[Chem. Pharm. Bull. 23(3) 686—687 (1975)]

UDC 547.466.1.05 : 615.355.011.5

Purification of Alkaline Phosphatase from Human Liver[1)]

Alkaline phosphatase from human liver was extracted by a modified Morton's butanol method and was purified by ammonium sulfate precipitation followed by diethylaminoethyl-cellulose, carboxymethyl-cellulose and Sephadex G-200 column chromatography.

Liver alkaline phosphatase thus purified to about 8900-fold with recovery of 10% was demonstrated to be homogeneous by polyacrylamide gel disc electrophoresis.

There is evidence that human alkaline phosphatase (orthophosphoric monoester phosphohydrolase E.C. 3.1.3.1) exists in multiple forms[2)] and that the forms found in different organs possess distinct properties[3)] by which they may be recognized. However, interpretations have not always been in agreement on the existence or definition of specific organ forms. Some of the difficulties may have been due to insufficiently purified enzyme preparations and to the limitations of using physical properties or differential substrate activity to define molecular differences.

It seems very interesting to us to clarify the enzymological properties and its structures of human organ specific alkaline phosphatase. As a series of studies on human alkaline phosphatase, we have already reported that purification of human placental alkaline phosphatase and its radioimmunoassay,[4)] and recently had a success in the purification of alkaline phosphatase from human intestinal mucosa.[5)]

In this paper, we report the purification of alkaline phosphatase from human liver.

Frozen human liver from metastatic liver cancer (tumor tissue was removed) 300 g was homogenized with water and butanol (liver: water: butanol=1: 3: 1). The supernatant was treated with 60% acetone in the cold to precipitate the protein and distilled water was added to the precipitate. The precipitate obtained at the concentration of 0.5—0.6 saturation of ammonium sulfate was collected and dialyzed against 10 mM Tris buffer (pH 7.4) containing 10 μM of $ZnCl_2$ and $MgCl_2$.

TABLE I. Purification Procedure of Alkaline Phosphatase from Human Liver

Procedure	Total activity (U)	Total protein (O.D. 280 nm)	Specific activity (U/O.D. 280 nm)
Crude enzyme	2000	8300	0.24
Fractional precipitation by ammonium sulfate	1400	255	5.49
DEAE-cellulose column chromatography	1070	29.2	36.6
CM-cellulose column chromatography	470	3.82	123
Sephadex G-200 gel filtration	200	0.093	2150

Activity was determined by use of Kind-King method; P.R.N. Kind and E.J. King, *J. Clin. Pathol.*, 7, 322 (1954)
Units of enzyme activity represent μmoles of phenol released per minute at 37°.

1) This paper forms part XCVI of "Studies on Enzymes"; preceding paper, Part XCV: M. Sugiura and M. Isobe, *Chem. Pharm. Bull.* (Tokyo), submitted.
2) *a*) K. Lorentz, B. Flatter and D. Heydrich, *Z. Klin. Chem. Klin. Biochem.*, 12, 81 (1974); *b*) H. Suzuki, M. Yamanaka, and T. Oda, *Ann. N.Y. Acad. Sci.*, 166, 811 (1969).
3) *a*) D.W Moss., *Clin. Chem. Acta*, 35, 413 (1971); *b*) W.H Fishman. and H.G Sie., *Clin. Chim. Acta*, 29, 339 (1970).
4) S. Iino, K. Abe, T. Oda, H. Suzuki, and M. Sugiura, *Clin. Chim. Acta*, 42, 161 (1972).
5) M. Sugiura, M. Isobe, K. Hirano, S. Iino, H. Suzuki and T. Oda, *Chem. Pharm. Bull.* (Tokyo), submitted.

The dialyzed enzyme solution was purified by the column chromatography with diethylaminoethyl (DEAE)-cellulose (3.0×10 cm). The enzyme solution was applied onto the column which had been equilibrated with the same buffer as used in dialysis. After washing the column, alkaline phosphatase was eluted by changing the concentration of NaCl gradiently from 0 to 0.3M, and the active fraction was collected and concentrated by the membrane filter.

The enzyme solution was dialyzed against 10 mM citrate buffer (pH 5.0) containing 10 μM $ZnCl_2$ and $MgCl_2$ for 6 hr. The solution was passed a column (2.0×30 cm) of carboxymethyl (CM)-cellulose which had been equilibrated with the same buffer as used in dialysis and the effluent was collected. After concentration, the solution was dialyzed against 10 mM Tris buffer (pH 8.0) containing 10 μM of $ZnCl_2$, $MgCl_2$ and 0.1M NaCl.

The dialyzed enzyme solution was purified on a column (2.5×100 cm) of Sephadex G-200 which had been equilibrated with same buffer as used in dialysis. Above purification procedures were summarized in Table I.

Human liver alkaline phosphatase was purified about 8900-fold based on the acetone precipitate level with recovery of 10%. The resulting enzyme gave a single band of protein by disc electrophoresis using 7.5% polyacrylamide gel at pH 9.4 as shown in Fig. 1.

Enzymological and immunological properties will be reported successively.

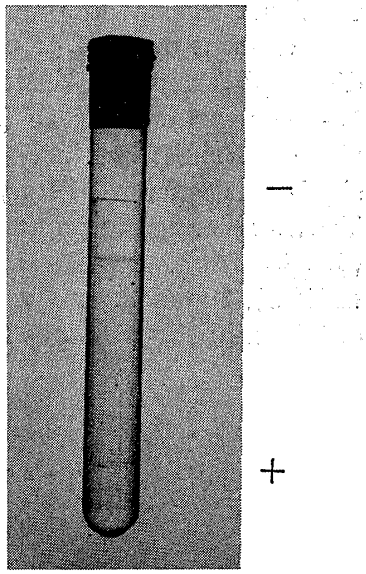

Fig. 1. Electrophoretic Pattern of Purified Alkaline Phosphatase from Human Liver

Electrophoresis was carried out at pH 9.4 under a constant current of 4 mA/tube for 70 min.

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Received October 30, 1974

[Chem. Pharm. Bull. 23(3) 687—689 (1975)]

UDC 547.677.2'717.04 : 547.562.1.04

Alkylation of Phenols by Phenanthrene 9,10-Oxide[1)]

The reactions of phenanthrene, 9,10-oxide (1) and phenols or phenoxides were investigated. In dipolar aprotic solvents, 1 alkylates only the oxygen atom of the ambident phenoxide. In protic solvents, C-alkylation of phenol is observed.

The recognition that arene oxides may play a significant role in carcinogenesis by polynuclear hydrocarbons is growing. The reaction between polycyclic arene oxides and biomolecules such as nucleic acid bases or protein residues, is thought to be responsible for the

1) Paper 4 in a series on the Chemistry of Carcinogenic Functional Groups. See the previous paper, K. Shudo and T. Okamoto, *Chem. Pharm. Bull.* (Tokyo), **21**, 2809 (1973).