BBA 75649

THE EFFECT OF DIMETHYLSULPHOXIDE ON THE PERMEABILITY OF THE LYSOSOMAL MEMBRANE

DONALD LEE

Division of Low Temperature Biology, Clinical Research Centre Laboratories, National Institute for Medical Research, London N.W.7 (Great Britain)

(Received November 16th, 1970)

SUMMARY

The latency of acid phosphatase in sucrose homogenates of rat liver and *Tetrahymena pyriformis* was found to decrease progressively on exposure to concentrations of dimethylsulphoxide between 5 and 25% (v/v). A progressive decrease in latency was also found in homogenates of rat liver prepared with potassium gluconate although, in this instance, the extent of the decrease was much less than with homogenates prepared in sucrose. With homogenates of rat liver prepared in β-glycerophosphate, dimethylsulphoxide in concentrations up to 10% had little effect on the latency of acid phosphatase, although it decreased on exposure to higher concentrations.

INTRODUCTION

MISCH AND MISCH have presented results[1,2] from which they claim that the permeability of the rat liver lysosomal membrane to β-glycerophosphate is increased, reversibly, in the presence of 25% dimethylsulphoxide. However, these conclusions were based on experiments in which there was marked loss of total acid phosphatase (orthophosphoric monoester phosphohydrolase, EC 3.1.3.2) during the centrifugation and washing procedures and no account was taken of accessible enzyme which was non-specifically adsorbed to the lysosomal membrane[3]. The results presented in this paper have been obtained using Triton X-100 to assess the latency of acid phosphatase and are not subject to the above criticisms.

The latency of acid phosphatase in sucrose homogenates of rat liver and *Tetrahymena pyriformis* decreased progressively after exposure to dimethylsulphoxide in concentrations between 5 and 25% (v/v). Nevertheless, dimethylsulphoxide in concentrations up to 10% had little effect on the latency of acid phosphatase in homogenates of rat liver prepared with β-glycerophosphate as non-penetrating solute. These results suggest that the decrease in latency found with sucrose is the consequence of increased permeability of the lysosomal membrane to sucrose, and there was no indication that the decrease in latency was reversible. The response of rat liver lysosomes in potassium gluconate was similar to that in β-glycerophosphate although the plateau in latency up to 10% dimethylsulphoxide was not present.

MATERIALS AND METHODS

Sucrose (Analar) was from Hopkin and Williams (Chadwell Heath, Essex). Sodium β-glycerophosphate (Grade 1), potassium gluconate (Grade 3) and Triton X-100 were from Sigma (London), while tryptone and yeast extract were supplied by Oxoid (London).

Culture and assay of protozoa

T. pyriformis, strain S, was grown in the dark, at room temperature, in 1% tryptone/0.05% yeast extract, harvested 5–7 days after inoculation and disrupted in the manner described previously[4].

Homogenisation of rat liver

The livers were removed from 150–250-g August strain rats after death by cervical dislocation and homogenised in ice-cold 0.25 M solutions of sucrose, β-glycerophosphate (pH 7.0) or potassium gluconate (pH 7.0).

Exposure of lysosomes to varying concentrations of dimethylsulphoxide

All exposures were conducted at 0°. Samples of homogenates prepared with sucrose, β-glycerophosphate or gluconate were diluted with equal volumes of similar solutions containing various concentrations of dimethylsulphoxide. After 30 min the sucrose homogenates were diluted with 4 vol. of 0.25 M sucrose and assayed for acid phosphatase activity[4] in reaction mixtures which contained 2.5% dimethylsulphoxide. Glycerophosphate homogenates were diluted with only 2.5 vol. of β-glycerophosphate–dimethylsulphoxide so that the non-penetrating solute could be used as substrate (0.05 M) in assay mixtures which thereby had to contain 5% dimethylsulphoxide. The conditions of assay used for gluconate homogenates were similar to those used for β-glycerophate homogenates although, in this instance, substrate had to be added at the same time as the pH was lowered to 4.75.

All assays were conducted as soon as possible after the dimethylsulphoxide had been diluted out and, although assays on homogenates of rat liver were conducted at 30°, those on homogenates of *T. pyriformis* were conducted at 0° because of instability of the lysosomes at temperatures greater than 10° (ref. 5). For determination of total activities Triton X-100 was present in assay mixtures at a concentration of 0.05% and latency was defined as the percentage of the total activity unmasked by the presence of Triton X-100.

