Behaviour and Properties of Membrane-bound Mouse Uterine Alkaline Phosphatase during Early Pregnancy

Louise E. Buxton and R. N. Murdoch

Department of Biological Sciences, University of Newcastle, Newcastle, N.S.W. 2308.

Abstract

Most of the alkaline phosphatase activity in the mouse uterus during early pregnancy was found to be membrane-bound and was associated with particulate material when homogenates were centrifuged at 105000 g. The activity of the enzyme increased in both the particulate and cytosol fractions of uterine homogenates during early pregnancy to reach maximum values on day 7 of pregnancy.

Studies of the enzyme in its membrane-bound and cytosolic forms before and after solubilization with Triton X-100, and n-butanol failed to detect any evidence that the membrane microenvironment or membrane binding per se exerts any regulatory effects on the enzyme, or that the active sites of the enzyme are deeply buried within the membranes of uterine cells. Thus, the properties of the enzyme in response to amino acids, inhibitors, and Mg²⁺ and Zn²⁺, and changes in pH, substrate concentration and temperature were essentially unaltered when the phosphatase was present in a membrane-bound or cytosolic form, or when fractions were treated with Triton X-100 and n-butanol.

Solubilized preparations of the enzyme from particulate and cytosol fractions of uterine homogenates displayed zones of activity with similar anodal migration rates during electrophoresis on cellulose acetate membranes suggesting that the cytosolic activity may arise from particulate material during homogenization of the tissue.

Several amino acids stimulated the activity of the phosphatase while cysteine, histidine, hom-arginine, Na₂HPO₄ and 4-(p-aminophenylazo)phenylarsonic acid were inhibitory. In addition, $K_m$ values for the enzyme from all uterine fractions hydrolysing p-nitrophenyl phosphate were temperature-dependent.

Introduction

Alkaline phosphatase (orthophosphoric monoester phosphohydrolase, EC 3.1.3.1) is a membrane-bound enzyme in mammalian tissues and is largely associated with the particulate material which sediments in homogenized preparations during centrifugation (Fernley 1971; Hung and Melnykovych 1976). This association in a number of instances influences the catalytic properties of the enzyme (Ey and Ferber 1977; Simon and Sutherland 1977). Although the alkaline phosphatase occurring 'free' in the cytosol of cells may, in some cases, represent a precursor for the membrane-bound activity, Simon and Sutherland (1977) suggest that the enzymes prepared from membrane and cytosol fractions of rat liver exist in different structural forms. Thus, membrane-binding of alkaline phosphatase may frequently involve modifications which may facilitate the widely speculated role of the enzyme in membrane transport processes (Fernley 1971).
In the mouse uterus, the bulk of the alkaline phosphatase activity is also associated with particulate material (Murdoch et al. 1978) and increases during the peri-implantation period of pregnancy when the stromal tissue is undergoing decidualization (Finn and Hinchliffe 1964; Finn and McLaren 1967; Smith 1973; Murdoch et al. 1978). It is not known, however, whether membrane binding affords regulation of the enzyme or influences its catalytic effectiveness at this time. In the present study an attempt was made to examine these aspects of uterine alkaline phosphatase. In particular, the activities of membrane and cytosol uterine preparations were examined during early pregnancy to gauge the effects of decidual cell induction and regression on the subcellular distribution of the enzyme. Various catalytic properties of the enzyme in sediment and supernatant fractions of uterine homogenates following centrifugation and after treatment with Triton X-100 or n-butanol or both were also studied to determine the effects of immobilization, solubilization, and the removal of membrane lipid components.

