J. Rubb. Res. Inst Malaysia, **24(4)** 233-236 (1976)

Lysozymes: Major Components of the Sedimentable Phase of Hevea brasiliensis Latex

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Two major basic proteins isolated from the sedimentable phase ('bottom fraction') of Hevea *latex have been found to have lysozyme activity. Their amino acid compositions show a close homology with lysozyme from fig* (Ficus) *latex. A novel zymographic procedure for lysozyme is described.*

Hevea brasiliensis latex is cytoplasmic and the sediment ('bottom fraction') obtained by centrifuging it consists largely of the lutoid particles, which have been shown to be lysosomes (PUJARNISCLE, 1968). They can also be considered as microvacuoles (WIERSUM, 1957; RIBAILLIER *et al.*, 1971) in agreement with Matile's proposal (MATILE, 1969) that vacuoles and lysosomes are equivalent in plant cells.

Hevea latex has long been known to have lysozyme (EC 3.2.1.17) activity (MEYER, 1948) which is concentrated in the bottom fraction (ARCHER *et al.*, 1969). Since this observation was made with washed bottom fraction, it was concluded (ARCHER *et al.*, 1969) that lysozyme activity is probably localised in the lutoid particles, *i.e.* in the lysosomes of the latex. Lysozymes from some (perhaps most) other sources are located in lysosomes (BARRETT, 1969); in rat kidney the enzyme accounts for 6% – 10% of the total lysosomal protein (BARRETT, 1969); lysozyme is also a major protein in some latices other than that of *Hevea, e.g.* in papaya *(Carica papaya)* (SMITH *et al.*, 1955). Lysozymes from animal and plant sources are basic (cationic) proteins (BARRETT, 1969; SMITH *et al.*, 1955; GLAZER *et al.*, 1969; JOLLES, 1960).

These facts suggested that one or more of the basic proteins already known to be present in bottom fraction (ARCHER *et al.*, 1969; ARCHER AND MCMULLEN, 1961; MOIR AND TATA, 1960; KARUNAKARAN *et al.*, 1961; TATA AND YIP, 1968; SOUTHORN AND EDWIN, 1968; TATA AND EDWIN, 1970; ARCHER, 1976) might be lysozymes and this has now proved correct.

Two major basic proteins have been isolated in crystalline form from freeze-dried bottom fraction and named 'hevamine fraction A' and 'hevamine fraction B'; they are almost indistinguishable in amino acid composition but can be separated by gel electrophoresis (ARCHER, 1976). We have found that the amino acid composition of the hevamines is remarkably similar to that of lysozyme isolated from fig latex (GLAZER *et al.*, 1969) even though the two genera of plants concerned belong to different families: *Hevea* in Euphorbiaceae and *Ficus* in Moraceae. Lysozyme from papaya latex has a similar amino acid composition (GLAZER *et al.*, 1969), but the homology is not so close *(Table 1)*.

We have also found that hevamines A and B have lysozyme activity in the standard assay at pH 6.2 using *Micrococcus lysodeikticus* cells as substrate (SHUGAR, 1952); hevamine A

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TABLE 1. AMINO ACID COMPOSITIONS OF HEVAMINES A AND B, FIG LYSOZYME AND PAPAYA LYSOZYME

Amino acid	Residues per 100 g of protein			
	Hevamine A (11)	Hevamine B (11)	Lysozyme (fig) (9)	Lysozyme (papaya) (9)
Ala	5.2	5.1	6.80	5.39
Arg	3.4	4.0	3.23	8.19
Asp	13.9	13.7	12.60	10.22
Cys/2	2.0	2.0	2.20	3.50
Glu	6.3	5.9	5.75	5.70
Gly	6.5	6.2	6.45	5.98
His	0.7	0.8	1.37	1.44
Ile	6.2	6.0	7.67	5.06
Leu	8.9	8.4	8.88	5.43
Lys	5.4	5.7	4.80	5.10
Met	1.2	1.1	1.37	2.06
Phe	4.6	4.5	3.37	6.47
Pro	5.5	5.5	4.61	7.11
Ser	7.2	7.2	5.78	5.33
Thr	4.3	4.4	4.73	5.36
Trp	3.0	2.4	5.51	5.46
Tyr	9.4	9.0	9.44	8.50
Val	3.5	3.5	4.00	2.71
Molecular weight	ca 25 000	ca 25 000	ca 29 000	ca 25 000

