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SEPARATION AND MOLECULAR WEIGHT DETERMINATION OF PROSTAGLANDIN B_1 OLIGOMERS BY SIZE-EXCLUSION CHROMATOGRAPHY

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SUMMARY

An efficient size-exclusion chromatographic method for the simultaneous separation and molecular weight (MW) determination of prostaglandin (PG) oligomers on Sephadex G-50 with borate buffer is described. The prostaglandins 15-keto-PGB_1 and 16,16-dimethyl-15-keto-PGB_1 were used for the synthesis of oligomers. MW determinations in the monomer to octamer range is based on the linear correlation between the partition coefficient and log (MW) of the oligomers. The essential role of the borate anion between the gel matrix and the oligomers is demonstrated.

INTRODUCTION

The polymeric prostaglandins PGB_x with a molecular weight (MW) of 2200–2500 (6–7 monomeric units), synthesized from 15-keto-PGB_1, has been shown to maintain oxidative phosphorylation during hypotonic degradation in aged mitochondria isolated from rat liver[1,2]. It also stimulates the release of Ca^{2+} from the fragmented sarcoplasmic reticulum and heart mitochondria. The observed ionophoric activity of PGB_x is approximately two orders of magnitude greater than that of other monomeric prostaglandins[3]. The complexity of an oligomeric mixture due to several reactive sites in the 15-keto-PGB_1 molecule led to the synthesis of 16,16-dimethyl-15-keto-PGB_1 oligomers. Oligomers of the 16,16-dimethyl analogue of 15-keto-PGB_1 also exhibit protection from the loss of oxidative phosphorylation in isolated rat liver mitochondria[4] and the Ca^{2+} ionophoric activity[5].

The separation of physiologically active oligomers from a crude polymerization mixture by size-exclusion chromatography on Sephadex LH-20 using methanol as an eluent has been described[2,6]. However, the quality of the separation on Sephadex LH-20 and/or LH-60 with methanol is not satisfactory owing to the absence of well defined peaks on the elution curve. As a result of the poor resolution, vapour pressure osmometric measurements were required in order to determine the average MW in each of the collected fractions. The first attempts to determine the MW of

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prostaglandin oligomers by gel chromatography have been reported previously[7]. In this paper, we describe a method of size-exclusion chromatography on Sephadex G-50 which gives an excellent resolution and permits the MW determination of prostaglandin B_1 (PGB_1) oligomers synthesized from 15-keto-PGB_1 and 16,16-dimethyl-15-keto-PGB_1.

EXPERIMENTAL

Synthesis of oligomers

Prostaglandins I and II (Fig.1) were synthesized starting from ethyl 5-oxo-1-cyclopentene-1-heptanoate[8]. The Michael addition of nitromethane to the enone unit followed by Nef reaction[9] and Wittig–Horner reaction with dimethyl 2-oxoalkylphosphonate gave the 15-ketoprostaglandins. Introduction of a double bond into the cyclopentane ring system was accomplished with copper(II) bromide according to Miller *et al.*[10]. The ^{13}C NMR chemical shifts in ppm downfield from internal tetramethylsilane standard are given in Fig. 1. The chemical shifts for II are consistent with the literature[11,12]. Assignments in I are based on the chemical shifts in II and multiplicities of the carbon-13 signals in monoresonance spectra.

Preparation and purification of dimers were accomplished according to Polis *et al.*[11]. Dimers are formed by base-catalysed Michael addition in which two nucleophilic (C-10, C-16) and two acceptor (C-13, C-14) sites of II[6] and one nucleophilic (C-10) and two acceptor (C-13, C-14) sites of I are active[5]. The presence of multiple reaction sites coupled with the formation of two new chiral centres for each new bond formed result in the formation of a complicated mixture of structural isomers further complicated by the presence of closely related stereoisomers[13]. The UV spectra of dimers of I and II showed two absorption peaks with maxima at 296 and 238 nm, observed to be the absorption maxima of the monomers (296 nm) and of the cyclopentenone unit (238 nm) formed by the disappearance of the 13,14-double bond in the Michael addition reaction[11]. Secondary ion mass spectrometric (SIMS) measurements of I dimer with glycerine, glycerine–NaCl and glycerine–$NaHCO_3$ liquid matrixes were carried out[14].