Materials and Methods

Chemicals

Tris(hydroxymethyl)aminomethane and Triton X-100 were obtained from Calbiochem (Australia) Pty. Ltd., Carlingford, N.S.W. Analytical grade glycine, zinc chloride, magnesium chloride, sodium carbonate, sodium bicarbonate, n-butanol, potassium chloride and sucrose were obtained from Ajax Chemicals, Sydney, N.S.W. The crystalline disodium salt of p-nitrophenyl phosphate was purchased from Boehringer Mannheim Australia Pty. Ltd., Mount Waverley, Vic. Disodium orthophosphate, methanol, and chloroform were obtained from BDH Chemicals (Australia) Pty. Ltd., Port Fairy, Vic., while 4-(p-aminophenylazo)phenylarsonic acid was purchased from the Aldrich Chemical Company, Inc., Milwaukee, Wisconsin, U.S.A. L-α-Lysophosphatidyl choline (from egg yolk), cholesterol, and Kit No. LAA-21 containing 21 L-amino acids were purchased from Sigma Chemical Co., St. Louis, Missouri, U.S.A. Experiments were carried out using demineralized and twice-distilled water.

Animals

Female Quackenbush strain mice, aged 8–12 weeks, were used in all experiments and were housed as previously described (Murdoch et al. 1978). Pregnancy was brought about by pairing the females with males of the same strain. The females were examined for copulation plugs each morning and the day of finding a plug was designated as day 1 or the first day of pregnancy. The mice were killed by cervical dislocation between 0900 and 1100 h on days 5–12 of pregnancy and were immediately dissected to remove the uterus free of fatty and connective tissue and of the attached oviducts and cervix. The uteri were then homogenized in 20 volumes of 0·25 M sucrose as described by Murdoch et al. 1978.

Centrifugation and Extraction of Uterine Homogenates

Homogenates of uteri, prepared individually from mice on days 5–12 of pregnancy, were assayed for alkaline phosphatase activity and then centrifuged at 105 000 g for 1 h at 4°C using a type A-321 rotor in a Damon B-60 ultracentrifuge. After centrifugation the supernatant, which contained the membrane-free cytosolic form of the enzyme, and designated as Sup 1, was retained and held at 4°C. The sediment or particulate fraction, which contained the membrane-bound form of the enzyme, and designated as Sed 1, was redispersed into a volume of 0·25 M sucrose equal to that of the supernatant removed, and also held at 4°C. The alkaline phosphatase activity of the supernatant and sediment fractions was determined immediately after centrifugation.

Solubilization of the enzyme was achieved by adding Triton X-100 (0·2% v/v) to the supernatant and sediment fractions and incubating at 37°C for 1 h with gentle agitation (Murdoch et al. 1978). The preparations were then centrifuged at 105 000 g for 1 h and the supernatants containing essentially all of the alkaline phosphatase activity were retained. These fractions from the original supernatant and sediment preparations were designated Sup 2 and Sed 2, respectively. The alkaline phosphatase activity of these fractions was again determined immediately after centrifugation.
In experiments designed to study the properties of alkaline phosphatase in sediment and cytosol fractions of homogenates, uteri were collected on day 7 of pregnancy (Murdoch et al. 1978) and processed in batches of 5–10 g wet weight. Supernatant and sediment fractions (viz. Sup 1, Sup 2, Sed 1, Sed 2) were prepared by homogenization, centrifugation and treatment with Triton X-100 as described above to permit the study of the enzyme in its membrane-bound cytosolic and solubilized forms. Because Hung and Melnykovich (1976) have indicated that lipid components of membranes may influence the activity of some alkaline phosphatase enzymes, the effects of removing lipids from the uterine preparations by extraction with n-butanol (Morton 1954) were also investigated. Aliquots of the fractions treated with Triton X-100 (viz. Sup 2 and Sed 2) were extracted with 20% (v/v) n-butanol as described by Murdoch et al. (1979). The aqueous extracts from the Sup 2 and Sed 2 fractions containing alkaline phosphatase activity after treatment with n-butanol were designated as Sup 3 and Sed 3, respectively. All fractions were dialysed against three changes of 10 volumes of 0.25 M sucrose for 24 h at 4°C and then stored at −20°C in stoppered plastic vials (capacity 5·0 ml). Preliminary studies revealed that storage under these conditions for 1 week did not significantly affect the release of bound enzyme or alter the activity of any of the preparations.

**Alkaline Phosphatase and Protein Analyses**

Unless otherwise stated, alkaline phosphatase activity was assayed by the spectrophotometric measurement of hydrolysis of p-nitrophenyl phosphate as described previously (Murdoch et al. 1979). Units of activity are defined as micromoles of substrate hydrolysed per minute and were calculated using a p-nitrophenolate molar extinction coefficient of 1.82 × 10³ at 405 nm.

