BINDING OF OESTRADIOL BY POLYOESTRADIOL

J. K. NORYMBERSKI
Unit for Endocrine Chemistry, Department of Zoology, University of Sheffield, Sheffield S10 2TN, England

Received 24 August 1979

1. Introduction

The preparation of polyoestradiol from oestradiol by the action of potassium ferricyanide, or horseradish peroxidase or lactoperoxidase and the partial characterisation of the product have been reported [1,2]. This communication concerns the binding of oestradiol by polyoestradiol and poses the question of the possible biological significance of that finding.

2. Materials and methods

[6,7-^{3}H]Oestradiol (44.9 Ci/mmol), [2,4,6,7-^{3}H]-oestrone (80 Ci/mmol) and [1,2,6,7-^{3}H]testosterone (80 Ci/mmol) were supplied by the Radiochemical Centre, Amersham; crystallised bovine serum albumin by British Drug Houses, Poole. Aqueous solutions of polyoestradiol (PEL) were prepared by dilution of its ethanolic solutions with water followed by the azeotropic removal of ethanol in vacuo at 35°C. Equilibrium dialysis was performed in Visking tubings (1–8/32 in.) for 72 h at 4–6°C (unless otherwise stated). In multiple dialysis experiments, aqueous solutions of PEL of different concentrations were placed in up to 6 inner compartments (1.6 ml in each) and an aqueous solution (50 ml) of the tritiated ligand was placed in the common outer compartment. Samples (0.2–0.4 ml) were taken from each compartment and, after admixture with Instagel, their radioactivity was counted in at least 6 cycles of 10 min (or to 10 000 cpm). Dialysis at different temperatures (see fig.1) were performed firstly at 20°C for 72 h; samples were then removed for counting, the remaining solutions were re-equilibrated at 4°C for 72 h and the procedure was repeated again at 40°C and finally at 6°C.

The rate of extraction of PEL from water (23 μg/ml) by ether (0.5 vol.) was determined from the extinction at 277 nm of the ethereal phase at different time intervals of vigorous mixing.

3. Results and discussion

Binding of oestradiol by polyoestradiol (PEL) was first examined by multiple equilibrium dialysis [3,4] at different concentrations of PEL, i.e., when the molar concentration of unbound oestradiol (U) remains constant whilst that of bound oestradiol (B) becomes a linear function of the concentration of PEL (c, g/l):

$$B = \frac{KUn/M}{1 + KU} \times c$$

where, K is the association constant; n is the no. binding sites/PEL molecule; and M is its mol. wt

The results (fig.1) show that binding of oestradiol by PEL increased when the temperature was lowered from 20°C to 4°C and that an irreversible partial loss of the binding affinity of PEL occurred at 40°C. The calculated difference (mean ± SE) between the B/c values determined at 4°C and at 20°C was (15.5 ± 1.8) × 10^{-10} mol/g and hence highly significant.

Using the same experimental design it was found that the combining affinity (B/Uc) of PEL with oestradiol:

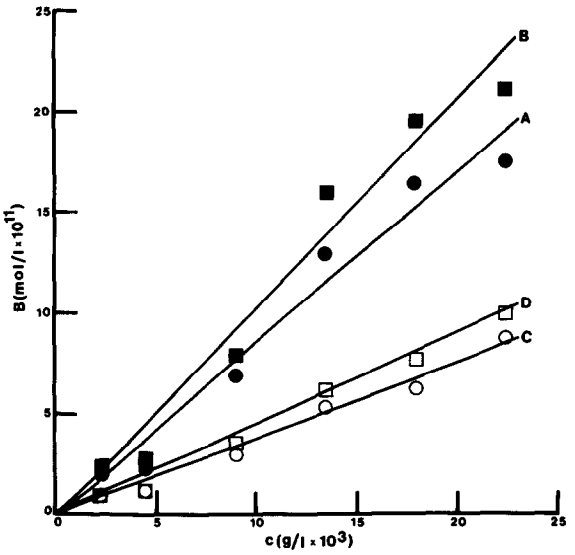

Fig.1. Concentration of bound oestradiol (B) as a function of the polyoestradiol (PEL) concentration (c). Equilibrations with [6,7-^{3}H]oestradiol (30 pg/ml) were carried out consecutively at: 20°C (line A, $y = 85 \times 10^{-10}\ x; r = 0.98$); at 4°C (line B, $y = 102.5 \times 10^{-10}\ x; r = 0.98$); at 40°C (line C, $y = 38 \times 10^{-10}\ x; r = 0.99$); at 6°C (line D, $y = 45 \times 10^{-10}\ x; r = 0.99$).

