Purification of Phosphodiesterase II from Rat and Guinea-Pig Intestinal Mucosa

By PETER R. FLANAGAN* and SIDNEY H. ZBARSKY

*Department of Biochemistry, University of British Columbia,
Vancouver, B.C. V6T 1W5, Canada*

(Received 12 December 1975)

Phosphodiesterase II from extracts of intestinal mucosa of rat and guinea pig was purified by chromatography on DEAE-cellulose, CM-cellulose and agarose. The rat enzyme was purified 350–550-fold, with recoveries ranging up to 46%. The best purification of the guinea-pig enzyme was 15-fold, and the recovery was only 2.6%, the large loss occurring during chromatography on DEAE-cellulose and agarose. The poor results with the guinea-pig enzyme reflect the difficulty in obtaining a truly soluble material. Repeated sonication of the crude guinea-pig preparations yielded material that was initially soluble but tended to re-aggregate quickly. Purification of the rat phosphodiesterase II increased its thermostability, the temperature of half-inactivation being increased from 54° to 60°C. Both enzymes had a K_m value of 4×10^{-5} M with thymidine 3'-(2,4-dinitrophenyl) phosphate as substrate and showed similar pH optima for activity. Both enzymes were inhibited slightly in 0.1 M-MgCl₂ or 2 M-urea and much more strongly in 2 M-(NH₄)₂SO₄ or 6 M-NaCl. The guinea-pig enzyme was usually inhibited more than the rat enzyme. The Arrhenius plots of the two enzymes differed slightly in slope, but both were biphasic, showing breaks between 30° and 40°C. It was concluded that the two enzymes were markedly similar in behaviour and that the differences found were related to the different degrees of purification attained by the procedures described.

We have shown that phosphodiesterase II (oligonucleotide 3'-nucleotidohydrolase, EC 3.1.4.18) did not display an identical subcellular location in the intestinal mucosa of rats and guinea pigs (Flanagan & Zbarsky, 1974). Whereas the bulk of the rat activity was found in the supernatant solution after ultracentrifugation of mucosal homogenates, the guinea-pig enzyme appeared to be located in the lysosomal fraction. In addition, the enzymes had different molecular weights, exhibited marked differences in stability when heated at 60°C, and differed in pH optima.

The above results were obtained with enzyme preparations that were relatively crude. It was considered that a more meaningful study and comparison of the properties of phosphodiesterase II from the two sources required more highly purified preparations. Accordingly, such purification of intestinal phosphodiesterase II from rat and guinea pig was undertaken as described below. A much higher degree of purification was achieved with the rat enzyme, but nevertheless the purified enzymes showed a greater similarity of behaviour than was observed previously with crude preparations. A brief preliminary account of some of this work has been presented (Flanagan & Zbarsky, 1972).

Experimental

Materials

The chemicals used were of analytical grade or of the purest grade available. Thymidine 3'-(2,4-dinitrophenyl) phosphate† was synthesized as previously described (Flanagan & Zbarsky, 1974). Whatman ion-exchange celluloses DE32 and CM22 were obtained from Mandel Scientific Co. Ltd., Montreal, P.Q., Canada. Sepharose 6B, Sephadex G-150, Blue Dextran 2000 and molecular-weight marker proteins were purchased from Pharmacia (Canada) Ltd., Dorval, P.Q., Canada, and Bio-Gel A-0.5m (100–200 mesh) was obtained from Bio-Rad Laboratories, Richmond, CA, U.S.A. Rats of the Wistar strain, weighing about 200 g each, and albino guinea pigs of the Hartley strain, weighing 300–350 g each, were from the animal unit of the University of British Columbia and were starved for 24 h before death.

Methods

Assay of phosphodiesterase II. This was carried out as described previously (Flanagan & Zbarsky, 1974). 'Method 2' (a fixed-time assay in a volume of 0.3 ml)

† Abbreviation: dTpN₂ph, thymidine 3'-(2,4-dinitrophenyl) phosphate.