Kₜₜₜ (Michaels constant) values, energy of activation values, and the effects of pH and temperature on the alkaline phosphatase activities of the various uterine preparations were determined as described by Murdoch et al. (1980) using a 50 mM glycine- NaOH buffer system at pH 10.5.

The effects of various L-α-amino acids and other compounds on the alkaline phosphatase activity of the sediment, supernatant and purified preparations were determined in a 50 mM carbonate-bicarbonate buffer (pH 9.8) system as follows. Each compound, dissolved in buffer to give final concentrations of 0, 1, 3 and 9 mM, was incubated with the enzyme preparation in a volume of 0.5 ml for 5 min at 37°C. Enzyme activity was then measured by adding 0.5 ml of buffer containing 4 mM (final concentration) substrate and incubating at 37°C for a further 15 min.

All assays were performed in duplicate and, where possible, were repeated using at least three different preparations.

The protein concentration of samples was determined by the method of Lowry et al. (1951) using standards of bovine serum albumin.

**Purified Enzyme and Apoenzyme Preparations**

Purified mouse uterine alkaline phosphatase was obtained as described in a previous paper (Murdoch et al. 1980). When used in conjunction with the various sediment and supernatant fractions described above, purified preparations were diluted with 0.25 M sucrose to ensure that the enzyme activity of all samples being studied simultaneously was approximately comparable.

Apoenzyme preparations containing no residual activity in the absence of added cations were obtained using the method described by Murdoch et al. (1980). The ability of the apoenzyme to reactivate in the presence of 2 mM MgCl₂ and 0.25 mM ZnCl₂ (when added separately and in combination) was tested in a system involving the direct spectrophotometric measurement of hydrolysis of p-nitrophenyl phosphate (Murdoch et al. 1980).

**Liposome Preparation**

Liposomes were prepared using the method of Shinitzky and Inbar (1974). Various proportions of lysophosphatidyl choline and cholesterol were dispersed in 1·0 ml of chloroform–methanol (2:1, v/v), evaporated to dryness under nitrogen, and then dispersed in 1·0 ml of 0·15 M KCl. Liposomes were formed by subjecting the mixtures to ultrasonic irradiation in an ice–water bath for 20 min with four 2-min interruptions. The preparations were then centrifuged at 30000 g for 20 min to remove any high-density particles.

To achieve binding of purified alkaline phosphatase to liposomes, 0.5 ml of purified enzyme in 0·01 M Tris- HCl buffer (pH 8·0) was added to 0·5 ml of the liposome preparation and the mixture was incubated in a shaking water bath at 37°C for 20 min. Enzyme activity was then assessed.
Cellulose Acetate Electrophoresis

Electrophoresis of alkaline phosphatase on Sepaphore III cellulose acetate membranes (Gelman Instrument Co., Artarmon, N.S.W.) was performed using the method previously described by Murdoch et al. (1979).

Statistical Analyses

Where necessary, the significance of results was assessed by a t-test using the interaction mean square from the analysis of variance (not published) to determine the standard error of the difference between means and the degrees of freedom associated with it. Data expressed as percentages were converted to angles before analysis. All values given in the text and tables are means ± standard error of the mean of unconverted data.

Results

Activity Associated with Uterine Particulate Material during Early Pregnancy

Table 1 shows the percentage distribution and specific activity of alkaline phosphatase in uterine homogenates following centrifugation at 105000 g. Between 73 and 75% of the total alkaline phosphatase activity in uterine homogenates was associated with particulate material on days 5, 9, 10, 11 and 12 of pregnancy. The percentage of the total activity in the particulate fraction increased significantly on day 6 of pregnancy and remained elevated until day 8. Between 81 and 85% of the enzyme activity was associated with the particulate material at these stages.

