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Dephosphorylation of MnDPDP and related compounds by acid and alkaline phosphatase

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Abstract

The enzymatic dephosphorylation of the magnetic resonance imaging contrast agent Teslascan® was studied in in vitro experiments with acid phosphatase (prostatic, from human semen) and alkaline phosphatase (from human placenta). The active component, MnDPDP (manganese (II)-*N*,*N*′-dipyridoxylethylenediamine-*N*,*N*′-diacetate-5,5′-bis(phosphate), was dephosphorylated by both enzymes to the monophosphate MnDPMP and the totally dephosphorylated compound MnPLED. The corresponding zinc compound, ZnDPDP (which is a result of in vivo metabolism), was also dephosphorylated by both enzymes to ZnDPMP and ZnPLED. In separate experiments, both enzymes dephosphorylated MnDPMP and ZnDPMP. With the same amount of enzyme units, alkaline phosphatase was almost four times more active than acid phosphatase in dephosphorylating MnDPDP and ZnDPDP with only minor differences whether the substrate contained Mn or Zn. A similar difference in enzymatic activity was seen with the monophosphates, MnDPMP and ZnDPMP. This, taken together with the approximately 50 times higher activity of alkaline phosphatase than acid phosphatase in serum shows that alkaline phosphatase is responsible for most of the dephosphorylation of MnDPDP and its metabolites in vivo.

Keywords: Acid phosphatase; Alkaline phosphatase; Teslascan®; Mangafodipir trisodium; MnDPDP; Metabolism

1. Introduction

Mangafodipir trisodium injection (Teslascan®; a proprietary pharmaceutical formulation of Nycomed Amersham plc, London, UK) contains MnDPDP (manganese (II)-*N*,*N*′-dipyridoxylethylenediamine-*N*,*N*′-diacetate-5,5′-*bis*(phosphate); structure shown in Fig. 1) as the active component [1]. The product is used as a contrast agent for magnetic resonance (MR) imaging to detect focal liver lesions [2]. The efficacy of MnDPDP is due to the greater Mn uptake by normally functioning hepatocytes than by abnormal or neoplastic hepatocytes or liver metastases of non-hepatocellular origin [3–5].

MnDPDP is metabolised by (i) dephosphorylation to the monophosphate MnDPMP (manganese (II)-*N*,*N*′-dipyridoxylethylenediamine-*N*,

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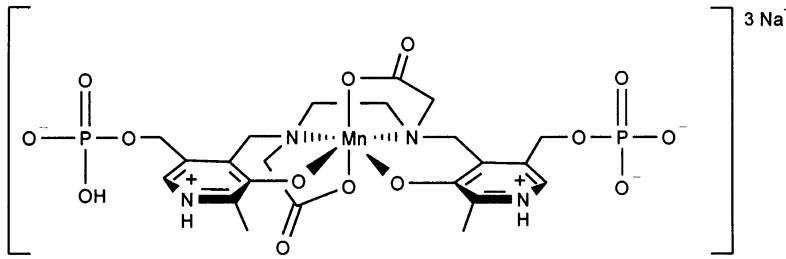

Fig. 1. Structure of the sodium salt of MnDPDP.

N′-diacetate-5-phosphate) and the fully dephosphorylated compound MnPLED (manganese(II)-*N*,*N*′-dipyridoxylethylenediamine-*N*,*N*′-diacetate) and (ii) simultaneous transmetallation to the corresponding zinc metabolites ZnDPDP, ZnDPMP and ZnPLED [6,7]. This metabolism, which is illustrated in Fig. 2, occurs rapidly in vivo, as there is nearly complete conversion of MnDPDP to ZnPLED within 2 h after administration of a clinical dose (5 μmol/kg body weight) in humans [6]. A similar metabolic profile for MnDPDP has been seen in the dog [8].