* Present address: The Hospital for Sick Children, Toronto, Ont. M5G 1X8, Canada.

was used as a routine to locate phosphodiesterase II activity in column effluents, one-tenth of the substrate concentration being used to conserve valuable material. For this reason the amounts of phosphodiesterase II activity in Fig. 1 are probably underestimated. An accurate determination of the activity present was obtained with an assay on the combined active fractions at each step by using 'Method 1' (continuous assay in a volume of 1 ml). A unit of enzyme activity is the amount that produces 1 μ mol of 2,4-dinitrophenol/h at 37°C.

Analytical methods. Protein was determined by the method of Lowry *et al.* (1951), with bovine serum albumin as the standard. In column effluents, however, eluted proteins were monitored by their absorbance at 280 nm. Measurement of salt concentrations was carried out with a Radiometer conductivity meter, type CDM2e. The measured conductivity was related to salt concentration by the use of standard curves prepared from solutions of known concentration.

Extraction of phosphodiesterase II. In some of the experiments the supernatant solution remaining after ultracentrifugation of homogenates of rat intestinal epithelial cells was used (fraction VI; Flanagan & Zbarsky, 1974). More often, however, rat mucosal scrapings were homogenized with 10 vol. of 0.3 M-sucrose/5 mM-sodium phosphate, pH 7, in a Potter-Elvehjem apparatus and the homogenate was centrifuged at 78 500 g_{av} for 60 min in a Spinco no. 30 rotor. The supernatant solution was kept and the sediment re-homogenized in a similar volume of sucrose/phosphate buffer and re-centrifuged. Occasionally the sediment was extracted for a third time. The combined supernatant solutions contained up to 70% of the total phosphodiesterase II activity in the original homogenate.

For extraction of the guinea-pig phosphodiesterase II the subcellular fractions that contain the bulk of the activity (fractions II+III+IV; Flanagan & Zbarsky, 1974) were used. These particulate fractions were resuspended in 10 vol. of 0.3 M-sucrose/5 mM-Tris/5 mM- NaH_2PO_4 , pH 7.6, and sonicated for 5 min at 20 kHz with a Bronwill sonicator. The product was centrifuged at 7380 g_{av} for 15 min in the SS-34 rotor of a Sorvall RC2-B centrifuge, and the supernatant kept. The pellet was suspended in 1 vol. of fresh sucrose/Tris/phosphate buffer equal to that of the supernatant and the material was sonicated as before and centrifuged to give a second supernatant that was combined with the first. In most experiments the sediment was extracted a third time and a 'high-speed' supernatant was prepared from the combined extracts by ultracentrifugation at 78 500 g_{av} for 60 min.

Purification of phosphodiesterase II. A three-step procedure, involving chromatography on DEAE-cellulose, CM-cellulose and agarose, was used.

Step 1. DEAE-cellulose chromatography was carried out at 4°C, on Whatman DE32, at either pH 6.5 or 7.6. For chromatography at pH 6.5, a column (2 cm $\times$ 20 cm) was equilibrated with 20 mM-sodium phosphate buffer, pH 6.5. The enzyme extract was dialysed against this buffer or, alternatively, diluted with cold (4°C) water until its conductivity was equal to that of the buffer. The protein solution was then applied to the DEAE-cellulose column, which was then washed with starting buffer (0.5–1.0 litre). More often, however, the protein adsorption was carried out by a batch technique. For this the DEAE-cellulose, pre-equilibrated as above with 20 mM-sodium phosphate buffer, pH 6.5, was added directly to the protein solution and the resulting suspension was stirred gently for 1 h at 4°C. The ion exchanger, with protein then adsorbed, was collected by filtration through a filter made from cotton laboratory-coat material, resuspended in the starting buffer and packed in the column, which was then washed as described above. The proteins adsorbed on these columns were eluted with a linear gradient composed of 500 ml of 20 mM-sodium phosphate buffer, pH 6.5, and 500 ml of 0.15 M-sodium phosphate buffer of the same pH. When chromatography at pH 7.6 was performed, the same basic technique was used, with the following exceptions: a larger column (3.2 cm $\times$ 28 cm) of DEAE-cellulose was used and the starting buffer was 5 mM-Tris/5 mM- NaH_2PO_4 , pH 7.6. The proteins were eluted from the column by means of a linear gradient composed of 1 litre of this Tris/phosphate buffer and 1 litre of this buffer made 1 M with respect to NaCl.