| Day of pregnancy | Activity in particulate fraction (as % of total) | 10^{-2} \times \text{Specific activity (units/mg protein)} in: |  \\
|------------------|------------------------------------------------|-------------------------------------------------|  \\
|                  |                                                  | Particulate fraction                             | Cytosol fraction                             |  \\
| 5                | 74 ± 0·81                                       | 82 ± 4·2                                        | 36 ± 3·2                                     |  \\
| 6                | 83 ± 1·32***                                    | 233 ± 12·1***                                  | 55 ± 2·1                                     |  \\
| 7                | 85 ± 0·40***                                    | 740 ± 15·3***                                  | 137 ± 6·0***                                 |  \\
| 8                | 81 ± 1·65**                                     | 665 ± 22·0***                                  | 115 ± 11·4***                                |  \\
| 9                | 75 ± 0·48                                       | 250 ± 17·1**                                   | 90 ± 3·0***                                  |  \\
| 10               | 74 ± 2·16                                       | 170 ± 10·0**                                   | 60 ± 5·3**                                   |  \\
| 11               | 74 ± 1·63                                       | 105 ± 5·4                                      | 40 ± 4·0                                     |  \\
| 12               | 73 ± 2·69                                       | 82 ± 1·3                                       | 36 ± 1·4                                     |  \\

The specific activity of alkaline phosphatase increased to a maximum on day 7 of pregnancy in both the particulate and cytosol fractions of uterine homogenates and then decreased to reach, on day 12, values similar to those recorded on day 5. These changes were more marked in the particulate fractions than in the cytosol fraction.

Treatment of the particulate fraction with 0·2% (v/v) Triton X-100 for 1 h at 37°C (results not shown) prevented the sedimentation of alkaline phosphatase activity when the samples were subsequently centrifuged at 105000 g. However, Triton X-100 had no significant effect on the total enzyme activity in either the particulate or cytosol fractions.
**Cellulose Acetate Electrophoresis**

Anodal migration of a single, well-defined zone of alkaline phosphatase activity on cellulose acetate membranes was a characteristic feature of all supernatant fractions (results not shown). Treatment of the supernatant fractions with Triton X-100 or n-butanol did not significantly alter the rate of migration. In fraction Sed 1 the enzyme activity remained at the origin and failed to migrate towards the anode on electrophoresis. However, treatment of this sediment fraction with Triton X-100 (Sed 2) or Triton X-100 and n-butanol (Sed 3) solubilized the enzyme and permitted a single, well-defined zone of activity to migrate towards the anode at a rate similar to that in the supernatant fractions.

**Effects of pH**

The response of the membrane-bound and cytosol fractions of alkaline phosphatase to changes in pH was examined using 6 mM substrate. No significant differences in response to pH values ranging from 9.0 to 11.0 were detected between any of the fractions studied and in all cases optimal activity occurred at pH 10.5. The patterns of activity very closely resembled those reported by Murdoch et al. (1980) for purified preparations of the enzyme.

**Reactivation of Apoenzyme Preparations**

Apoenzyme preparations of alkaline phosphatase in the membrane-bound, cytosol and purified forms displayed no detectable enzyme activity in the absence of added cations (results not shown). However, the addition of 2.0 mM Mg\(^{2+}\) and 0.25 mM Zn\(^{2+}\) together restored activity back to the original level in all cases. None of the apoenzyme preparations were reactivated to any extent by the separate addition of the cations.

**Effects of Temperature**

In this experiment the thermostability of alkaline phosphatase in the various particulate and cytosol fractions was examined. No significant differences were detected between the particulate and cytosol fractions in their response to heat and the enzyme in all fractions, including those treated with Triton X-100 and n-butanol, remained stable following heating for 15 min at temperatures of 35, 40 and 45°C. Fractions Sed 1 and Sup 1 still remained stable following heating at 50°C while fractions that had been treated with Triton X-100 (viz. Sed 2 and Sup 2) and with n-butanol (viz. Sed 3 and Sup 3) lost about 35% of their activity at this temperature. Fractions treated with the detergent and n-butanol continued to be more thermostable than the untreated Sed 1 and Sup 1 fractions at 55°C, and at 60°C total inactivation was achieved in all cases.