Alkaline phosphatase is present in practically all tissues in the body as a membrane-bound metalloenzyme consisting of a group of isoenzymes, all glycoproteins [9,10]. The enzyme is bound to cell membranes through a COOH-terminal glycan-phosphatidylinositol anchor [10]. Although the natural substrates for the enzyme have not been identified [11], the enzyme is capable of dephosphorylating a wide variety of naturally occurring and synthetic substrates [9].

The name acid phosphatase refers to a group of similar or related enzymes, which includes all phosphatases with optimal activity below pH 7.0 [12,13]. The enzyme with greatest clinical importance is that derived from the prostate. The acid phosphatases are present mainly in lysosomes, which are organelles present in all mammalian cells except mature erythrocytes, but extralysosomal acid phosphatases are also present in many cells [12,13]. This enzyme is also capable of dephosphorylating a number of different substrates [12].

The purpose of the present study, was to investigate whether the acid and/or alkaline phosphatase were responsible for the dephosphorylation of MnDPDP and its phosphate containing metabolites (MnDPMP, ZnDPDP and ZnDPMP) at physiological pH. The results show that alkaline phosphatase causes most of the dephosphorylation of MnDPDP and its metabolites.

2. Materials and methods

2.1. Chemicals and reagents

MnDPDP, MnDPMP, MnPLED, ZnDPDP, ZnDPMP and ZnPLED were supplied by Nycomed Imaging AS, Oslo, Norway. The synthesis and characterisation of MnDPDP has been described previously [14]. Water was purified by reverse osmosis, ion exchange and filtration through an 0.45-μm filter using a Milli-Q system (Millipore, Bedford, MA). To minimise air oxidation of the Mn, water used to prepare calibration standards and control samples was degassed with N_2-gas for 30 min. Acid phosphatase (prostatic, from human semen; P 1649) and alkaline phosphatase (from human placenta; P 3895) was obtained from Sigma. Other chemicals were of analytical grade.

2.2. Incubations

The four phosphate containing substrates (MnDPDP, MnDPMP, ZnDPDP and ZnDPMP) were solubilised in 20 mM sodium 5,5-diethylbarbiturate, pH 7.2, to a final concentration of 1 mM. To 1 ml of these solutions were added 5 μl buffer containing 0.5 units of either acid or alkaline phosphatase and the solutions were incubated at 37°C. Similar incubations were performed using the four substrate containing solutions, but without addition of enzyme. Following incubation times of 30, 60 and 120 min, aliquots of 50 μl were removed from the incubation solutions and added to 450 μl of an ice-cold solution of the mobile phase used for the HPLC analysis (see below). This mixture was stored on ice until 200 μl was transferred to the HPLC vials; 50 μl was injected into the HPLC system.

2.3. HPLC analysis

The HPLC method was based on separation on a mixed-bed column with both anion exchange and reversed-phase functions (OminiPac™ PAX-500, 4 × 250 mm, 8.5 μm from Dionex, Sunnyvale, CA) and has been described in detail elsewhere [15]. Briefly, an isocratic elution was used with 0.2 M sodium phosphate buffer containing 3% (v/v) acetonitrile as an organic modifier and 1.5 mM tetrabutylammonium phosphate as an ion pairing agent. The pH in the mobile phase was adjusted with 1 M NaOH to pH 9.12. The flow rate of the mobile phase was 1.2 ml/min and the concentrations of the substrates and products were estimated by calibration curves for the different substances generated by UV detection at 310 nm [15].

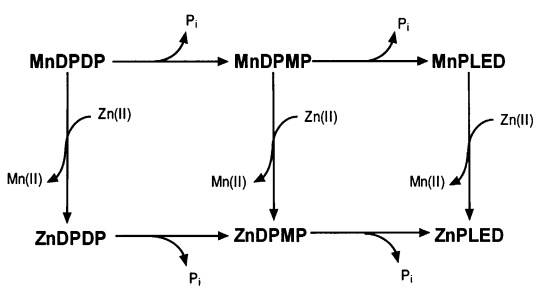

Fig. 2. Schematic representation of the dephosphorylation and transmetallation of MnDPDP.