Step 2. The active fractions from Step 1 were combined and acidified with acetic acid to pH 5. Occasionally a precipitate formed at this stage, and this was removed by centrifugation before proceeding. Columns (2 cm $\times$ 20 cm) of CM-cellulose (Whatman CM22) were equilibrated at 4°C in 50 mM-sodium acetate buffer, pH 5. The phosphodiesterase II-rich solution from Step 1 was generally not dialysed against this starting buffer before application to the column, since it was found that the phosphodiesterase activity was adsorbed very strongly to the CM-cellulose once the pH of the solution was lowered to 5.0; the presence of salts up to an ionic strength of 0.15 mol/litre did not affect this adsorption. After the column had been washed with 200–400 ml of starting buffer, the adsorbed proteins were eluted with a linear gradient composed of 500 ml of starting buffer and 500 ml of starting buffer which was made 0.5 M (or occasionally 1.0 M) with respect to NaCl.

Step 3. Phosphodiesterase II was found to adsorb strongly to commercial agarose preparations. Bio-Gel A-0.5 m was used in all the experiments described here, but very similar results have been obtained with Sepharose 6B. Columns (3.2 cm $\times$ 15 cm) of the agarose beads were equilibrated at 4°C with 50 mM-

sodium acetate-50mM-NaCl, pH5. The NaCl concentration in the combined active fractions from Step 2 was decreased to about 0.05M by the addition of up to 4 vol. of cold (4°C) water. Alternatively the solution of combined phosphodiesterase II-rich fractions from step 2 was concentrated by ultrafiltration to 50–100ml in an Amicon apparatus (model 402) equipped with either a UM20E or a PM30 Diaflo membrane. The concentrated solution was dialysed against several changes of starting buffer, and applied to the agarose column, which was then washed with about 200 ml of starting buffer. The adsorbed phosphodiesterase II was eluted by increasing the NaCl concentration of the eluting buffer from 20mM to 0.2M or greater.

Results and Discussion

Purification of the rat enzyme

The elution behaviour of protein and phosphodiesterase II activity during a typical purification procedure are shown in Fig. 1 and the results of three such experiments are summarized in Table 1. Rat intestinal phosphodiesterase II was purified 350–550-fold by this procedure in good yield. The enzyme was adsorbed only weakly to DEAE-cellulose but quite strongly to CM-cellulose, as indicated by the ionic strength of the buffers used to elute the enzyme in Steps 1 and 2. Although substantial loss of enzyme activity occurred during Step 1, this treatment was necessary to remove large amounts of protein and to enable greater purification with CM-cellulose and agarose.

The most significant purification of the enzyme occurred during Step 3. As shown in Fig. 1, only very small amounts of protein were adsorbed to the agarose, whereas 70–95% of the phosphodiesterase II activity was bound under the conditions used.

Since it is known that even the purest preparations of agarose probably still contain small numbers of sulphate groups (Hjerten, 1962), and since sulphate is a potent inhibitor of some nucleases (Rauenbusch & Altman, 1960), it was thought at first that the interaction between phosphodiesterase II and the agarose might be the result of a specific affinity of the enzyme for sulphate ion. However, such an explanation is probably unlikely, since sulphate appears to have little effect on phosphodiesterase II activity (Flanagan & Zbarsky, 1974). The behaviour of phosphodiesterase II on agarose columns might be better explained by simple ion-exchange effects, with the sulphate-containing agarose acting as a weak cation exchanger.