RESULTS

Samples of homogenates which had not been exposed to dimethylsulphoxide were assayed for acid phosphatase activity in the presence and absence of appropriate concentrations of this substance in order to assess its effect on this part of the procedure. In the absence of dimethylsulphoxide the mean latency values obtained were 83.7 ± 0.5, 83.7 ± 4.3 and 78.6 ± 0.3% for rat liver homogenates prepared with sucrose, β-glycerophosphate and potassium gluconate respectively (± S.E.; $n = 4$). In the presence of dimethylsulphoxide these values fell to 81.9 ± 1.3.

81.2 ± 4.4 and 77.4 ± 1.4%, respectively, but they are not significantly different from those obtained in the absence of dimethylsulphoxide.

The mean of the latency obtained with homogenates of *T. pyriformis* in sucrose was 59.0 ± 2.1% and, in the presence of dimethylsulphoxide, this fell to 56.6 ± 2.7% although, once again, this value was not significantly different from that found in the absence of dimethylsulphoxide.

Decreasing latencies of acid phosphatase were found when homogenates of rat liver in sucrose were exposed to increasing concentrations of dimethylsulphoxide prior to enzyme assay (Fig. 1). The response with homogenates in β-glycerophosphate was quite different and a noticeable decrease in latency did not occur until exposure to dimethylsulphoxide in concentrations greater than 10%; even after exposure to 20% dimethylsulphoxide, 70% of the original latency was maintained although the corresponding figure for homogenates in sucrose was only 38%.

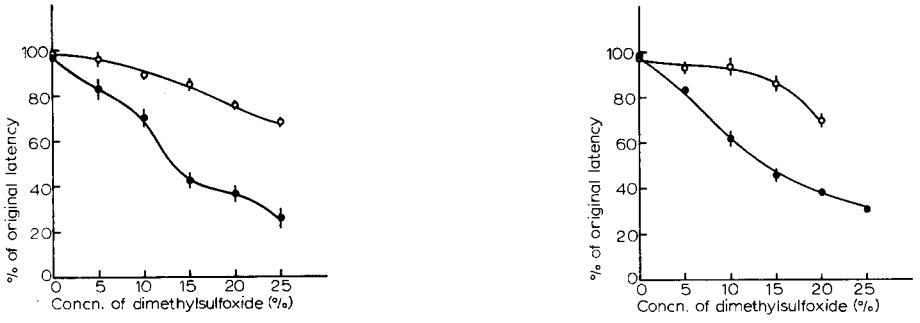

Fig. 1. Percentage of the latency of acid phosphatase remaining after exposure of homogenates of rat liver to different concentrations of dimethylsulphoxide. ●, homogenates prepared with 0.25 M sucrose; ○, homogenates prepared with 0.25 M β-glycerophosphate. In both figures, points represent the mean of four determinations and the vertical lines represent the standard errors although, in some cases, these were too small to be shown.

Fig. 2. Percentage of the latency of acid phosphatase remaining after exposure of homogenates to different concentrations of dimethylsulphoxide. ●, homogenates of *T. pyriformis* prepared with 0.25 M sucrose; ○, homogenates of rat liver prepared with 0.25 M potassium gluconate.

The latency of acid phosphatase in homogenates of rat liver prepared with potassium gluconate also decreased on exposure to increasing concentrations of dimethylsulphoxide (Fig. 2). Its behaviour on these occasions was more like that found with homogenates prepared in β-glycerophosphate than in sucrose, however, the plateau in latency found with β-glycerophosphate was not present.

Fig. 2 also shows that the response with homogenates of *T. pyriformis* in sucrose was similar to that found with homogenates of rat liver in spite of the differences in properties of the lysosomes from the two sources[5,6].

DISCUSSION

Fig. 1 shows clearly that the latency of acid phosphatase in sucrose homogenates of rat liver decreased progressively after exposure for 30 min to increasing concentrations of dimethylsulphoxide. Only 30% of the original latency remained

after exposure to 25% dimethylsulphoxide and, although this is much lower than the values obtained by MISCH AND MISCH[1,2], the difference is most probably explained by a longer time of exposure to dimethylsulphoxide in this work and by adsorption of accessible enzyme to the membranes of the particulate fraction spun down by these authors. The fact that dimethylsulphoxide in concentrations up to 10% had little effect on the latency of acid phosphatase when β-glycerophosphate was used as non-penetrating solute suggests that the decrease in latency with sucrose homogenates is due to penetration of sucrose through the lysosomal membrane in the presence of dimethylsulphoxide. The decreases in latency would therefore result from a permanent change in the properties of the lysosomes, *i.e.* total disruption or sufficient distension of the membrane to allow access of substrate to the enclosed enzyme. The decreases found in the presence of gluconate and β-glycerophosphate are much less than those with sucrose and it seems unlikely that the large decreases found with the disaccharide could result from transient osmotic pressure gradients arising from dilution of dimethylsulphoxide.

