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# BIOCHEMICAL PROPERTIES OF LATICES FROM THE EUPHORBIACEAE*

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**Key Word Index**—*Euphorbia*; Euphorbiaceae; latex, protease, esterase, phosphatase, *N*-acetyl-β-glucosaminidase, mannosidase, galactosidase, amylase.

**Abstract**—Latex sera from 18 *Euphorbia* species were surveyed for protein, carbohydrate and total solid contents. Protease, esterase, alkaline and acid phosphatase, *N*-acetyl-β-glucosaminidase, α-mannosidase, α- and β-galactosidase, α- and β-glucosidase, α-amylase, lysozyme, leucine amino peptidase and cellulase activities were also measured. The effects of nine protease inhibitors were assayed as were haemagglutinating (lectin) contents. Two-dimensional (isoelectric focusing and SDS) PAGE maps of the sera were made. The results obtained show that the latices have widely varying biochemical properties.

## INTRODUCTION

Latex is a milky fluid composed of a liquid serum which holds, either in solution or suspension, a mixture of substances. It is contained in specialized cells called lacticifers. These are internal secretory structures which are either simple, non-articulated single cells or articulated structures of a compound type. Latices may contain a variety of cellular components, among them nuclei, mitochondria, ribosome-like particles and lutoids (lysosome analogues) as well as nucleic acids. Apart from the agglomerative low-density materials which are common in latices and can give them a wound-sealing capacity, various enzymes, terpenes, alkaloids, vitamins, carbohydrates, lipids and free amino acids have all been identified as components.

Latex has been reported to occur in 12 000 plant species belonging to 900 genera [1]. In a given family not all genera are latex-bearing, which complicates definition of a role for latex in plants. However, the wide-spread occurrence of lacticifers shows that their existence can confer some advantage, and examination of them may elucidate an understanding of that phenomenon. Further, latex-bearing plants, notably *Hevea brasiliensis*, which is the major source of natural rubber, are of significant commerical value and so again comprehension of the function of latices in the plants is of interest.

The genus *Euphorbia* is composed essentially of latex-bearing species, and recently we have used a number of these plants as well as *Elaeophorbia drupifera* (also of the Euphorbiaceae) as sources of proteases (known as euphorbains [2–7]), phosphatases [8], lectins [9] and lysozymes [K. R. Lynn, unpublished work]. During that work we surveyed latices of 18 Euphorbia species and *Elaeophorbia drupifera* for properties such as pH, protein and carbohydrate contents, enzymatic activities, lectin contents ([9]; haemagglutinating activities against both sheep and human blood) and the effects of various protease inhibitors. The data are presented here.

The wound-sealing property of latices was noted above, and other functions have been discussed: the presence of proteases [2–7] may suggest metabolic and transport roles, or even a protective function for latex. The presence of lysozymes with chitinase properties in latex from e.g. papaya and ficus [10, 11] further support the latter possibility, as does the identification of irritant constituents [12 and refs. therein]. The data reported here, however, do not lend themselves to a simple and singular function for latices: a wide variety of enzymatic activities are shown to be present in varying amounts in the materials examined which, furthermore, also contain widely varying quantities of other proteins, including lectins [9].

Some latex constituents of members of the Euphorbiaceae have been examined for taxonomic purposes [13]. We have included here some data from two-dimensional electrophoresis, which were collected during this work to investigate the taxonomic value [14] of a technique by which protein constituents are separated on the basis of charge (by isoelectric focusing) and $M_r$ (by SDS electrophoresis).

## RESULTS AND DISCUSSION

The plant species discussed here may conveniently be divided into two groups: *E. characias*, *E. cyparissias*, *E. esula*, *E. helioscopia*, *E. lathyris* and *E. platyphylla* are categorized as 'leafy' euphorbs, while *E. coerulescens*, *E. cylindrifolia*, *E. globosa*, *E. hermentiana*, *E. lactea*, *E. lactea cristata*, *E. mammillaris*, *E. splendens*, *E. stapelioides*, *E. tirucalli* with *E. trigona* are classified as 'succulents'. *Elaeophorbia drupifera* is placed in the latter group.