Purification of guinea-pig enzyme

Since the bulk of the phosphodiesterase II activity in guinea-pig intestinal preparations is found in the

Fig. 1. Chromatographic purification of rat intestinal phosphodiesterase II

Details of the preparation of the extract (five rats were used) and the chromatographic steps are described in the Experimental section. The recoveries of protein and enzyme activity at the various steps are given in Table 1 (Expt. 1). (a) Step 1: chromatography on DEAE-cellulose. Elution was carried out with a linear gradient formed from 500ml of 20mM-sodium phosphate and 500ml of 0.15M-sodium phosphate, both at pH6.5. Fractions (20ml) were collected. (b) Step 2: chromatography on CM-cellulose. Elution was by linear gradient formed by 500ml of 50mM-sodium acetate buffer, pH5, and 500ml of buffer made 0.5M with respect to NaCl. Fractions (20ml) were collected. (c) Step 3: chromatography on Bio-Gel A-0.5m. Elution was by linear gradient formed by 500ml of 50mM-sodium acetate/80mM-NaCl and 500ml of 50mM-sodium acetate/0.5M-NaCl. Fractions (10ml) were collected. —, Phosphodiesterase II (units/ml); ----, E_{280} of eluate; ····, molar concn. of phosphate (a) or NaCl (b and c) in eluate.

lysosomal fraction of this tissue (Flanagan & Zbarsky, 1974), several methods were tested in attempts to solubilize the enzyme. These are shown in Table 2. Although none was completely effective, the most successful treatment was repeated sonication, as described under 'Methods'. With this treatment, 90% of the initial activity was recovered, and of this over

Table 1. *Purification of rat intestinal phosphodiesterase II*

The experiments were carried out as outlined in the text by using the chromatographic procedures described in the Experimental section. The column chromatography for Expt. 1 is shown in Fig. 1. The number of rats in each experiment is given in parentheses.

Expt. no.	Purification step	Total activity (units)	Total protein (mg)	Specific activity (units/mg of protein)	Overall recovery (%)	Purification (fold)
1 (5)	Supernatant	710	920	0.77	100	1
	DEAE-cellulose	500	117	4.3	70	6
	CM-cellulose	434	20.8	21	61	27
	Agarose	305	0.7	420	43	540
2 (10)	Supernatant	1800	1930	0.93	100	1
	DEAE-cellulose	615	225	2.7	34	3
	CM-cellulose	502	156	3.2	28	3.5
	Agarose	351	1.0	338	20	360
3 (20)	Supernatant	1680	6540	0.65	100	1
	DEAE-cellulose	879	283	3.1	52	5
	CM-cellulose	574	36.0	16	34	25
	Agarose	546	1.5	355	33	550

Table 2. *Solubilization of guinea-pig phosphodiesterase II*

The particulate preparation was obtained by combining fractions II+III+IV (Flanagan & Zbarsky, 1974). The particles were resuspended in 5ml of either 20mm-sodium acetate, pH 5, or 20mm-Tris/HCl, pH 8, containing the appropriate addition and left at 24°C for 30min. Incubations with trypsin, neuraminidase and phospholipase C were carried out as described by Henning *et al.* (1973). A 3.5h incubation with phospholipase D at pH 5 and 37°C in the presence of 0.07M-CaCl₂ was used. At the end of each treatment the particulate matter was sedimented by centrifugation and resuspended in 20mm-Tris/HCl, pH 8 (5ml). Phosphodiesterase II activity in the supernatant and sediment was determined by using 'Method 1.'

Treatment	Activity recovered (%)	Percentage of recovered activity in	
		Supernatant	Sediment
20mm-Tris/HCl, pH 8	100	21	79
20mm-Sodium acetate, pH 5	100	2	98
Sonication three times (pH 8)	90	72	28
50mm-Mercaptoethanol	89	41	59
50mm-EDTA	85	8	92
10% 2-Methyl-2-butanol	65	48	52
3M-NaCl	56	18	82
1% Deoxycholate	49	38	62
Trypsin (100 µg/ml)	80	41	59
Neuraminidase (5 units/ml)	83	11	89
Phospholipase C (100 µg/ml)	73	20	80
Phospholipase D (100 µg/ml)	66	18	82
4M-Urea	73	51	49
1% Triton X-100	111*	37*	63
1% Tween-80	141*	65*	35

* Interference in phosphodiesterase II assay.

