyme (a highly purified lysosomal fraction) to the incubation mixture which contained the corresponding substrate and heparin in 50 mM-sodium acetate buffer, pH 4.0, always at a final heparin/lysosomal protein ratio of about 3. Reaction times varied between 2 and 20 min and were carried out in a shaking water bath (150 rev./min). Results are expressed as percentage of the activity obtained under identical incubation periods except that no heparin was present in the incubation mixture. Values are the means of three different experiments with separate lysosomal preparations.

Enzymes	Time of contact at 37°C (min) ...	Activity (% of control)				
		2	5	10	15	20
Peroxidase		87	71	61	54	49
Lysozyme		8	7	7	7	7
β -Glucuronidase		23	19	15	15	15
<i>N</i> -Acetyl- β -glucosaminidase		29	24	21	20	20
Cathepsin C		28	25	24	24	25
α -Mannosidase		26	22	22	20	21
β -Galactosidase		36	30	30	30	30

common condition they must have in order to interact with sulphated glycosaminoglycans is a pI value at or greater than pH 5.0 in order to be positively charged at lower pH values. This seems indeed to be the case for the basic isoenzymes (pI > 5.0) of many acid

hydrolases, at least in other animal tissues (Goldstone & Koenig, 1974; Hultberg *et al.*, 1974; Mersmann *et al.*, 1974; Romeo *et al.*, 1975; Needleman *et al.*, 1975).

From our previous results it can be hypothesized

that, during the phagocytic process, the fusion of the primary lysosomes with the phagosomes would carry protein-rich extracellular medium into secondary lysosomes, which in addition to a dilution of the intralysosomal milieu, would induce changes in the intravacuolar pH towards more alkaline pH values (5.0–7.0), so favouring the activity of the lysosomal neutral proteinases and activating the whole of the lysosomal enzymes (either by ending the glycosaminoglycan-induced inhibition or by reaching the optimal pH of most of the lysosomal enzymes). Later on, the existence of a lysosomal membrane-linked ATP-driven proton pump (Mego, 1975) coupled to the existence of a characteristic lysosomal adenosine triphosphatase (Schneider, 1974) would again decrease the intravacuolar pH (Jensen & Bainton, 1973), so leading to acidification of the intravacuolar milieu and to a new glycosaminoglycan-lysosomal protein interaction, which would finally lead to enzyme inhibition.

As in leucocytic secondary lysosomes the glycosaminoglycans might be partly degraded during intralysosomal digestion, this could be solved by: (a) fusion with another primary lysosome in order to obtain new intact glycosaminoglycans or (b) elimination of the glycosaminoglycan-deficient organelle through exocytosis.

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