It should be noted that the latices of the 'leafy' plants were collected from populations numbering hundreds to thousands. A total of about 50 specimens of *E. tirucalli* were sampled in the course of this work and we also had access to five *E. lactea*, *E. lactea cristata*, *Elaeophorbia drupifera* and *E. splendens* plants. However, latex from only two *E. stapelioides* could be obtained, and single specimens of *E. trigona*, *E. hermentiana*, *E. coerulescens*, *E. cylindrifolia*, *E. globosa* and *E. mammillaris* were

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available to us. While the use of single specimens opens the possibility of the data obtained from them being atypical of their species, we have no evidence from the results collected that this is so: plants of, for example *E. lathyris*, when sampled individually, gave results in close accord with those obtained with latex collected from a large population. As noted in the experimental section, latices were commonly obtained from mature plants. When we examined the proteases and lectins of latex from *E. lathyris* over a period of 1 year, there was no evidence of age-related behaviour in either constituent.

It is evident in Table 1 that latices of all 'leafy' members are comparatively rich in protein, while the 'succulents' vary widely in the protein contents of their latices. Neither of the two categories shows a consistent pattern on measuring carbohydrate content, or the total solids present in the latex sera, but three of the 'leafy' specimens, namely *E. characias*, *E. cyparissias* and *E. esula*, have significant amounts of carbohydrate in their latices. The total solids measured after freeze-drying samples of the latices would be made up of protein, carbohydrate, lipid, resin, triterpene, steroid components, pigments and metals etc. Clearly these vary considerably—by a factor of 7 in the samples reported in Table 1. All of the latices measured here have pHs between 4.6 and 5.5, which is of interest, as many of the proteolytic enzymes isolated from them have pH maxima above that range [2–7]. This may suggest that these proteases react not in the latices, which may be only a transport mechanism for the proteases, but elsewhere in the plants. Alternatively, the enzymes may react in the latex, but in a state which modifies their response to pH.

As it is known [2–7] that the latices commonly contain protease activity attributable to the presence of serine-centred enzymes, the effects of a number of peptidyl inhibitors for trypsin-like enzymes were examined. The results are collected in Table 2, and they are supplemented with comparable data collected for three other inhibitors of cysteine-centred (antipain, leupeptin [15, 16]) and carboxyl-centred (pepstatin A [17]) enzymes.

It is apparent in Table 2 that no clear division of 'leafy' from 'succulent' euphorbs is made by the inhibitors used. Potato I inhibitor is the most generally effective compound reported here, but even that causes, maximally, only 62% inhibition. While leupeptin and pepstatin A have negligible effects on the esterolytic capacities of the latices discussed, antipain was notably inhibitory for the enzymes in *E. cyparissias* and *E. esula*. Proteases from the former of these were also inhibited by iodoacetic acid [5], suggesting that as well as the essential serines of the active site, a cysteine residue may play a role in the catalysis by euphorbain from this source.

The latices examined had only minor leucine aminopeptidase activities, but $\beta$-glucosaminidase, $\alpha$-mannosidase and $\alpha$- as well as $\beta$-galactosidase activities were commonly observed (Table 3). However we were unable to detect $\alpha$- or $\beta$-D-glucopyranosidase activities in latices from *E. characias*, *E. coerulescens*, *E. cyparissias*, *E. helioscopia*, *E. hermentiana*, *E. lactea cristata*, *E. lathyris*, *E. splendens*, *E. tirucalli* or *E. trigona*. $\alpha$-Amylase activities have been reported [13] in latices from *E. heterophylla*, *E. marginata* and *E. tirucalli* and were found, also, in this work in *E. cyparissias* and *E. lathyris*. Similarly, a peroxidase was isolated [18] from the latex of *E. characias*. Low levels of lysozyme activity were measured in the latices of *E. lathyris*, *E. lactea* and *Elaeophorbia drupifera* (Table 3), and no significant amounts of cellulase activities were detected in latices from the sources listed in Table 3.

In that table are collected data from surveys of enzymatic activities of 11 of the Euphorbiaceae latices discussed here. Protease acitivity was measured with azocoll, azocasein and bovine serum albumin (BSA). It is evident that the collagen substrate is preferred, and that the majority of latices have proteolytic activity with each of the proteins, though the relative degrees of activity vary depending on the enzyme-source examined. Thus, for