had about 2200 units per milligram and hevamine B 3000 units per milligram, compared with 22 000 units per milligram for a commercial sample of egg-white lysozyme (Sigma Chemical Company) assayed under the same conditions. These figures for the activity of the hevamines are not maximal, however, since we have not yet established optimum assay conditions for them but have some evidence that their pH optima are around 4.0 to 4.5, not 6.2. Also, the hevamine samples were several years old.

The samples also showed lysozyme activity by a new zymographic technique developed during the present work. A sheet of polyacrylamide gel containing dispersed cells (2 mg per millilitre) of *M. lysodeikticus* is superimposed on one slice of a starch gel electropherogram (SMITHIES, 1955; TATA AND MOIR, 1964) cut in the usual way (SMITHIES, 1955) after the electrophoretic run. After 2 to 3 h at room temperature, clear zones develop in the polyacrylamide immediately above the zones of lysozyme in the starch. When the acrylamide gel is photographed on a black background the lysozyme bands appear darker than the rest of the gel, which is white and semi-

opaque. By direct staining of the complementary slice of the starch gel with naphthalene black, the correspondence between a protein band and its lysozyme activity can be seen *(Figure 1)*.

The homology of amino acid composition with fig lysozyme, the activity in conventional lysozyme assays and the data presented in *Figure 1* indicate that hevamines A and B are two isozymes of *Hevea* lysozyme. Although it had one main component, the hevamine A sample was slightly heterogeneous; this may have been due to deterioration or polymerisation (ARCHER, 1976) in storage but it is also possible that the main 'impurity' is a third isozyme, less cationic than hevamine A or B *(Figure 1)*. This will require further investigation.

Since fig and papaya lysozymes have marked chitinase (EC 3.2.1.14) activity (GLAZER *et al.*, 1969; HOWARD AND GLAZER, 1967; HOWARD AND GLAZER, 1969) it seemed likely that the hevamines accounted, at least in part, for the chitinase already reported briefly as present in the bottom fraction of *Hevea* latex (RUBBER RESEARCH INSTITUTE OF MALAYA, 1966). This is also currently under investigation; preliminary results suggest that the hevamines do have chitinase activity.