The rate of hydrolysis of p-nitrophenyl phosphate at pH 10.5 increased with increasing temperatures between 0.5 and 35°C, but again no significant differences in response to these temperatures were detected between the various particulate and cytosol fractions. Thus, the activation energy calculated from Arrhenius plots was essentially the same in all fractions studied and was valued at 46.4 ± 2.6 kJ mol\(^{-1}\).

Apparent \(K_a\) values for the enzyme hydrolysing p-nitrophenyl phosphate at pH 10.5 were also found to be temperature-dependent. However, no significant
differences in the value of $K_m$ were found between any of the particulate, cytosol, or purified enzyme preparations studied. In general, $K_m$ values decreased as the temperature increased and values of $1.64\pm0.15$, $1.45\pm0.04$, $1.17\pm0.07$ and $0.86\pm0.04$ mm were recorded at 15, 22, 29 and 36°C, respectively. The values at 29 and 36°C differed significantly ($P < 0.01$) from that recorded at 15°C.

Table 2. Effects of amino acids and other compounds on the activity of mouse uterine alkaline phosphatase

Values are expressed as relative to the activity in the absence of any added compound. *, **, *** Significantly different from activity (100%) in the absence of any added compound, $P < 0.05$, $P < 0.01$, $P < 0.001$, respectively

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration of compound</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 mm</td>
</tr>
<tr>
<td>Glycine</td>
<td>113 ± 7.6</td>
</tr>
<tr>
<td>Alanine</td>
<td>109 ± 5.5</td>
</tr>
<tr>
<td>Valine</td>
<td>117 ± 9.6</td>
</tr>
<tr>
<td>Leucine</td>
<td>95 ± 5.2</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>108 ± 9.9</td>
</tr>
<tr>
<td>Serine</td>
<td>113 ± 8.7</td>
</tr>
<tr>
<td>Threonine</td>
<td>113 ± 12.2</td>
</tr>
<tr>
<td>Cysteine</td>
<td>13 ± 4.2***</td>
</tr>
<tr>
<td>Methionine</td>
<td>99 ± 4.7</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>133 ± 14.4</td>
</tr>
<tr>
<td>Asparagine</td>
<td>103 ± 3.7</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>100 ± 4.9</td>
</tr>
<tr>
<td>Glutamine</td>
<td>106 ± 4.1</td>
</tr>
<tr>
<td>Arginine</td>
<td>94 ± 2.1</td>
</tr>
<tr>
<td>Lysine</td>
<td>102 ± 4.4</td>
</tr>
<tr>
<td>Histidine</td>
<td>110 ± 10.2</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>108 ± 6.2</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>113 ± 5.1</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>112 ± 6.0</td>
</tr>
<tr>
<td>Proline</td>
<td>114 ± 9.9</td>
</tr>
<tr>
<td>Hydroxyproline</td>
<td>109 ± 6.6</td>
</tr>
<tr>
<td>Homoaarginine</td>
<td>88 ± 4.6*</td>
</tr>
<tr>
<td>4-(p-Aminophenyl azo)-phenylarsonic acid</td>
<td>23 ± 2.8***</td>
</tr>
<tr>
<td>Na$_2$HPO$_4$</td>
<td>97 ± 2.9</td>
</tr>
<tr>
<td>Mannitol</td>
<td>102 ± 2.3</td>
</tr>
</tbody>
</table>

Effects of Amino Acids and Inhibitors

Table 2 shows the effects of various amino acids, 4-(p-aminophenylazo)phenylarsonic acid, and Na$_2$HPO$_4$ on the activity of mouse uterine alkaline phosphatase. As in previous experiments, all fractions including purified preparations were examined individually but since no significant differences in responses were detected between any of the preparations the values shown in Table 2 represent combined results of all assays.

Cysteine, histidine, homoarginine, Na$_2$HPO$_4$ and 4-(p-aminophenylazo)phenylarsonic acid significantly inhibited enzyme activity when present in concentrations of 1–9 mm. In contrast, glycine, alanine, glutamic acid, serine, threonine, aspartic acid and proline significantly stimulated the activity of the enzyme to varying extents.
The addition of valine, leucine, isoleucine, methionine, asparagine, glutamine, arginine, lysine, phenylalanine, tyrosine, tryptophan, hydroxyproline or mannitol did not significantly alter enzyme activity. In addition, incubation in carbonate-bicarbonate buffers ranging from 25 to 100 mm, pH 9·8, in the absence of any added compound failed to significantly alter the activity of the enzyme in any fraction studied (results not shown).