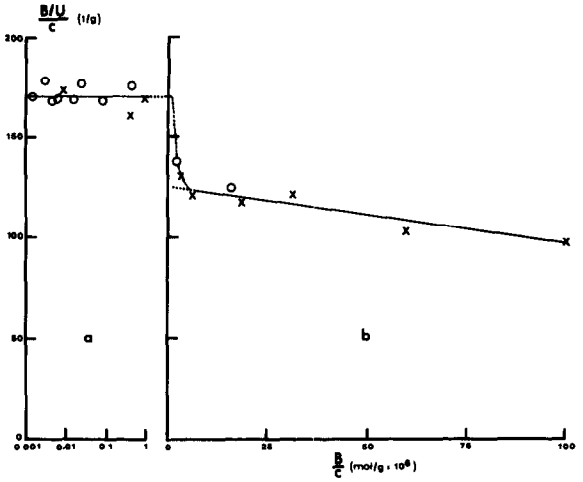

Fig.2. Combining affinity (B/Uc) of oestradiol with polyoestradiol (PEL) as a function of specific binding (B/c). [6,7-^{3}H]Oestradiol (3.6–18 pg/ml) and inert oestradiol (0–360 ng/ml) in 10 ml water equilibrated with PEL ((○) 24.7 μg/ml; (x) 12.35 μg/ml) in 1.0 ml water at 6°C. Solid line in (a): mean of 11 points, $\bar{y} = 171 \pm 5$ (SD). Solid line in (b): regression line of last 6 points, $y = -2.8 \times 10^5\ x + 126$ ($r = 0.915$).

(a) Was of the same magnitude as that with oestrone;
(b) Exceeded that with testosterone by a factor of 10–20;
(c) Was decreased by 70% when an aqueous solution of PEL was refluxed for 1 h and only by 20% when solid PEL was kept overnight at 110°C in vacuo;
(d) Exceeded that of bovine serum albumin with oestradiol by a factor of 85.

The binding properties of PEL were next examined at different concentrations of oestradiol. The results, presented in a modified Scatchard plot (fig.2), show that with increasing binding (expressed in terms of moles oestradiol bound by 1 g PEL; B/c) the combining affinity (B/Uc) remained at first constant at 171 ± 5 l/g over a wide range of B/c values ($0.0015–0.9 \times 10^{-6}$ mol/g), then dropped sharply over a narrow range of B/c values ($1–4 \times 10^{-6}$ mol/g) and finally declined slowly without reaching saturation at B/c of 10^{-4} mol/g. These results reveal the presence of at least two types of binding sites, one of low and the other of high binding affinity. The slope of the regression line derived from the last 6 experimental points of fig.2 (see also legend to fig.2) indicates that the low affinity sites have $K < 2.8 \times 10^5\ M^{-1}$. The magnitude of the association constant of the high affinity binding sites cannot be assessed from the available data. An alternative interpretation is that there exists an equilibrium between two forms (α and β) of PEL and that in the presence of oestradiol a new equilibrium is formed between oestradiol, the two binding species and the two resultant complexes; with increasing concentration of the ligand, that equilibrium is shifted towards the α-PEL–oestradiol complex until the shift is arrested whilst the formation of the β-PEL–oestradiol complex continues. On this basis it can be expected that the lower the initial concentration of α-PEL, the larger its association constant.

Some evidence for the existence of an equilibrium between two species, though not necessarily binding species, was obtained from the distribution of ultraviolet-absorbing material on mixing an aqueous solution of PEL with ether. At short mixing time (~1 min) the same amount (~5%) of PEL was

extracted irrespective of the volume ratio of the solvents whilst on prolonged mixing (10–330 min) the amount extracted (E, %) and time of mixing (t, min) were related by:

$$E = 12.2 \log t + 0.5 \; (r = 0.99; N = 8)$$

Oestrogen-induced uterine peroxidase transforms oestradiol in vitro to a mixture of products most of which are soluble in water and not extractable by ether [5–7]. As both horseradish peroxidase and lactoperoxidase transform oestradiol to PEL [1,2] and as peroxidases are nonspecific towards phenolic substrates, it seems feasible that under appropriate conditions uterine peroxidase can bring about the same transformation. Whether it does so in vivo and, if so, whether the oestrogen-binding properties of the formed PEL exert a physiological function are the main questions arising from the present findings.

Acknowledgement

I thank Mrs E. Hews for technical assistance.

References

[1] Norymberski, J. K. (1977) FEBS Lett. 76, 231–234.
[2] Norymberski, J. K. (1978) FEBS Lett. 86, 163–166.
[3] Daughaday, W. H., Kozak, I. and Biederman, O. (1959) J. Clin. Invest. 38, 998–999.
[4] Westphal, U., Ashley, B. D. and Selden, G. L. (1961) Arch. Biochem. 92, 441–448.
[5] Klebanoff, S. J. (1965) Endocrinology, 76, 301–311.
[6] Jellinck, P. H., McNabb, T., Cleveland, S. and Lyttle, C. R. (1976) Adv. Enzyme Regul. 14, 447–465.
[7] Keeping, H. S. and Jellinck, P. H. (1978) J. Steroid Biochem. 9, 1049–1054.