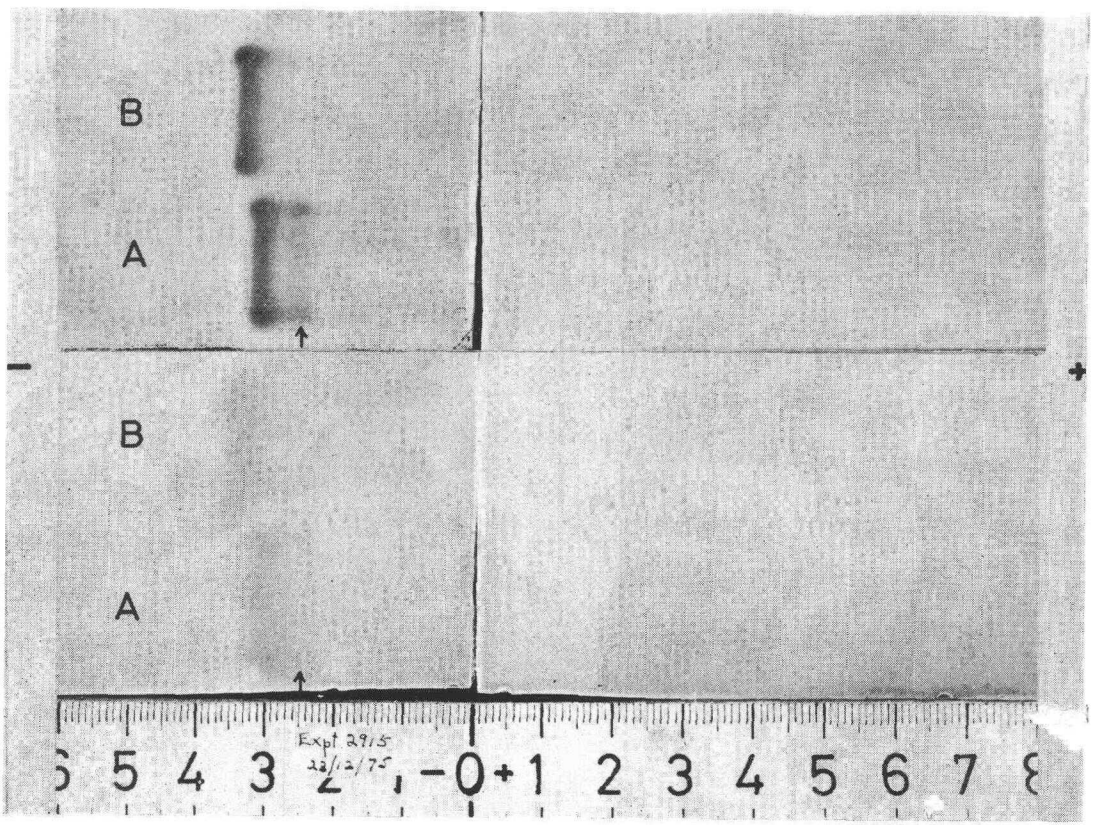

Figure 1. Lysozyme activity of hevamines. The upper photograph shows hevamines A and B stained with naphthalene black on a starch gel electropherogram. The lower photograph shows the corresponding zymogram prepared in a sheet of polyacrylamide as described in the text. Both hevamines show lysozyme activity; the 'impurity' in hevamine A (arrows) also appears to be active.

The basic proteins in the bottom fraction of *Hevea* latex are of particular interest because of their supposed involvement in the physiology of latex flow (SOUTHORN AND EDWIN, 1968; SOUTHORN AND YIP, 1968; SOUTHORN, 1969). It is not yet known whether their lysozyme activity is important in this connection.

ACKNOWLEDGEMENT

We thank Encik G. F. J. Moir and Professor Ph. Matile for their advice and interest in this work and Encik-Encik S. Sivanayagam, Lee Yat Hoong, K. Vivayganathan, M. Gopalsami and N. Sedambram for their technical assistance.

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April 1976

REFERENCES

ARCHER, B.L. (1976) Hevamine: a crystalline basic protein from *Hevea brasiliensis* latex. *Phytochemistry*, **15**, 297.

ARCHER, B.L., AUDLEY, B. G., MCSWEENEY, G.P. AND TAN, C.H. (1969) Studies on composition of latex serum and 'bottom fraction' particles. *J. Rubb. Res. Inst. Malaya*, **21(4)**, 560.

ARCHER, B.L. AND MCMULLEN, A.I. (1961) Some recent studies of the non-rubber constituents of natural rubber latex. *Proc. nat. Rubb. Res. Conf. Kuala Lumpur 1960*, 787.

BARRETT, A.J. (1969) *Lysosomes in Biology and Pathology (Dingle, J.T. and Fell, H.B. ed.)*, vol. 2, p. 245. Amsterdam: North-Holland.

GLAZER, A.N., BAREL, A.O., HOWARD, J.B. AND BROWN, D.M. (1969) Isolation and characterization of fig lysozyme. *J. biol. Chem.*, **244**, 3583.

HOWARD, J.B. AND GLAZER, A.N. (1967) Studies of the physicochemical and enzymatic properties of papaya lysozyme. *J. biol. Chem.*, **242**, 5715.