Table 1. Protein, carbohydrate and 'total solid' contents of latex sera. pHs are listed

| Source of latex | Protein (mg/ml) | Carbohydrate (mg/ml) | Total solids (mg/ml) | pH of latex serum |
|---|---|---|---|---|
| *E. characias* | 18.6 | 15.5 | 90 | n.m. |
| *E. cyparissias* | 59.7 | 28.8 | 139 | 5.3 |
| *E. esula* | 43.4 | 16.3 | n.m.* | 5.3 |
| *E. helioscopia* | 55.3 | 2.1 | n.m. | n.m. |
| *E. lathyris* | 49.4 | 0.9 | 71 | 4.9 |
| *E. platyphylla* | 36.8 | 3.6 | 110 | n.m. |
| *E. coerulescens* | 42.3 | 2.3 | n.m. | 5.1 |
| *E. cylindrifolia* | n.m. | n.m. | n.m. | 4.7 |
| *E. globosa* | 40.0 | n.m. | n.m. | 4.6 |
| *E. hermentiana* | 25.8 | 1.3 | 44 | 4.9 |
| *E. lactea* | 19.1 | 0.9 | n.m. | 4.8 |
| *E. lactea cristata* | 9.7 | 0.4 | 40 | n.m. |
| *E. mammillaris* | 5.1 | 0.9 | n.m. | 4.8 |
| *E. splendens* | 34.7 | 3.0 | 50 | 5.5 |
| *E. stapelioides* | 10.0 | n.m. | n.m. | 5.0 |
| *E. tirucalli* | 4.3 | 0.8 | 100 | 4.9 |
| *E. trigona* | 13.0 | 0.7 | 40 | 4.9 |
| *Elaeophorbia drupifera* | 24.5 | 1.5 | 65 | 5.0 |

*Not measured.

Table 2. Effects of various peptide inhibitors of proteases on latex sera of euphorbs: percentage inhibition CGN-hydrolysing ability of latex-serum with 50 nmol inhibitor

| Source of latex | Antipain | 1-α-Antitrypsin | Bovine pancreatic trypsin inhibition | Chymostatin | Elastatinal | Leupeptin | Ovomucoid trypsin inhibitor | Pepstatin A |
|---|---|---|---|---|---|---|---|---|
| *E. cyparissias* | 52 | 22 | 10 | 55 | 71 | 0 | 0 | 0 |
| *E. esula* | 52 | 56 | 5 | 16 | 0 | 0 | 0 | 0 |
| *E. helioscopia* | 0 | 42 | 11 | 9 | 0 | 0 | 0 | 0 |
| *E. lathyris* | 12 | 0 | 0 | 27 | 21 | 8 | 0 | 9 |
| *E. platyphylla* | 0 | 8 | 20 | 0 | 0 | 0 | 0 | 0 |
| *E. portlaudica* | 18 | 0 | 0 | 0 | 0 | 12 | 9 | 0 |
| *E. lactea* | 0 | 40 | 0 | 0 | 0 | 0 | 0 | 0 |
| *E. splendens* | 32 | 0 | 0 | 32 | 0 | 11 | 0 | 0 |
| *E. tirucalli* | 20 | 0 | 0 | 28 | 17 | 11 | 0 | 4 |
| *Elaeophorbia drupifera* | 0 | 0 | 0 | 11 | 0 | 6 | 25 | 4 |

Table 3. Enzymatic activities of crude latex sera (units/mg protein)

| | Esterase (CGN) | Protease (azocasein) | Protease (azocoll) | Protease (BSA) | Acid phosphatase | Alkaline phosphatase | *N*-Acetyl-β-glucosaminidase | α-Mannosidase | Leucine-amino peptidase | α-Galactosidase | β-Galactosidase | Lysozyme |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *E. cyparissias* | 6.2 | 0.06 | 20.1 | 0.3 | 0.72 | 0.06 | 7.9 | 0.10 | 0.01 | 0.09 | 0.07 | 0 |
| *E. esula* | 2.4 | 0.21 | 25.3 | 0.05 | 0.92 | 0.11 | 5.2 | 0.06 | 0.01 | 0.06 | 0.03 | n.m. |
| *E. helioscopia* | 4.5 | 0.12 | 19.0 | 0.25 | 1.07 | 0.07 | 1.3 | 0.05 | 0.04 | 0.12 | 0.06 | 0 |
| *E. lathyris* | 2.1 | 0.03 | 1.5 | 0.06 | 0.31 | 0.01 | 9.1 | 0.05 | 0.01 | 0.03 | 0.03 | $14 \times 10^{-5}$ |
| *E. coerulescens* | 0.41 | 0.08 | 6.8 | n.m. | 1.0 | 0.02 | 0.9 | 0.10 | 0.01 | 0.05 | 0.02 | 0 |
| *E. hermentiana* | n.m. | 0.01 | n.m. | 0.05 | 0.31 | 0.02 | 0.2 | 0 | 0.01 | 0.03 | 0.01 | 0 |
| *E. lactea* | 4.9 | 0.04 | 10.1 | 0.12 | 0.30 | 0.02 | 1.8 | 0 | 0.01 | 0.08 | 0.01 | $65 \times 10^{-5}$ |
| *E. splendens* | 3.4 | 0.05 | 9.3 | 0.22 | 0.84 | 0.02 | 4.7 | 0.2 | 0.01 | 0.04 | 0.02 | 0 |
| *E. tirucalli* | 8.3 | 0.10 | 17.0 | 0.11 | 1.25 | 0.11 | 3.8 | 0.12 | 0.07 | 0.19 | 0.13 | 0 |
| *E. trigona* | n.m. | 0.00 | 0.8 | n.m. | 0.51 | 0.02 | 3.0 | 0 | 0.02 | 0.05 | 0.02 | 0 |
| *Elaeophorbia drupifera* | 2.9 | 0.02 | 3.0 | 0.02 | 0.37 | 0.01 | 1.1 | 0.01 | 0 | 0.01 | 0.01 | $52 \times 10^{-5}$ |

