ACTION OF POLYETHYLENE GLYCOLS WITH LINEAR STRUCTURE AND DIFFERENT MOLECULAR WEIGHTS ON RAT LIVER LYSOSOMES

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The action of nonpolar detergents – polyethylene glycols with linear structure and differing in molecular weight from 700 to 15,000 – on rat liver lysosomes was studied. Triton X-100 was found to activate acid phosphatase of the total lysosome fraction more than the other polyethylene glycols. The action of polyethylene glycols on activation of acid phosphatase in lysosomal subfractions was inconstant, evidently because of the morphological and biochemical heterogeneity of the lysosomes. On the basis of comparison of these observations with data in the literature the equal resistance of lysosomes to the action of polyethylene-glycol derivatives in vitro and in vivo is postulated.

KEY WORDS: polyethylene glycols; lysosomes; acid phosphatase.

In connection with the role of the lysosomal system of malignant cells in the production of the antitumor effect [4], the action of nonpolar detergents, including polyethylene glycols (PEGs) on the lysosomal membrane [7] and the successful use of PEG in the treatment of metastases of experimental neoplasms [8, 9], it was decided to study the action of PEGs with a linear structure on isolated lysosomes of the rat liver.

EXPERIMENTAL METHOD

The degree of action of PEGs with molecular weights of 700-800 (Triton X-100), 3000, 4000, and 15,000 was judged from changes in the level of "bound" and "free" activity of acid phosphatase (APase, EC 3.1.3.2), a marker enzyme of lysosomes. Total activity was determined by the action of 0.1% Triton X-100, "free" activity without Triton, and "bound" activity as the difference between "total" and "free" activity. Experiments were carried out on the total lysosomal fraction (L_0) isolated from the liver of male albino rats [3]. Since lysosomes are organelles of varied morphological structure, with varied biochemical properties [6], the action of PEGs on subfractions of lysosomes sedimented at 12,000, 18,000, and 27,000 g consecutively, was studied in a special series of experiments. These subfractions were called L_1, L_2, and L_3, respectively. Activity of APase in each experiment was determined in total samples of the liver of three rats. The fractions were incubated in tris-acetate buffer, pH 5, with 0.7 M sucrose and the PEGs to be tested for 15 min.

Triton X-100 (Kebo) and PEG (Schuchardt) were used. The remaining reagents were of Soviet origin.

EXPERIMENTAL RESULTS AND DISCUSSION

The results of the action of PEGs of different molecular weights on L_0 are shown in Fig. 1. Clearly an increase in APase activity was observed after the use of all PEGs in concentrations starting from $9 \cdot 10^{-8}$ M, and the APase activity in PEG in a concentration of $3 \cdot 10^{-7}$ M reached its highest level. A further increase in the concentration of the detergents did not lead to activation of the enzyme. Within the concentration range from $2.8 \cdot 10^{-7}$ to $10 \cdot 10^{-6}$ M the APase activity in the presence of Triton X-100 was almost double that determined in the presence of the other PEGs; PEGs with molecular weights of 3000, 4000, and

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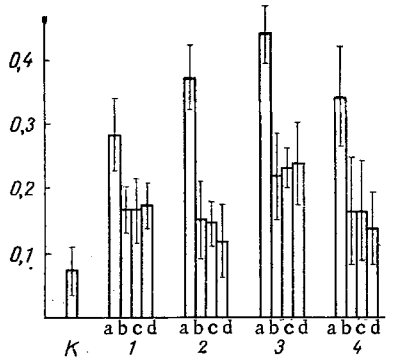

Fig. 1. Activity of APase in total fraction (L_0) of lysosomes in the presence of polyethylene glycols. Ordinate, APase activity (in μmoles p-nitrophenol/mg protein after incubation for 30 min at 25° C and pH 5.0). Incubation medium (1 ml) contained 1 μmole p-nitrophenyl phosphate, 1 μmole magnesium acetate, 45 μmoles tris-acetate buffer, 0.77 mole sucrose; protein content in samples 400-800 μg; K) control; a) Triton X-100; b) PEG with molecular weight 3000; c) PEG with molecular weight 4000; d) PEG with molecular weight 15,000; 1, 2, 3, 4) PEG concentration $9 \cdot 10^{-8}$, $2.8 \cdot 10^{-7}$, $3 \cdot 10^{-7}$, and 10^{-6} M, respectively.