70% appeared to be solubilized. However, if the solubilized guinea-pig fractions were kept for a few days at 4°C, as much as 40% of their activity could then be sedimented by ultracentrifugation. It was considered that the enzyme was not in truly soluble form, but remained bound to very small fragments resulting from the sonication procedure. Further

evidence of this was seen when a guinea-pig preparation was purified by chromatography on DEAE-cellulose, CM-cellulose and agarose, as described under 'Methods', and compared with a rat extract containing similar amounts of protein and purified on an identical set of columns under similar conditions. The results are summarized in Table 3. In

Table 3. *Comparison of purification of phosphodiesterase II from guinea-pig and rat intestine*

Extracts from ten guinea pigs and ten rats were prepared. The experiments were performed in a manner similar to that described in Table 1, except that DEAE-cellulose chromatography was carried out at pH 7.6 as described under 'Methods.'

Fraction	Total activity (units)	Total protein (mg)	Specific activity (units/mg of protein)	Overall recovery (%)	Purification (fold)
Rat					
Supernatant	1150	1600	0.71	100	1
DEAE-cellulose	725	360	2.0	63	2.8
CM-cellulose	598	50	9.9	52	14
Agarose	493	2.4	207	43	300
Guinea pig					
Supernatant	767	1500	0.51	100	1
DEAE-cellulose	308	194	1.6	40	3.1
CM-cellulose	232	58	4.0	30	7.8
Agarose	20	2.7	7.4	2.6	15

Fig. 2. *Chromatography of partially purified rat and guinea-pig phosphodiesterase II on agarose*

The active fractions from the CM-cellulose column were combined, concentrated by ultrafiltration, dialysed against several changes of 50 mM-sodium acetate/50 mM-NaCl, pH 5 (the eluting buffer), and chromatographed on agarose columns as described under 'Methods'. The adsorbed phosphodiesterase II was eluted by increasing the NaCl concentration of the eluting buffer to 0.5 M. Other details are given in Table 3. (a), rat; (b), guinea pig; ●, phosphodiesterase II; ○, E_{280} .

purification Steps 1 and 2, the behaviour of the guinea-pig enzyme was reasonably similar to that of the rat enzyme, although less guinea-pig phosphodiesterase activity was recovered. However, a major difference was noted in Step 3, as shown in Fig. 2. Whereas the rat enzyme was almost entirely adsorbed, only 10% of the guinea-pig phosphodiesterase remained on the agarose, the balance appearing in the flow-through volume with the bulk of the unadsorbed protein (Fig. 2). The resultant poorer yield and lower degree of purification were almost certainly related to the fact that the guinea-pig enzyme was not completely soluble. Further, in contrast with the rat extracts, the solubility of the guinea-pig preparations seemed to be dependent on pH. For example, with the latter it was necessary to carry out the chromatography on DEAE-cellulose at pH values higher than 7, because the bulk of the proteins in the guinea-pig extract were insoluble below this value. It was also noted that in the steps performed at pH 5 (CM-cellulose and agarose) the guinea-pig preparations were quite opalescent, indicating that not all the protein was soluble at this pH value.

pH optimum

The pH-activity curves of the partially purified enzymes were very similar to those displayed by the crude preparations (Flanagan & Zbarsky, 1974). A prominent shoulder of activity noticeable at lower pH values with the guinea-pig enzyme was probably due to contaminating deoxyribonuclease activity (Flanagan & Zbarsky, 1974). This would be consistent with the lower degree of purification attainable for the guinea-pig enzyme. The optimum pH for each of the enzymes was not appreciably affected by increasing the concentration of the buffers, but the absolute amount of activity found under these conditions was diminished considerably. For example,

high concentrations of sodium succinate buffer ($>0.4\text{M}$) inhibited both enzyme activities, the guinea-pig enzyme to a slightly greater extent than the rat enzyme. A similar inhibition by high succinate concentrations was reported by Razzell & Khorana (1961) for spleen phosphodiesterase. High concentrations of phosphate buffers also inhibited both enzymes.