example, the latex of *E. esula* is relatively active against azocoll and azocasein, but not against BSA. The synthetic substrate, carbobenzoxy-glycine *p*-nitrophenyl ester (CGN), has been commonly used in the purification of proteases from the latices of Euphorbiaceae [2–7] and is seen, in Table 3, to be a suitably general substrate. Other esterase activities were also measured in 10 latices using *p*-nitrophenyl esters of acetic, propionic, *n*-butyric, valeric, caproic, caprylic, capric, lauric, myristic and palmitic acids. These data are summarized in Table 4, and it is clear that the esters of acids of higher chain-length than C-8 (caprylic) are unaffected by the latices examined. It should be noted that these esterase activities do not result from action of latex proteases: this was checked using the purified proteases [2–7].

The latices studied possess, also, both alkaline and acid phosphatase activities, and representatives of these latter enzymes have lately been isolated from *E. lathyris*, *E. trigona* and *Elaeophorbia drupifera* [8].

It is apparent that the latices investigated contain a wide variety of enzymatic activities. One of the richest sources of enzymes among those described here is the latex of *E. tirucalli*, though this contains relatively small amounts of protein (Table 1). From the variety of enzymatic activities described in Tables 3 and 4, each plant species apparently uses the latex for a variety of enzymological purposes unless, as suggested above, that medium acts principally as a transport mechanism.

No pattern of enzymatic properties is observed in the latices listed in Tables 3 and 4, even at the elementary level of distinguishing between 'leafy' and 'succulent' euphorbs.

In Table 5 are presented data from a survey [9] of the lectin contents of a number of latices of the Euphorbiaceae. While it is apparent that these haemagglutinins are widely distributed in this family, again no pattern of relationships can be discerned. However, of the seven lectins isolated as homogeneous proteins, five, comprising those from *E. hermentiana*, *E. lactea*, *E. lactea cristata*, *E. trigona* and *Elaeophorbia drupifera*, were shown to have amino acid compositions which are related to each other [9].

Two-dimensional maps on polyacrylamide gel sheets

Table 4. Esterolytic activity to aliphatic *p*-nitrophenyl esters of *Euphorbia* latices measured in 100 mM Tris-HCl, pH 7.0

| Latex | Activity ($A_{410}$/min/mg protein) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Acetate | Propionate | Butyrate | Valerate | Caproate | Caprylate | Caprate | Laurate | Myristate |
| *E. characias* | 3.4 | 3.1 | 1.7 | 1.7 | 1.31 | 1.8 | 0.45 | 0.28 | 0.16 |
| *E. cyparissias* | 0.07 | 0.02 | 0.11 | 0.21 | 0.15 | 0.38 | 0 | 0.07 | 0 |
| *E. helioscopia* | 0.27 | 0.55 | 0.48 | 1.0 | 2.2 | 2.4 | 0.41 | 0.33 | 0 |
| *E. lathyris* | 0.04 | 0.06 | 0.12 | 0.10 | 0.31 | 0.62 | 0.06 | n.m. | 0 |
| *E. coerulescens* | 0 | 0.35 | 0.12 | 0.06 | 0.46 | n.m. | 0 | 0 | n.m. |
| *E. hermentiana* | 0 | 0 | 0.01 | 0.02 | 0.06 | 0.09 | n.m. | n.m. | n.m. |
| *E. lactea cristata* | 0.02 | 0 | 0.02 | 0.04 | 0.06 | 0.19 | 0.06 | 0.02 | 0 |
| *E. splendens* | 0.09 | 0.07 | 0.17 | 0.24 | 0.58 | 1.0 | 0.10 | 0.14 | 0.04 |
| *E. tirucalli* | 4.0 | 4.0 | 4.0 | 9.0 | 5.0 | 4.0 | 0 | 0 | n.m. |
| *E. trigona* | 0 | 0.04 | 0.4 | 0.1 | 0.18 | 0.12 | 0.04 | n.m. | 0.02 |