15,000 caused no significant differences in the level of APase activation.

In the next series of experiments the action of 0.1% PEG on the subfractions of lysosomes was studied (Tables 1 and 2). The results show that the levels of free APase activity differed both in the same experiment, in different subfractions, and in the same subfraction but in different experiments, and that 0.1% Triton X-100 as a rule produced greater activation of APase than the other PEGs. No consistent pattern in the change of APase activity under the influence of 0.1% PEG on the lysosomal subfractions could be detected.

The conflicting data obtained by the study of the action of PEGs on the lysosomal subfractions can evidently be explained by the morphological and biochemical heterogeneity of these organelles, with their ability to convert granules of one type into another, the rapid change in their functional state, and the associated change in their biochemical properties [6]. This suggests that in each experiment the lysosomal subfractions contained different quantities of morphological and biochemical variants of the organelles, and this evidently lay at the basis of differences in the resistance of the subfractions of the lysosomes to the action of PEGs. Similar arguments have been put forward previously as regards mitochondrial subfractions [2].

The level of free and bound activity must be influenced by the localization of the enzyme in the lysosomes, for experiments have shown [1, 6] that APase is present in both the matrix of the lysosomes and the membranes. In experiment 1 (Table 1, L_2), for instance, no bound APase activity could be detected, evidently because the enzyme was located in the membrane. In experiment 5 (Table 1, L_1), the APase activity changed sharply in the presence of Triton X-100, possibly indicating the intralysosomal location of the enzyme. Another possibility is that, for example, in experiments 3 and 4 (Table 1, L_1 and L_3) the enzyme was located partly in the matrix and partly in the membrane.

The possibility that PEG may influence the activity of the enzyme must be taken into account when Table 2 is analyzed. In particular, PEGs with molecular weights of 3000, 4000, and 15,000 may perhaps convert the enzyme into a more active conformational state than Triton (compare Table 2, experiment 3, L_3), or may inactivate the enzyme (Table 2, experiment 1, L_1, PEG with molecular weights of 4000 and 15,000). These results point to differences in the resistance of APase in different experiments to the action of PEG, most probably on account of the molecular heterogeneity of the APase. This hypothesis is supported by recent observations showing differences in the sensitivity of isoforms of several enzymes to the action of inhibitors and activators [5].

TABLE 1. Free APase Activity in Subfractions of Lysosomes (free activity expressed in % of total, determined in the presence of 0.1% Triton X-100 and taken as 100%)

Experiment No.	L_1	L_2	L_3
1	39	105	64
2	20	96	100
3	64	54	71
4	71	0	55
5	1	70	0

TABLE 2. APase Activity in Subfractions of Lysosomes in the Presence of 0.1% PEG

Experiment No.	Subfraction of lysosomes	Control (without detergents)	Triton	PEG with molecular weight of		
				3000	4000	15 000
1	L_1	0,050	0,060	0,070	0,030	0,022
	L_2	0,047	0,100	0,070	0,032	0,050
	L_3	0,025	0,044	0,049	0,020	0,022
	Total		0,204	0,189	0,082	0,094
2	L_1	0,010	0,040	0,009	0,010	0,012
	L_2	0,003	0,010	0,009	0,005	0,002
	L_3	0,005	0,008	0,005	0,006	0,006
	Total		0,058	0,023	0,021	0,020
3	L_3	0,010	0,010	0,014	0,022	0,016

Legend. Conditions of determination of APase activity indicated in caption to Fig. 1.

PEGs with molecular weights of 4000, 5000, and 7000, if given to animals in near-equimolar doses, produce changes of the same type (developing at virtually the same time) in the lysosomal apparatus [7]. The present experiments showed that PEGs with molecular weights of 3000, 4000, and 15,000, in equimolar concentrations, had practically equal destructive actions on the total lysosome fraction (L_0). It can accordingly be concluded that the resistance of the total lysosomal fraction to the action of PEGs with molecular weights of 3000 and above in vivo and in vitro is the same.

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