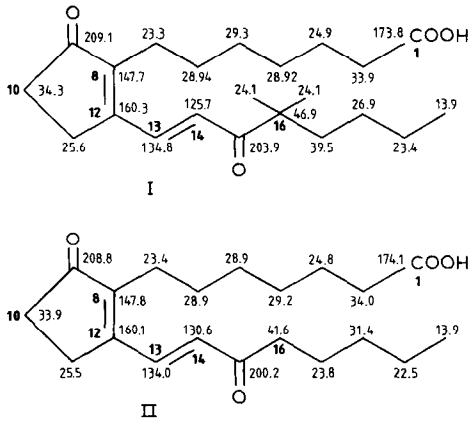

Fig. 1. Monomeric prostaglandins 16,16-dimethyl-15-keto-PGB_1 (I) and 15-keto-PGB_1 (II) as precursors of corresponding oligomers. ^{13}C NMR chemical shifts in ppm downfield from internal TMS are shown.

The abundant ions at m/z 725 (M + H), 747 (M + Na), 769 (M + 2Na − H) and 791 (M + 3Na − 2H) show that the I dimer with three acidic hydrogens (two carboxylic and one presumably at C-10) was obtained.

The treatment of 16,16-dimethyl-15-keto-PGB_1 (I) and/or 15-keto-PGB_1 (II) with 1 *M* 50% ethanolic KOH (2.5 mg/ml PG) for 30 min at 20°C gave a crude reaction product. After neutralization with 3 *M* HCl, the oligomers were extracted with ethyl acetate. The organic layer was washed with saturated KCl solution and oligomers were extracted into 50 m*M* borate buffer (pH 8.9). The final concentration of oligomers was about 5 mg/ml. The remaining trace amounts of ethyl acetate were removed by vacuum evaporation. The dimeric and monomeric prostaglandins were also dissolved in 50 m*M* borate buffer (pH 8.9) at a concentration of 5 mg/ml.

Chromatographic materials and equipment

Sephadex G-25, G-50, G-75, LH-20 and LH-60 gels were purchased from Pharmacia and dextran blue from Fluka. Methanol, KH_2PO_4, $KHCO_3$, H_3BO_3, KOH and KCl were obtained from Reachim; methanol was distilled before use. Doubly distilled water was used to prepare the buffer solutions. An LKB gel filtration apparatus including a Varioperpex peristaltic pump, a two-channel Uvicord III ultraviolet detector, a RediRac fraction collector, a two-channel 2210 recorder and 150 × 2.8 cm I.D. glass columns with a volume of 920 ml were used.

Chromatographic conditions

Sephadex G-25, G-50 and G-75 columns were eluted with 50 m*M* borate, 50 m*M* carbonate or 50 m*M* phosphate buffer solutions at pH 8.9, 9.0 and 8.0, respectively. Methanol was used as the eluent with Sephadex LH-20 and LH-60 columns. The sample size did not exceed 6 ml. The flow-rate was adjusted to 40 ml/h. The time interval between fractions was 6 min. The elution process was monitored by a UV detector simultaneously at 254 and 206 nm. Elution volumes (V_e) were calculated from the printed-out retention times. The void volumes (V_0) were determined by using dextran blue. The partition coefficients (K_{av}) were calculated using the equation $K_{av} = (V_e - V_0)/(V_t - V_0)$, where V_t is the total volume of the chromatographic bed[15].

RESULTS AND DISCUSSION

The elution curves of 15-keto-PGB_1 oligomers on Sephadex LH-type columns are presented in Fig. 2. The appearance of several maxima on the elution curve obtained with LH-60 instead of LH-20 suggest some improvement in resolution. Further investigations on Sephadex G-50 demonstrated that the fractions collected from the main peak on the LH-60 elution curve contain oligomers from the dimer to the hexamer.