Effect of inhibitors

A number of compounds known to affect nuclease activity were tested against both enzymes. Thus Hilmoie (1960) reported that Mg^{2+} inhibits phosphodiesterase II, whereas EDTA is supposed to activate the enzyme. In the present experiments both MgCl_2 and EDTA, at a concentration of 0.1M , inhibited rat and guinea-pig phosphodiesterase slightly. Although we previously showed that low concentrations of SO_4^{2-} do not affect phosphodiesterase II (Flanagan & Zbarsky, 1974), at higher concentrations inhibition is observed. For instance, $2\text{M}-(\text{NH}_4)_2\text{SO}_4$ inhibited rat phosphodiesterase II by 55%, and the guinea-pig enzyme by 72%. However, the inhibition may be caused by the increased ionic strength of these solutions, since $6\text{M}-\text{NaCl}$ also inhibited the enzyme activities by 59% and by 68% respectively.

Finally, although Razzell & Khorana (1961) reported that calf spleen phosphodiesterase II is inhibited by 50% in the presence of 2M -urea, we found that the intestinal enzymes from rat and guinea pig are inhibited only by 16 and 13% respectively.

Heat stability

Fig. 3 shows that 5 min at 64°C was needed to half-inactivate the partially purified rat enzyme, whereas the corresponding temperature for the guinea-pig preparation was 61°C . With crude preparations in similar experiments, the temperatures for half-inactivation were 54°C for the rat and 60°C for the guinea pig. Purification appeared not to affect the thermostability of the guinea-pig enzyme, whereas that of the rat enzyme was significantly increased. However, it should be remembered that the degree of purification was not the same in both cases, the rat enzyme having been purified to a much greater extent.

Kinetics of hydrolysis of dTpN_2ph

Although von Tigerstrom & Smith (1969) and Menon & Smith (1970) have previously used dTpN_2ph as a substrate for spleen and testis phosphodiesterase II respectively, the appropriate Michaelis constants were not given. Our experiments performed with the partially purified rat and guinea-pig enzymes

Fig. 3. Heat inactivation of crude and purified rat and guinea-pig phosphodiesterase II

Portions of the crude (open symbols) and agarose-purified (closed symbols) preparations described in Table 3 were heated in 5mM -Tris/ 5mM - NaH_2PO_4 , pH 7.6, at the temperature indicated for 5 min. They were then cooled rapidly in ice/water and samples were assayed for remaining phosphodiesterase II activity by using 'Method 1'. $\square$ and $\blacksquare$, rat; $\circ$ and $\bullet$, guinea pig. The results obtained with the crude guinea-pig preparation were indistinguishable from those obtained with the purified fraction and are not shown.

indicate that the apparent K_m values, obtained by Lineweaver & Burk (1934) plots, were both of the order of $4 \times 10^{-5}\text{M}$. It is noteworthy that the K_m values for intestinal phosphodiesterase II are one-tenth to one-eightieth of those published for the enzymes from spleen (Razzell & Khorana, 1961) or *Lactobacillus acidophilus* (Fiers & Khorana, 1963), although some of this difference is probably due to the difference in affinity of the enzymes for thymidine 3'-(nitrophenyl) phosphate, the substrate used in the previous studies, and dTpN_2ph , the substrate used in the present work.

The effect of temperature on the hydrolysis of dTpN_2ph by the enzymes was also studied. When the initial velocities of both phosphodiesterase reactions were determined at temperatures from 12° to 60°C the data shown in the Arrhenius plots in Fig. 4 were obtained. An apparent break in the curve for each enzyme was observed. The activation energy for the enzyme-catalysed hydrolysis of dTpN_2ph decreased as the reaction temperature was increased; a decrease from 54 to 31 kJ/mol was observed for the rat enzyme and from 73 to 50 kJ/mol for the guinea-pig enzyme. According to Kumamoto *et al.* (1971) temperature breaks in Arrhenius plots are a consequence of a phase change in either the enzyme or the reactants.