The isolation of lysosomes *in vitro* requires the presence of a non-penetrating solute to provide osmotic protection and, although suggestions have been made that lysosomes do not behave as simple osmometers[7], it seems likely that non-penetrating solutes will provide osmotic protection for lysosomes within a cell. The question naturally arises as to which substances could provide such protection.

Obviously, uncharged molecules with dimensions similar to, or greater than, those of sucrose could provide the necessary protection in rat liver cells[8], but it appears doubtful whether such molecules could occur in the cytosol in sufficient concentration. The lactate anion would probably provide little protection[8,9], although it seems possible that it could be provided collectively by phosphate esters and carboxylate anions larger than lactate. This concept is also attractive since any decrease in intracellular pH would suppress ionisation of acidic groups and thereby increase their permeability to the lysosomal membrane.

It is difficult to assess the contribution of carboxylate anions to the osmotic protection of lysosomes but the swelling and enlargement of Golgi vesicles, acid phosphatase-containing cytosomes and lysosomes in rat liver tissue during O_2 deprivation[10–13] could indicate a correlation with the cellular content of phosphate esters. In addition, CROKER *et al.*[14] have studied cell necrosis in the toad bladder and have found that swelling of Golgi vesicles is associated with a pronounced fall in tissue levels of ATP.

A dramatic reduction in the level of hepatic ATP can be induced by injection of ethionine[15] or fructose[16] and, while the former also causes an increase in the size of liver cytosomes[17], the latter does not[18]. These observations apparently weigh against osmotic protection of lysosomes by phosphate esters but the position can be rationalised when one considers that administration of fructose to the rat results in accumulation of phosphate esters in the liver[16,19] while administration of ethionine probably results in the accumulation of inorganic phosphates[20].

It must be admitted that segregation of plasmatic material within the vacuoles of rat liver has also been observed in response to hypoxia[13] and, while a decrease in the intracellular content of phosphate esters may not be the only reason for the swelling of the vacuolar system during O_2 deprivation, it seems to merit consideration as one of the major causes.

ACKNOWLEDGMENTS

It is a pleasure to thank Miss Jacqueline Osborne for her excellent technical assistance and Dr Audrey U. Smith of the Medical Research Council's External Staff for her valuable criticism of the manuscript.

REFERENCES

1 D. W. MISCH AND M. S. MISCH, *Proc. Natl. Acad. Sci. U.S.*, 58 (1967) 2462.
2 D. W. MISCH AND M. S. MISCH, *Nature*, 221 (1969) 862.
3 J. BERTHET, L. BERTHET, F. APPELMANS AND C. DE DUVE, *Biochem. J.*, 50 (1951) 182.
4 D. LEE, *J. Cell. Physiol.*, 76 (1970) 17.
5 D. LEE, *J. Protozool.*, 18 (1971) 173.
6 M. MÜLLER, P. BAUDHUIN AND C. DE DUVE, *J. Cell. Physiol.*, 68 (1966) 165.
7 H. KOENIG, in J. T. DINGLE AND G. B. FELL, *Lysosomes in Biology and Pathology*, Vol. 2, North Holland, Amsterdam, 1969, p. 111.
8 J. B. LLOYD, *Biochem. J.*, 115 (1969) 703.
9 D. LEE, *Biochim. Biophys. Acta*, 211 (1970) 550.
10 B. F. TRUMP, P. J. GOLDBLATT AND R. E. STOWELL, *Lab. Invest.*, 11 (1962) 986.
11 M. BASSI AND A. BERNELLI-ZAZZERA, *Exptl. Mol. Pathol.*, 3 (1964) 332.
12 J. F. R. KERR, *J. Pathol. Bacteriol*, 90 (1965) 419.
13 F. M. BACCINO, *Brit. J. Exptl. Pathol.*, 50 (1969) 150.
14 B. P. CROKER, A. J. SALADINO AND B. F. TRUMP, *Am. J. Pathol.*, 59 (1970) 247.
15 K. H. SHULL, *J. Biol. Chem.*, 237 (1962) PC1734.
16 P. H. MÄENPÄÄ, K. O. RAIVIO AND M. P. KEKOMÄKI, *Science*, 161 (1968) 1253.
17 P. J. GOLDBLATT AND G. M. WILLIAMS, *Am. J. Pathol.*, 57 (1969) 253.
18 P. J. GOLDBLATT, H. P. WITSCHI, M. A. FRIEDMAN, R. J. SULLIVAN AND H. K. SHULL, Unpublished results quoted in ref. 17.
19 E. PITKÄNEN AND J. PERHEENTUPA, *Ann. Paediat. Fenn.*, 8 (1962) 245.
20 J. A. STEKOL, *Advan. Enzymol.*, 25 (1963) 369.