the surface pressure result at urea concentrations of 1.5–6.0 M, the range in which we observed the activating effects of urea. With 8.0 M urea or 0.1 M bisulfite there are rather large increases in surface pressure and marked inactivation of the enzyme. The action of urea or sulfite to unfold or expand lysozyme molecules spread in monolayers at the air–water interface has been reported[5,6]. However, treatment with guanidine hydrochloride at 1.0 or 4.0 M also induces moderate increases in surface pressure but nearly complete inactivation of the enzyme. Hence it seems that moderate and apparently reversible unfolding of lysozyme can either enhance or greatly reduce activity depending upon the agent employed. Greater unfolding such as is produced apparently by 8.0 M urea or reduction with bisulfite is associated with inactivation. It would be desirable to confirm the effects of urea or other denaturants by assaying with a better-defined substrate such as an oligosaccharide of *N*-acetylglucosamine.

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Inorganic pyrophosphatase activity of human placental alkaline phosphatase

Alkaline phosphatase (orthophosphoric monoester phosphohydrolase, EC 3.1.3.1) has been reported to be capable of inorganic pyrophosphate phosphohydrolase activity[1–5]. In other studies with alkaline phosphatase pyrophosphatase activity has not been observed, and consequently, there is uncertainty whether the enzyme reacts with inorganic pyrophosphate[6–8]. The present studies which were conducted with a highly purified human placental alkaline phosphatase demonstrate both inorganic pyrophosphate phosphohydrolase and phosphotransferase activities as properties of this enzyme.

The procedures for purification of placental alkaline phosphatase have been

described[9]. In brief, alkaline phosphatase was extracted from homogenates of human placentae with *n*-butyl alcohol and was purified successively by acetone precipitation (60%, v/v), chromatography on DEAE-cellulose eluting with a linear gradient between 0.03 and 0.10 M NaCl in 0.01 M Tris–HCl buffer, pH 7.4, and gel filtration through Sephadex G-200. The purity of the resultant enzyme preparation was evaluated by polyacrylamide gel disc electrophoresis and by ultracentrifugal analysis. The preparation gave a single coincident band when stained for both enzyme activity and protein after separation on a 7.5% gel at pH 9.5 (ref. 10) and was a single protein band (not stained for enzyme) after separation on a 7.5% gel at pH 4.3 (ref. 11). After sedimentation equilibrium experiments[12] the plots of ln *c versus* r^2 were linear, indicating homogeneity, and the molecular weight was calculated to be 116 000 (ref. 13). The specific activity was 285 μmoles of *p*-nitrophenol liberated from *p*-nitrophenyl phosphate per min per mg protein at 37° and pH 11.5.

In the present experiments the reaction of purified placental alkaline phosphatase with PP_i was compared with its reactions with a monophosphate ester. Phosphohydrolase and phosphotransferase activities were measured, using either PP_i or *p*-nitrophenyl phosphate as substrates. Phosphohydrolase activity was evaluated by measuring *p*-nitrophenol[14] or inorganic phosphorus[15]. Phosphotransferase activity was evaluated using 2.0 M glycerol and 2.0 M glucose as phosphoryl acceptors and measuring the products of the reaction, α-glycerophosphate and glucose 6-phosphate. Glucose 6-phosphate was measured by the reduction of $NADP^+$ in the presence of glucose-6-phosphate dehydrogenase, and α-glycerophosphate by the reduction of NAD^+ in the presence of α-glycerophosphate dehydrogenase. Both reactions were measured by fluorescence spectrophotometry (λ excitation, 360 mμ, λ emission, 410 mμ).

The optimum conditions for phosphohydrolase and phosphotransferase activities were determined for each substrate. Each reaction was evaluated at 25°, 37°, and 56° at optimum pH and substrate concentration. Under these conditions the pH optima were unaffected by temperature. With PP_i as substrate, the pH optimum for both phosphohydrolase and phosphotransferase activities was pH 8.5. With *p*-nitrophenyl phosphate as substrate, the pH optimum for phosphohydrolase activity with no acceptor or with glycerol as acceptor was pH 11.5. Experiments with glucose as acceptor were performed at pH 10.0 because above this pH side products were formed which interfered with the enzymic reaction. The reaction rate for both phosphohydrolase and phosphotransferase activities increased up to 56° with either substrate. Both activities were stable at 56° for 30 min with each substrate; partial inactivation (50%) occurred at 75°.