**Table 3. Effect of cholesterol-lysolecithin liposomes on the activity of purified mouse uterine alkaline phosphatase**

<table>
<thead>
<tr>
<th>Ratio of cholesterol to lysolceithin</th>
<th>Specific activity (units/mg protein) Before treatment</th>
<th>After treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 : 4</td>
<td>767 ± 37</td>
<td>501 ± 59**</td>
</tr>
<tr>
<td>1 : 2</td>
<td>798 ± 73</td>
<td>498 ± 66**</td>
</tr>
<tr>
<td>1 : 1</td>
<td>794 ± 71</td>
<td>535 ± 46**</td>
</tr>
<tr>
<td>2 : 1</td>
<td>775 ± 66</td>
<td>498 ± 42**</td>
</tr>
<tr>
<td>0 : 1</td>
<td>762 ± 34</td>
<td>498 ± 49**</td>
</tr>
</tbody>
</table>

A Subjection to ultrasonic irradiation.

Liposome Studies

Table 3 shows the effects of liposomes composed of varying proportions of cholesterol and lysolecithin on the activity of purified preparations of alkaline phosphatase. The activity of the enzyme was significantly \((P < 0.01)\) depressed in the presence of sonicated liposome preparations with the effect being independent of the liposome composition.

Discussion

The results of the present investigation confirm previous observations (Murdoch et al. 1978) that the alkaline phosphatase activity of the mouse uterus is largely membrane-associated and increases during early pregnancy reaching a maximum on day 7. Although increases in the specific activity of the enzyme were evident both in the particulate and cytosol fractions of uterine homogenates, the response was more marked in the former than in the latter fractions. This difference in magnitude of response between the two fractions was due to a small but significant increase (11%) in the proportion of alkaline phosphatase activity which remained associated with particulate material in the uterine homogenate following centrifugation. The increased cell division accompanying decidual cell transformation of the uterine stromal tissue during early pregnancy (Finn and Martin 1967) would provide newly formed lipoprotein membranes (Yunghans et al. 1979) to accommodate the enzyme and account for increases in the proportion of the activity remaining associated with particulate material. This is consistent with the observation that the proportion of enzyme activity associated with the particulate fractions decreased on day 9 of pregnancy when decidual involution is normally initiated (Hetherington 1968; Murdoch et al. 1978).
Murdoch et al. (1978) failed to detect any significant change in the proportion of alkaline phosphatase associated with the particulate fractions of the uterus when decidual cells were induced during pregnancy or pseudopregnancy. This discrepancy between results can be explained by differences in the experimental conditions. The earlier studies involved comparisons only between virgin and day 7 pregnancy or pseudopregnant mice using homogenates sedimented and subsampled progressively at 500, 10,000 and 105,000 g. In the present study pregnant mice only were used and homogenates were sedimented and subsampled after a single centrifugation at 105,000 g.

Although most of the alkaline phosphatase activity in the mouse uterus is clearly membrane-associated and there is a significantly higher proportion of the enzyme in this bound form on days 6, 7 and 8 of pregnancy, it is unlikely that the membrane microenvironment or membrane binding per se exerts any regulatory effect on the enzyme. Thus the present results show that, in general, the catalytic properties of the uterine alkaline phosphatase are not significantly different when the enzyme is present in a membrane-bound or cytosol form, and furthermore are not altered following solubilization with Triton X-100 or when lipids are extracted with n-butanol.

Hung and Melnykovych (1977) claimed that binding of alkaline phosphatase from HeLa cells to phospholipid membrane models or liposomes containing a 1:1 lysophosphatidyl choline to cholesterol ratio, enhanced enzyme activity. In the present study, however, association of mouse uterine alkaline phosphatase with liposomes decreased enzymic activity regardless of the proportion of cholesterol present. The reason for this discrepancy is not clear and requires further investigation.