2.4. Data handling

PA Nelson ACCESS*CHROME GC/LC data sampling system, v. 1.8, was used for sampling and integration of the chromatograms. GraphPad Prism™, v. 2.0, was used for the preparation of calibration curves, calculating the metabolite concentrations and preparing graphs.

3. Results and discussion

The data obtained following incubation of the diphosphates MnDPDP and ZnDPDP with the same amount of enzyme units of either the acid or alkaline phosphatase are shown in Fig. 3. As can be seen the alkaline phosphatase was more active than the acid phosphatase in dephosphorylating both MnDPDP and ZnDPDP. The relative areas of the diphosphates decreased to 23 and 25% for MnDPDP and ZnDPDP, respectively after 120 min of incubation with alkaline phosphatase, whereas the figures for acid phosphatase were 69 and 76% for MnDPDP and ZnDPDP, respectively. Accordingly, there was no significant difference between the rate of dephosphorylation for the Mn or Zn compounds. The data in Fig. 3 also show some dephosphorylation of the monophosphates (MnDPMP and ZnDPMP) to the totally dephosphorylated compounds (MnPLED and ZnPLED), but it is difficult to compare the relative rate of dephosphorylation of the monophosphates based on these data. Separate experiments with the monophosphates as substrates (Fig. 4) show that the effect of the enzymes on MnDPMP and ZnDPMP are similar to that observed for the diphosphates (Fig. 3) although the difference between the enzymes is less for the monophosphates (Table 1). In control experiments performed with incubation in the absence of enzyme, no dephosphorylation of any of the four compounds was observed (data not shown).

The data presented were obtained using the same amount of units for both enzymes. Enzyme