Fig. 4. Arrhenius plots of the rat and guinea-pig phosphodiesterase II-catalysed hydrolysis reactions

Enzyme preparations purified up to and including step 1 were used. Assays were by 'Method 1', except that the dTpN₂ph concentration was 0.8 mM and the temperature of the reaction was varied as indicated. In each case the reaction was initiated by the addition of the enzyme. Rat phosphodiesterase II, ■; guinea pig, ●. Velocity was measured as units of enzyme activity as defined in text.

Although no firm data on the structure of rat and guinea-pig phosphodiesterase II are available, some evidence was discussed earlier in the present paper that these enzymes, especially the enzyme from the guinea pig, can exist in aggregated or insoluble forms under some conditions. It would be interesting to know whether these properties of the enzymes were responsible for the breaks in the Arrhenius plots noted above. Since the breaks were always observed between 30° and 40°C it is not likely that lipid interactions were responsible, because in such situations a much lower transition temperature is observed (Raison, 1973).

Previous work (Flanagan & Zbarsky, 1974) suggested that if intestinal phosphodiesterase II is localized in the lysosomes in both guinea pig and rat, it may be more readily released from the granules of the rat tissue during the homogenization procedures than is the case with the guinea-pig tissue. The present work further indicates the difficulty in obtaining the

guinea-pig enzyme in soluble form. As a consequence the guinea-pig enzyme could not be purified to the same degree as the soluble phosphodiesterase II from rat intestinal mucosa by the chromatographic procedures described in the present paper. Nevertheless, in many of their properties the enzymes are remarkably similar. Both enzymes were inhibited by solutions of high ionic strength, exhibited maximal activity in the pH range 6–7, had K_m values of about 4×10^{-5} M for binding to dTpN₂ph and showed discontinuities between 30° and 40°C in their Arrhenius plots. It is speculated that the small differences noted in these properties of the partially purified enzymes were related to some extent to the differences in degree of purification and that despite the difference in subcellular location, rat and guinea-pig intestinal phosphodiesterase II are closely similar if not identical proteins.

This work was supported by the Medical Research Council of Canada. P. R. F. was the recipient of a Medical Research Council Studentship and a University of British Columbia Graduate Fellowship.

References

- Fiers, W. & Khorana, H. G. (1963) *J. Biol. Chem.* **238**, 2780–2788
- Flanagan, P. R. & Zbarsky, S. H. (1972) *Proc. Can. Fed. Biol. Soc.* **15**, 517
- Flanagan, P. R. & Zbarsky, S. H. (1974) *Biochem. J.* **142**, 545–553
- Henning, R., Plattner, H. & Stoffel, W. (1973) *Biochim. Biophys. Acta* **330**, 61–75
- Hilmoe, R. J. (1960) *J. Biol. Chem.* **235**, 2117–2121
- Hjerten, S. (1962) *Biochim. Biophys. Acta* **62**, 445–449
- Kumamoto, J., Raison, J. K. & Lyons, J. M. (1971) *J. Theor. Biol.* **31**, 47–51
- Lineweaver, H. & Burk, D. (1934) *J. Am. Chem. Soc.* **56**, 658–666
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- Menon, K. M. J. & Smith, M. (1970) *Biochemistry* **9**, 1584–1592
- Raison, J. K. (1973) *Bioenergetics* **4**, 285–309
- Rauenbusch, E. C. & Altman, K. L. (1960) *Proc. Soc. Exp. Biol. Med.* **104**, 385–388
- Razzell, W. E. & Khorana, H. G. (1961) *J. Biol. Chem.* **236**, 1144–1149
- von Tigerstrom, R. & Smith, M. (1969) *Biochemistry* **8**, 3067–3070