Table 5. Measurement of agglutination of neuraminidase-stripped sheep and human A, B, AB and O erythrocytes using latex-sera from 18 euphorbs and from *Elaeophorbia drupifera*

| Source of Latex | Protein (mg/ml) | Titres | | | | |
|---|---|---|---|---|---|---|
| | | Sheep | Human A | Human B | Human AB | Human O |
| *E. characias* | 18.6 | 0 | 1600 | 1600 | 1600 | 3200 |
| *E. cyparissias* | 59.7 | 128 | 800 | 800 | 800 | 800 |
| *E. esula* | 43.4 | 64 | 256 | 256 | 256 | 256 |
| *E. helioscopia* | 55.3 | 4 | 16 | 16 | 8 | 32 |
| *E. lathyris* | 49.4 | 800 | 3200 | 3200 | 1600 | 3200 |
| *E. platyphylla* | 36.8 | 16 | 256 | 256 | 256 | 2048 |
| *E. aphylla* | | 16 | | | | |
| *E. coerulescens* | 42.3 | 12800 | 128000 | 64000 | 6400 | 6400 |
| *E. cylindrifolia* | | | 128000 | 256000 | 128000 | 128000 |
| *E. globosa* | | 0 | 0 | 0 | 0 | 0 |
| *E. hermentiana* | 25.8 | 3200 | 102400 | 102400 | 102400 | 102400 |
| *E. lactea* | 19.1 | 1600 | 6400 | 6400 | 6400 | 6400 |
| *E. lactea cristata* | 9.7 | 10240 | 6400 | 12800 | 12800 | 51200 |
| *E. mammillaris* | 5.1 | | 0 | 0 | 0 | 0 |
| *E. splendens* | 34.7 | 700 | 6400 | 6400 | 6400 | 12800 |
| *E. stapelioides* | | | 0 | 0 | 0 | 0 |
| *E. tirucalli* | 4.3 | 16 | 64 | 32 | 32 | 64 |
| *E. trigona* | 13.0 | 12800 | 256000 | 256000 | 128000 | 256000 |
| *Elaeophorbia drupifera* | 24.5 | 25600 | 32000 | 32000 | 32000 | 32000 |

were prepared, with separation in one dimension being by charge (isoelectric focusing) and in the second by $M_r$ (SDS). Latices from the following 18 members of the Euphorbiaceae were inspected with this technique using staining for both protein content [2, 19] and carbohydrate ([20]; glycoprotein): *E. characias*, *E. cyparissias*, *E. esula*, *E. helioscopia*, *E. lathyris*, *E. platyphylla*, *E. coerulescens*, *E. cylindrifolia*, *E. globosa*, *E. helioscopia*, *E. hermentiana*, *E. lactea cristata*, *E. mammillaris*, *E. splendens*, *E. stapelioides*, *E. tirucalli*, *E. trigona* and *Elaeophorbia drupifera*. The maps obtained from the two staining procedures were, in all cases, essentially identical and showed that the proteins present in the various latices were mainly glycoproteins. Of the three proteinous groups isolated from some of the species discussed here, namely proteases [2–7] phosphatases [8] and lectins [9] all that were analysed were found to be glycoproteins, so offering some confirmation of the above observations.

No relationships between the two-dimensional maps, which could be correlated with accepted taxonomy, were discerned. Thus maps of the latex proteins of several members of the genus *Euphorbia* [21], sub-genus *Esula* and sections *esula* (*E. esula* and *E. cyparissias*), *lathyris* (*E. lathyris*) and *tithymalus* (*E. helioscopia*) were examined. While two varieties of *E. esula*, originating from Austria and Montana (U.S.A.), were similar, they were clearly different. They also had no apparent relationship with the maps for the taxonomically related *E. cyparissias*, *E. lathyris* or *E. helioscopia*. It should be noted that the maps discussed here were stained for only two attributes: the presence of proteins and of glycoproteins. Comparison of the two-dimensional maps, then, was made only as that of gross patterns, each of which was unique for the two properties examined. If greater discrimination between the maps were possible, by, for example, quantitating several properties of the separated glycoproteins *in situ*, a

more effective comparison could be undertaken. However, the data in the tables above suggest that, even then, the electrophoretic separation of latex proteins would be of no taxonomic value [14].