A comparison of the phosphohydrolase and phosphotransferase activities of the enzyme with each substrate is presented in Table I. Enzyme activity for each reaction was greater with *p*-nitrophenyl phosphate as substrate. Both glycerol and glucose at 2.0 M concentration slightly inhibited phosphohydrolase activity. Glycerol was a better phosphoryl acceptor than glucose. Relative phosphohydrolase activity, *p*-nitrophenyl phosphate:PP_i, was 38:1; and relative phosphotransferase activities, *p*-nitrophenyl phosphate:PP_i, were 13:1 with glycerol as acceptor and 12:1 with glucose as acceptor. These ratios were constant at each temperature tested.

These experiments demonstrate that human placental alkaline phosphatase reacts with PP_i and catalyzes both pyrophosphate phosphohydrolase and pyrophosphate phosphotransferase reactions. It is unlikely that these reactions were from a

TABLE I

COMPARISON OF MONOESTER PHOSPHATE AND INORGANIC PYROPHOSPHATE PHOSPHOHYDROLASE AND PHOSPHOTRANSFERASE ACTIVITIES OF PURIFIED HUMAN PLACENTAL ALKALINE PHOSPHATASE

The reaction mixture contained in 0.5 ml final volume, 30.0 μmoles of glycine or citrate as buffer; 2 μmoles of *p*-nitrophenyl phosphate as substrate and 0.05 μmole of $MgCl_2$ as cofactor or 0.5 μmole PP_i as substrate and 0.25 μmole of $MgCl_2$ as cofactor. In phosphotransferase experiments, the reaction mixture contained in addition 1000 μmoles of glycerol or glucose as acceptor. The pH of the reaction was pH 8.5 with PP_i (citrate buffer), pH 11.5 with *p*-nitrophenyl phosphate with no acceptor or with glycerol and pH 10.0 with glucose as acceptor (glycine buffers). Reactions were performed at 37°. Each reaction was started by addition of 1 μg of purified placental alkaline phosphatase (spec. activity, 285 μmoles *p*-nitrophenol liberated per mg per min at 37° and pH 11.5). The reaction was stopped with 0.05 M H_2SO_4 at 3 min with *p*-nitrophenyl phosphate as substrate and at 90 min with PP_i as substrate. Glucose 6-phosphate was determined in 1 ml final volume containing 500 μmoles of triethanolamine as buffer at pH 7.8, 0.5 μmole of $NADP^+$ and 0.6 I.U. glucose-6-phosphate dehydrogenase. α-Glycerophosphate was determined in 1 ml final volume containing 500 μmoles of glycine, 200 μmoles of hydrazine, 2.5 μmoles of EDTA as buffer at pH 9.6, 0.5 μmole of NAD^+ and 1.5 I.U. α-glycerophosphate dehydrogenase.

Reactants	*Phosphohydrolase activity**		*Phosphotransferase activity***	
	PP_i	*p-Nitrophenyl phosphate*	*PP_i*	*p-Nitrophenyl phosphate*
Substrate; no acceptor	7.5	285	—	—
Substrate + 2.0 M glycerol	6.0	265	0.4	5.2
Substrate + 2.0 M glucose	5.5	190	0.1	1.2

* nmoles of either P (PP_i substrate) or *p*-nitrophenol (*p*-nitrophenyl phosphate substrate) liberated per min per reaction volume (0.5 ml).

** nmoles of either α-glycerophosphate or glucose 6 phosphate formed per min per reaction volume (0.5 ml).

contaminant pyrophosphatase in the enzyme preparation because, in addition to the established purity of the enzyme preparation, the reactions with pyrophosphate were heat stable and a constant ratio was obtained comparing activity with *p*-nitrophenyl phosphate to PP_i as substrates at 25°, 37°, and 56°.

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