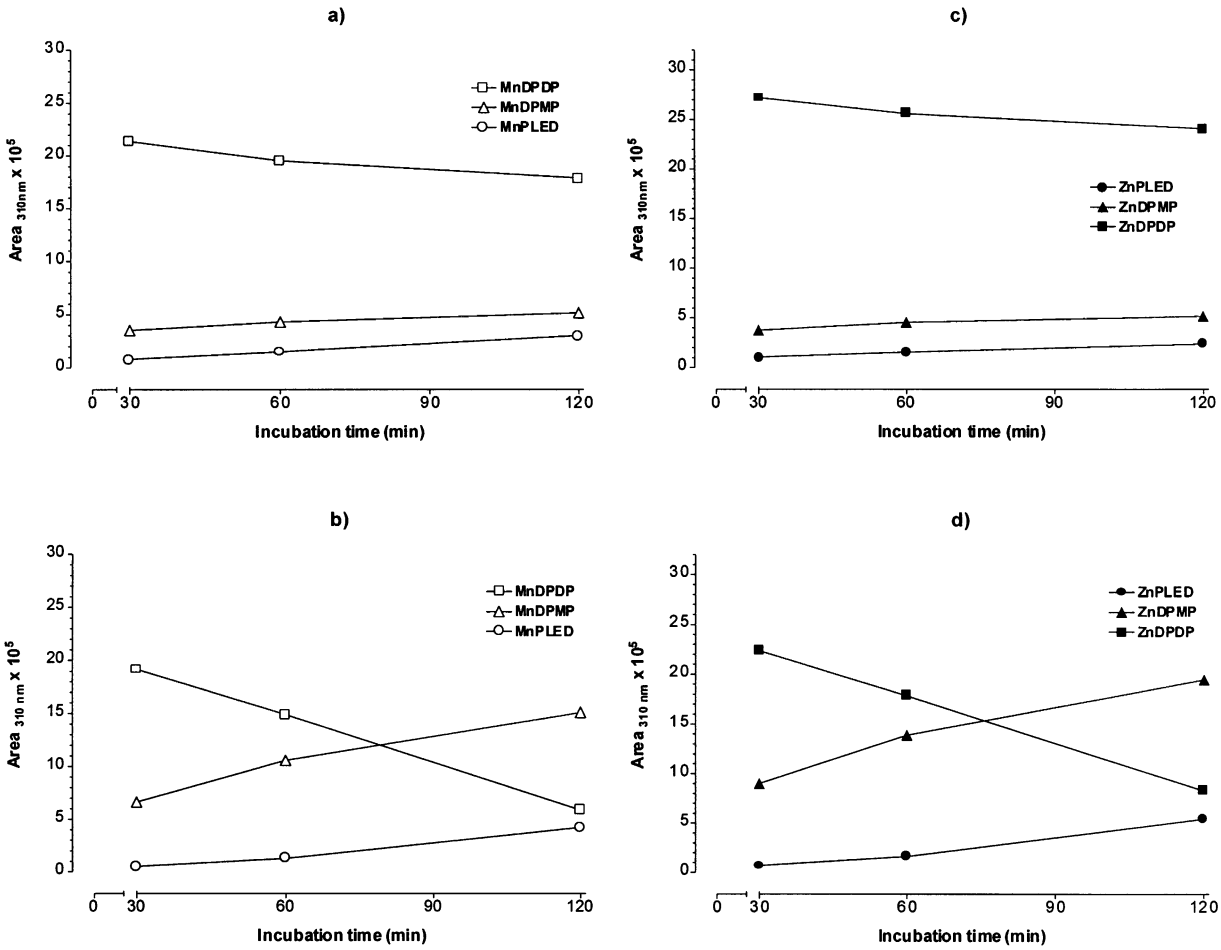

Fig. 3. Dephosphorylation of the diphosphates MnDPDP and ZnDPDP by acid phosphatase (ACP) and alkaline phosphatase (ALP) as a function of time. The left figures show the data obtained with MnDPDP as the substrate; (a) in the presence of ACP; (b) in the presence of ALP. The right figures show the data obtained with ZnDPDP as the substrate; (c) in presence of ACP; (d) in presence of ALP.

activity was checked with the substrate *para*-nitrophenylphosphate for both enzymes (data not shown). Under these conditions the alkaline phosphatase was more active in dephosphorylating MnDPDP and its metabolites MnDPMP, ZnDPDP and ZnDPMP than the acid phosphatase. Moreover, the activity of alkaline phosphatase is approx. 50 times the activity of acid phosphatase in serum, as the reference range of alkaline phosphatase and acid phosphatase is 25–100 and 0.5–1.9 enzyme units/l, respectively [9,12].

Taken together, these data show that the alkaline phosphatase is mainly responsible for the in vitro dephosphorylation of MnDPDP and its metabolites in blood [7]. As discussed earlier [7] the dephosphorylation of these compounds is more rapid in vivo [6]. The reason for a faster dephosphorylation in vivo is not known. This difference, however, may be due to a reduced activity in vitro by inactivation/inhibition of the phosphatases, or increased activity in vivo by the activity in plasma being supplemented by tissue bound phosphatases that are present at or in the cell membranes in practically all tissues of the