The similar anodal migration rates of the solubilized enzyme preparations during electrophoresis on cellulose acetate membranes suggests that the membrane-bound and cytosol forms of the enzyme do not represent different molecular species and confirms previous observations (Murdoch et al. 1979) that alkaline phosphatase in the mouse uterus exists as a single isozymic form. It is considered therefore that much of the activity in the cytosol is due to enzyme released from the particulate fraction during homogenization of the tissue. In contrast, Simon and Sutherland (1977) claim that the alkaline phosphatases from membrane and cytosol fractions of rat liver exist in different structural forms.

The responses of the uterine enzyme to the cations, Mg$_2^+$ and Zn$_2^+$, and to changes in pH are similar to those reported by Murdoch et al. (1980). The activities of all apoenzyme preparations were completely restored by the addition of both Mg$_2^+$ and Zn$_2^+$ and none were reactivated to any extent by the separate addition of the cations. This observation is in contrast to that of Simon and Sutherland (1977) who found that the different molecular forms of the apoenzymes of alkaline phosphatase in rat liver have different divalent cation requirements. Ey and Ferber (1977) claim that the membrane-bound form of calf thymus alkaline phosphatase is more resistant to inactivation by loss of essential metal ions in EDTA than purified preparations of the enzyme. In the present study, the association of mouse uterine alkaline phosphatase with decidual cell membranes did not prevent the successful preparation of an inactive apoenzyme following dialysis against EDTA. This finding lends support to the proposal (Murdoch et al. 1978) that the active sites of the mouse uterine enzyme are not deeply buried within the membranes of these cells. Apparent $K_m$ values for the enzyme hydrolysing p-nitrophenyl phosphate were also
unaltered by membrane association providing further evidence that the active sites of the enzyme are not masked or in any way influenced by membrane components and are readily accessible to substrate.

Membrane association also failed to influence the thermostability of the enzyme in the present study and the loss of all enzyme activity after heating for 15 min at 60°C is consistent with previous results (Murdoch et al. 1980). The relationship between temperature and the apparent $K_m$ value for the enzyme hydrolysing p-nitrophenyl phosphate is similar to that reported for pig kidney alkaline phosphatase (Hiwada and Wachsmuth 1974). Silvius et al. (1978) claimed that such a relationship may frequently introduce artefacts into Arrhenius plots in the form of a transition and thereby influence the determination of the energy of activation. This problem was not encountered in the present study and values calculated for the energy of activation were in close agreement with those previously determined for purified preparations of the uterine enzyme (Murdoch et al. 1980).

The similar responses of membrane-bound, cytosolic and purified enzyme preparations to amino acids and other compounds described in Table 2 further supports the proposal that alkaline phosphatase exists as a single isozymic form in the uterus of the mouse and is not profoundly influenced by the membrane microenvironment. The insensitivity of the mouse uterine enzyme to phenylalanine and its inhibition by homoarginine places this enzyme in the liver-type class of alkaline phosphatase (Fishman and Sie 1971). The mechanism whereby various amino acids stimulate the activity of the enzyme remain to be elucidated. No inhibitory effects of the carbonate–bicarbonate buffer system are involved and because the phosphoryl acceptor, mannitol, failed to affect activity, the amino acids are not considered to stimulate the enzyme by accepting liberated phosphoryl groups (Fernley 1971). Ghosh et al. (1977) also failed to detect any evidence of transphosphorylation with alkaline phosphatase in glycine buffer, although activity was greater in glycine buffer than in Tris buffer.

In conclusion, the results indicate that the membrane microenvironment is unlikely to be a major factor involved in the regulation of preformed uterine alkaline phosphatase during the decidual cell transformation accompanying early pregnancy in the mouse. However, mouse uterine alkaline phosphatase is a glycoprotein (Murdoch et al. 1980). Thus membrane-associated glycosylation events that occur in the endoplasmic reticulum and Golgi apparatus (Cook 1973) when the enzyme is being formed cannot be ruled out by the present study as areas for further consideration.

Acknowledgments

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References


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