In comparison with other Sephadex G-type media, the best results were obtained using a Sephadex G-50 column and 50 m*M* borate buffer at pH 8.9 as the eluent (Fig. 3). The distribution coefficients, K_{av}, for monomers and dimers were measured by eluting them separately. They both gave a single peak and the K_{av} values for 15-keto-PGB_1 and its dimer were 0.655 and 0.47 and for 16,16-dimethyl-15-keto-PGB_1 and its dimer 0.735 and 0.51, respectively. The K_{av} values were plotted against log (MW), assuming that the third, fourth and fifth peaks on the elution curve

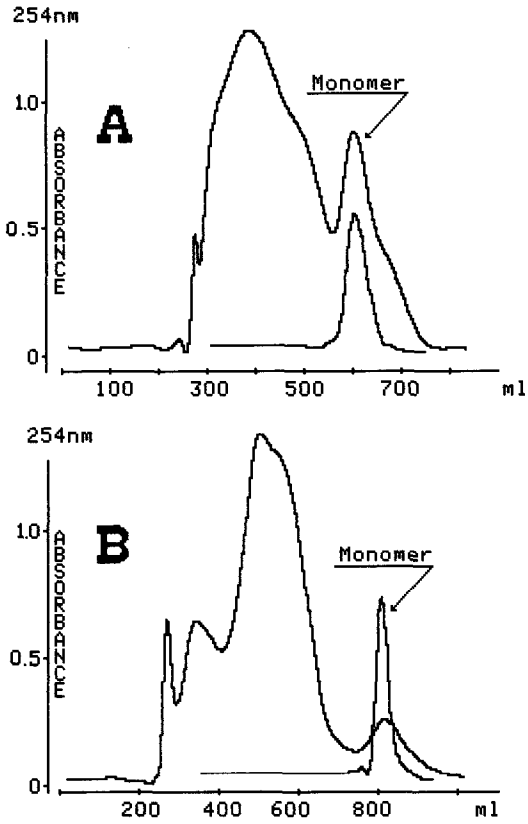

Fig. 2. Chromatography of 15-keto-PGB$_1$ oligomers on Sephadex LH-20 (A) and LH-60 (B) with methanol as eluent.

correspond to the trimer, tetramer and pentamer, respectively. The correlation coefficient, R, and the linear regression coefficients, k_1 and k_2, were obtained by least-squares fitting. Their values in the equation $K_{av} = k_1 - k_2 \log (MW)$ are presented in Fig. 4. A linear correlation between K_{av} and log (MW) with correlation coefficient $R = 0.999$ confirms that the third peak corresponds to the trimer, the fourth peak to the tetramer, etc. This permits the simultaneous separation and MW determination of oligomers. MW determinations up to the octamer are possible after extrapolation of the linear relationship between K_{av} and log (MW).

The resolution on Sephadex G-50 was strongly affected when borate buffer was replaced with sodium phosphate buffer (*cf.*, Figs. 3 and 5). Similar results were obtained by using carbonate buffer. This suggests that the borate anion plays a critical role in the separation process. We propose that, under our conditions, complexation of the borate and Sephadex occurs. There is other evidence that the borate anions give complexes with dextrans as it gives rise to undesirable phenomena in the traditional gel filtration of proteins[16]. Interestingly, the addition of 0.2 M NaCl to the borate buffer in an effort to increase the ionic strength leads to the disappearance of individual peaks on the elution curve (Fig. 6). The good linear correlation ($R = 0.999$) between K_{av} and