We may summarize the results reported here and elsewhere [9] as showing that within the same genus, the latices of the several species of *Euphorbia* examined differ widely in such properties as protein and carbohydrate contents, in the variety and amounts of enzymes present, and in their haemagglutinating activity for both human and sheep blood. Two-dimensional gel-electrophoresis maps confirmed those observations, as the sera of each *Euphorbia* latex examined gave a unique pattern on staining for both protein and carbohydrate.

## EXPERIMENTAL

*Reagents.* Except for Potato I inhibitor, prepared as described in [22], these were obtained from common commerical sources. Reagents and methods for assays for the lectins have been described elsewhere [9].

*Latices.* These were collected from incisions in the stems of mature plants. The milky product was clarified by centrifugation at 20000 *g* for 60 min at 4° and the clear aq. layer separated and stored at −10° until required. The conditions of growth, and the sources of the plants have been described [9].

*Methods. Total solids* in the latices were measured after freeze drying; *protein content* estimated by the Folin method [23] with bovine serum albumin as standard and *carbohydrate content* assayed with the procedure of Dubois [24].

*Esterase activities* were measured with CBZ glycine *p*-nitrophenyl ester (CGN) as previously described [2]. Activities with *p*-nitrophenyl esters of the following aliphatic acids were measured under the same conditions: acetic, propionic, butyric, valeric, caproic, caprylic, capric, lauric, myristic and palmitic, and again 1 unit produces 1 μmol *p*-nitrophenol/min. *Protease activities* were measured with azocollagen, azocasein and bovine serum albumin, using previously described methods [2–7].

*Inhibition measurements.* Latex-serum (10 μl) was assayed for esterolytic activity with CGN as described above in the absence, then presence, of 50 nmol of inhibitor, at pH 7.0.

*Phosphatase activities* were measured with *p*-nitrophenyl phosphate using 10 μl latex serum and 200 μl buffer (sodium acetate, 100 mM, pH 5.4 for acid phosphatases; Tris–HCl, 100 mM, pH 8.5 for alkaline phosphatases) and 400 μl 3 mM substrate in the same buffer. After incubation at 37° for 10 min, 1.0 ml 1 M NaOH was added and absorption at 410 nm measured. 1 unit $= A_{410} \times 10^2$.

*Glycosidase activities.* Assays for α- and β-glucosidase, α- and β-galactosidase, α-mannosidase and *N*-acetyl-β-glucosaminidase activities were all made using the respective *p*-nitrophenyl derivatives of the substrate sugars in acetate buffer, 100 mM, pH 5.0, at 37°. The procedure was essentially that of Li and Li [25]; 1 unit is the amount of enzyme hydrolysing 1 μmol *p*-nitrophenyl derivative/min, using 10 μl latex serum.

*Leucine aminopeptidase.* Latex serum (20 μl) in 400 μl 100 mM NaPi buffer, pH 7.5, was mixed with 100 μl L-leucine-2-naphthylamide in $Me_2CO$ (1 mM). After 3 hr incubation at 37°, coupling reagents were added and absorption at 540 nm measured; 1 unit $= A_{540}$/min.

*Amylase activity.* The method has been described [13], and employed soluble starch as the substrate and 10 μl latex serum in 10 mM NaOAc, pH 4.8.

*Lysozyme activity.* Measurements were made with dried cell walls of *Micrococcus lysodeikticus* following Howard and Glazer [10]. Latex serum (10 μl) was incubated with a 1 ml of a suspension (0.2 mg per ml) of cell wall in 100 mM NaOAc, pH 4.6, at 37° for an appropriate time. Reaction was terminated by addition of 100 μl of 4 M NaOH; a unit is $A_{440}$/min.

*Cellulase activity* was measured with carboxymethyl cellulose as substrate [26]. *Lectin activity* was measured as described [9].

*Two-dimensional gel electrophoresis.* Isoelectric focusing on polyacrylamide disc gels was by the method of Righetti and Drysdale [27] at 250 V, for 16 hr at 20°. The rods so obtained were attached to 9 cm square SDS-polyacrylamide (12%) plates by agarose bridges and subjected to electrophoresis using the Laemli procedure for 5 hr [28]. The gels were stained for protein using silver [19] or Coomassie Blue [2], and for glycoprotein by the method of Zacharius [20].

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