INFLUENCE OF PYRIDOXAL 5′-PHOSPHATE ON THE DNA BINDING ACTIVITY OF STEROID HORMONE RECEPTORS AND OTHER DNA BINDING PROTEINS

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1. Introduction

The interaction of steroid hormone receptors with the genome of the target cells has attracted much attention. This interaction is considered responsible for the regulation of specific gene expression by steroid hormones. In [1,2] pyridoxal 5′-phosphate was observed to inhibit the binding of steroid hormone receptors to immobilized DNA or nucleotides [1,2]. Pyridoxal phosphate may also operate in vivo modulating the interaction of the glucocorticoid receptor with the hepatic cell nuclei [3]. The 4′-aldehyde group of pyridoxal 5′-phosphate was shown to be required for the interaction with the glucocorticoid receptor [4]. Reduction with $NaBH_4$ leads to irreversible loss of DNA binding activity, suggesting the formation of a Schiff base intermediate [5].

Very little is known, however, on the protein specificity of this pyridoxal phosphate effect. In fact pyridoxal 5′-phosphate could have a general inhibitory effect on DNA binding proteins simply due to its ability to react with amino groups in the active site, thus interferring with electrostatic interactions. To explore this question we have investigated the influence of pyridoxal phosphate on DNA binding activity of purified hepatic glucocorticoid receptor [6], of the *Escherichia coli lac*-repressor, and of other DNA-binding proteins present in liver cytosol. We have found that similar concentrations of pyridoxal 5′-phosphate inhibit specific as well as non-specific binding to DNA.

2. Materials and methods

[^{3}H]Triamcinolone acetonide (25 Ci/mmol), d[^{3}H]-CTP (30 Ci/mmol) and d[α-^{32}P]CTP (400 Ci/mmol) were purchased from the Radiochemical Center, Amersham. *E. coli* DNA polymerase grade I and pyridoxal 5′-phosphate, were obtained from Boehringer, Mannheim. *lac*-Operator DNA, the purified *lac*-repressor and isopropylthiogalactoside (IPTG) were kindly gifted by K. Beyreuther, Köln. Nick-translation of operator DNA and λ-DNA was done as in [7] using either d[^{3}H]CTP or d[^{32}P]CTP as label. The specific activity of the DNA probes is indicated in the legend to the corresponding experiments.

The activated glucocorticoid receptor of rat liver was purified as previously reported [6].

2.1. *DNA-binding assay*

The reactions were done in 25 μl assays, containing binding buffer (10 mM Tris–HCl (pH 7.4), 10 mM KCl, 10 mM $MgCl_2$, 0.5 mM dithioerythritol, 0.1 mg/ml bovine serum albumin), DNA-binding proteins and the radioactive DNA probes. When the effect of pyridoxal 5′-phosphate was tested, the proteins were incubated for 5 min at room temperature with various concentrations of the drug prior to addition of the labelled DNA. Incubation was then continued for 20 min at room temperature and the reaction mixture was filtered through nitrocellulose filter (Gelman GN-6, 0.45 μm, 6 mm diam.). Prior to the binding assay the filters were boiled 10 min in binding buffer minus bovine serum albumin, and washed several times in complete binding buffer. After binding the filters were washed twice with 50 μl binding buffer, dried and counted in a toluene-based scintillator.

3. Results

In preliminary experiments the amount of glucocorticoid receptor required to retain 20–30% of the labelled DNA on the filter disc was determined. When

1 ng DNA was used 2–5 ng receptor were needed to obtain this percentage of retention. The influence of pretreatment of the receptor with pyridoxal phosphate on its binding to λ-DNA in solution is shown in fig.1a. These findings confirm results obtained with DNA-cellulose and crude receptors preparations [4], and show that mM levels of pyridoxal phosphate are sufficient to inhibit the DNA binding activity of the purified receptor.