HOWARD, J.B. AND GLAZER, A.N. (1969) Papaya lysozyme. Terminal sequence and enzymatic properties. *J. biol. Chem.*, **244**, 1399.

JOLLES, P. (1960) *The Enzymes (Boyer, P.D., Lardy, H. and Myrbeck, K. ed.)*, vol. 4A p. 431. London, New York: Academic Press.

KARUNAKARAN, A., MOIR, G.F.J. AND TATA, S.J. (1961) The proteins of *Hevea* latex: ion exchange chromatography and starch gel electrophoresis. *Proc. nat. Rubb. Res, Conf. Kuala Lumpur 1960*. 798.

MATILE, PH. (1969) Plant lysosomes. *Lysosomes in Biology and Pathology (Dingle, J.T. and Fell, H.B. ed.)*, vol. 1, p. 406. Amsterdam: North-Holland.

MEYER, K. (1948) *Modern Trends in Ophthalmology (Sorsby, A. ed.)*, vol. 2, p. 71. London: Butterworth.

MOIR, G.F.J. AND TATA, S.J. (1960) The proteins of *Hevea brasiliensis* latex. Part 3. The soluble proteins of 'bottom fraction'. *J. Rubb. Res. Inst. Malaya*, **16(4)**, 155.

PUJARNISCLE, S. (1968) Caractére lysosomal des lutoides du latex d'*Hevea brasiliensis* Mull. Arg. *Physiol. Vég.*, **6**, 27.

RIBAILLIER, D., JACOB, J-L AND D'AUZAC, J. (1971) Sur certains caractéres vacuolaires des lutoides du latex d'*Hevea brasiliensis* Mull. Arg. *Physiol. Vég.*, **9**, 423.

RUBBER RESEARCH INSTITUTE OF MALAYA (1966) *Rep. Rubb. Res. Inst. Malaya 1965*, 68.

SHUGAR, D. (1952) The measurement of lysozyme activity and the ultraviolet inactivation of the enzyme. *Biochem. Biophys Acta*, **8**, 302.

SMITH, E.L., KIMMEL, J.R., BROWN, D.M. AND THOMPSON, E.O.P. (1955) Isolation and properties of a crystalline mercury derivative of a lysozyme from papaya latex. *J. biol. Chem.*, **215**, 67.

SMITHIES, O. (1955) Zone electrophoresis in starch gels: group variations in the serum proteins of normal human adults. *Biochem. J.*, **61**, 629.

SOUTHORN, W.A. (1969) Physiology of *Hevea* (latex flow). *J. Rubb. Res. Inst. Malaya*, **21(4)**, 494.

SOUTHORN, W.A. AND EDWIN, E.E. (1968) Latex flow studies II. Influence of lutoids on the stability and flow of *Hevea* latex. *J. Rubb. Res. Inst. Malaya*, **20(4)**, 187.

SOUTHORN, W.A. AND YIP, E. (1968) Latex flow studies III. Electrostatic considerations in the colloidal stability of fresh *Hevea* latex. *J. Rubb. Res. Inst. Malaya*, **20(4)**, 201.

TATA, S.J. AND EDWIN, E.E. (1970) *Hevea* latex enzymes detected by zymogram technique after starch gel electrophoresis. *J. Rubb. Res. Inst. Malaya*, **23(1)**, 1.

TATA, S.J. AND MOIR, G.F.J. (1964) The proteins of *Hevea brasiliensis* latex. Part 5. Starch gel electrophoresis of C serum proteins. *J. Rubb. Res. Inst. Malaya*, **18(3)**, 97.

TATA, S.J. AND YIP, E. (1968) A protein fraction from B-serum with strong destabilizing activity on latex. *Res. Archs Rubb. Res. Inst. Malaya Docum. 59*.

WIERSUM, L.K. (1947) Enkele latexproblemen. *Vakbl. Biol.*, **37**, 17.