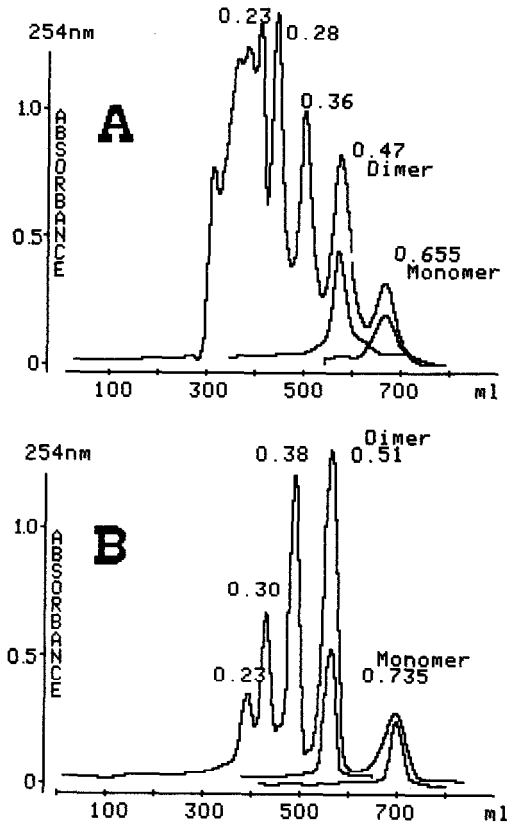

Fig. 3. Chromatography of 15-keto-PGB_1 oligomers (A) and 16,16-dimethyl-15-keto-PGB_1 oligomers (B) on Sephadex *G-50* with *50* m*M* borate buffer (pH *8.9*) as eluent. Numbers at the peaks are K_{av} values.

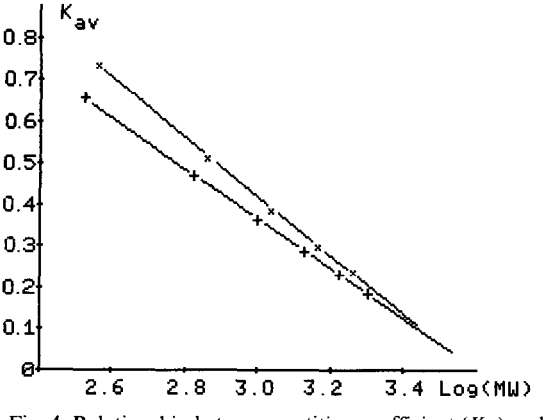

Fig. 4. Relationship between partition coefficient (K_{av}) and molecular weight (MW) of 15-keto-PGB_1 (+) and 16,16-dimethyl-15-keto-PGB_1 (×) oligomers on Sephadex *G-50* with *50* m*M* borate buffer (pH *8.9*) as eluent. Coefficients k_1 and k_2 in the equation $K_{av} = k_1 - k_2 \log(MW)$ were obtained by least-squares fitting. ×: $k_1 = 2.58 \pm 0.03$; $k_2 = 0.72 \pm 0.01$; $r = 0.9997$. +: $k_1 = 2.20 \pm 0.02$; $k_2 = 0.61 \pm 0.01$; $r = 0.9998$.

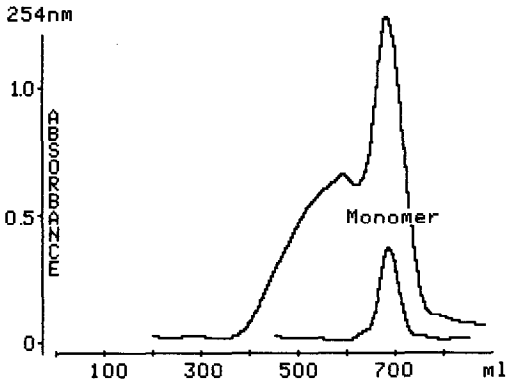

Fig. 5. Chromatography of 15-keto-PGB_1 oligomeric mixture on Sephadex G-50 with 50 m*M* phosphate buffer (pH 8.0) as eluent.

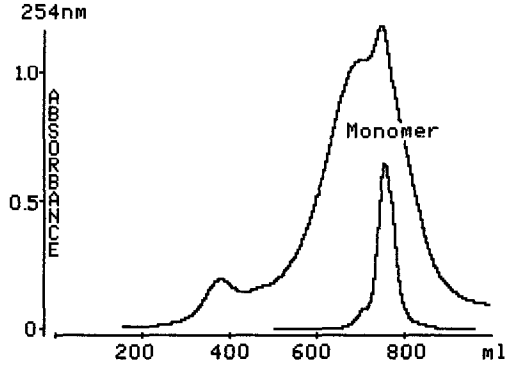

Fig. 6. Chromatography of 15-keto-PGB_1 oligomers on Sephadex G-50 with 50 m*M* borate buffer–0.2 *M* NaCl (pH 8.9) as eluent.

log (MW) suggests that only the molecular sieving process take place with borate buffer. Replacing the buffer anion or adding NaCl results in a decrease in resolution and therefore we conclude that borate buffer with a low molarity is required to prevent secondary, perhaps hydrophobic, interactions between the prostaglandin oligomers and the gel matrix.

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