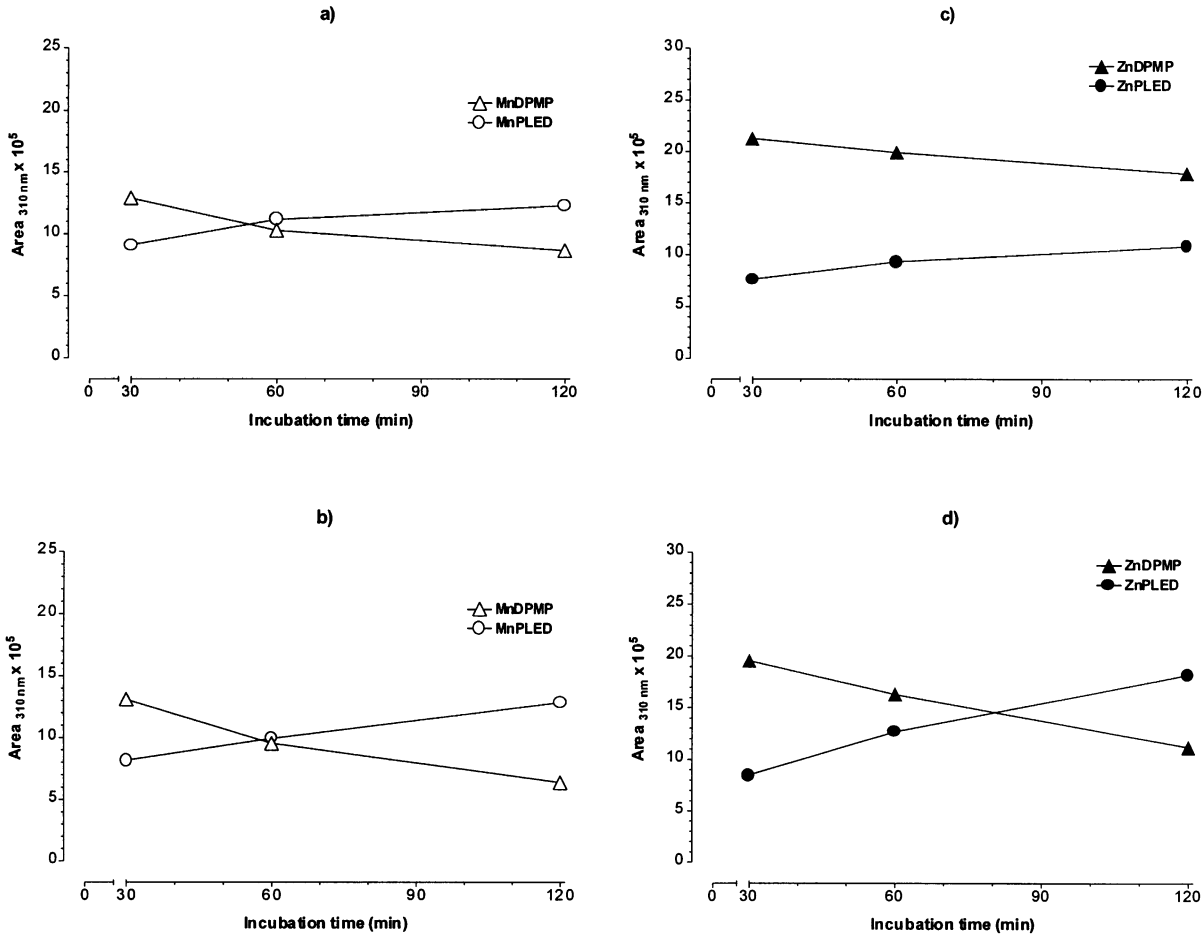

Fig. 4. Dephosphorylation of the monophosphates MnDPMP and ZnDPMP by acid phosphatase (ACP) and alkaline phosphatase (ALP) as a function of time. The left figures show the data obtained with MnDPMP as the substrate; (a) in presence of ACP; (b) in presence of ALP. The right figures show the data obtained with ZnDPMP as the substrate; (c) in presence of ACP; (d) in presence of ALP.

Table 1
Relative dephosphorylation rates from 30 to 120 min[a]

Enzyme	Diphosphates		Monophosphates	
	MnDPDP (%)	ZnDPDP (%)	MnDPMP (%)	ZnDPMP (%)
Acid phosphatase	25	22	50	41
Alkaline phosphatase	94	100	80	100

[a] The rates calculated as decrease in area units/min from Figs. 3 and 4, are transformed to % of the highest dephosphorylation rate for diphosphates and monophosphates, respectively.

body [9]. Since similar metabolic profiles of Mn-DPDP have been seen in the dog [8] and man [6], it is likely that the findings of this study apply to several mammalian species.

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