To answer the question whether other DNA-binding proteins present in liver cytosol are also affected by pyridoxal phosphate, we first deprived the cytosol of activated receptor by passing it through a phosphocellulose column [6], then tested the influence of pyridoxal phosphate on the DNA-binding activity of the flow-through fraction (fig.1a). There is a considerable DNA binding activity in this fraction, in fact much more than in the phosphocellulose-bound fraction containing the activated receptor. As with the purified receptor, mM levels of pyridoxal phosphate inhibit the DNA binding activity of this crude hepatic preparation. To make sure that the DNA binding activity in the crude protein fraction is not due to the residual non-activated receptor, we compared the thermal stability of both functions: steroid binding and DNA-binding (fig.2). It is clear that whereas the receptor is rather thermolabile and looses its radioactive steroid between 40–45°C, the DNA binding activity of the crude fraction is not reduced at this temperature. In addition, the DNA-binding activity of the purified

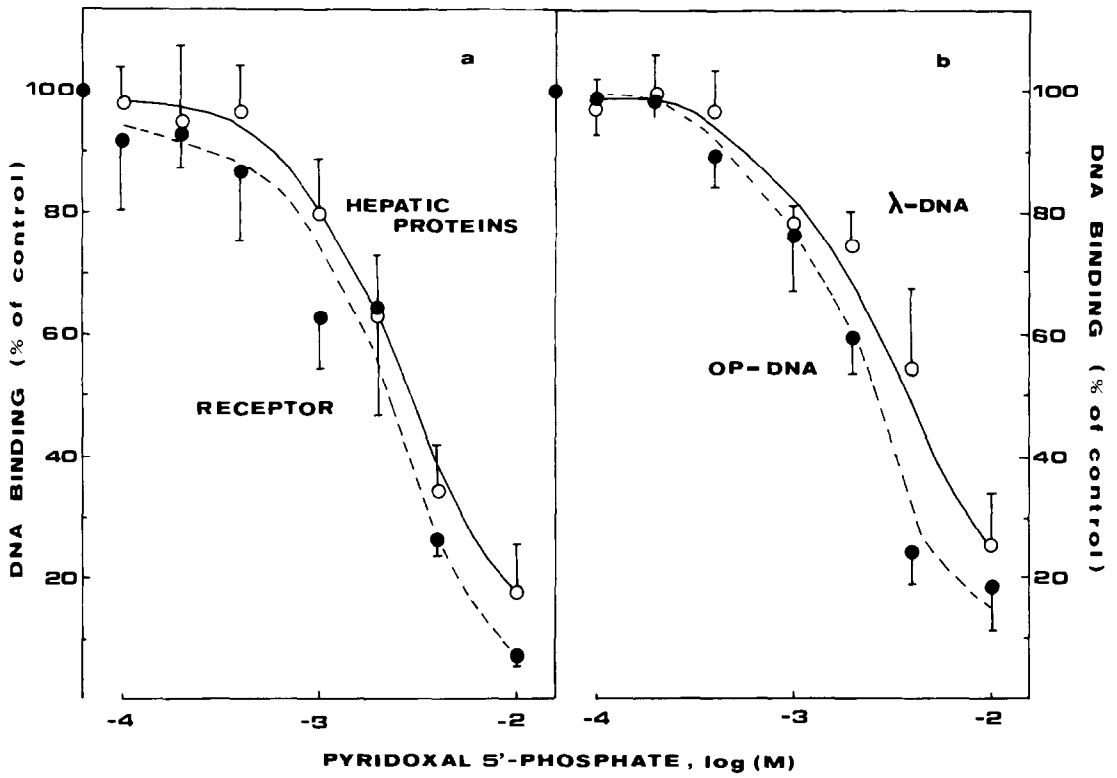

Fig.1. Effect of pretreatment with pyridoxal 5'-phosphate on the DNA-binding activity of hepatic proteins and the *lac*-repressor. (a) Purified activated glucocorticoid receptor from rat liver (2 ng) (•), or the flowthrough fraction of a phosphocellulose column (4 μg) (○) were incubated with several concentrations of pyridoxal 5'-phosphate, and their DNA-binding activity was determined using a filter binding assay and 1 ng λ-DNA labelled with ^{32}P to spec. act. 2×10^7 cpm/μg. (b) Purified *lac*-repressor from *E. coli* (50 ng) was incubated with several concentrations of pyridoxal 5'-phosphate and its DNA-binding activity was tested with operator DNA (•) (40 ng, labelled with ^{3}H to spec. act. 10^5 cpm/μg) and with λ-DNA (○) (50 ng, labelled with ^{3}H to spec. act. 1.7×10^5 cpm/μg). The abscissa shows the final concentration of pyridoxal 5'-phosphate in the 25 μl assays, and the ordinate the DNA bound to the filter as percentage of the control assay in the absence of pyridoxal phosphate. The blanks observed with DNA alone, were <5% of these control values and have been deduced from all experimental values. These blanks were not influenced by pyridoxal phosphate. The results represent the average and standard error of 2 expt carried out in duplicate.

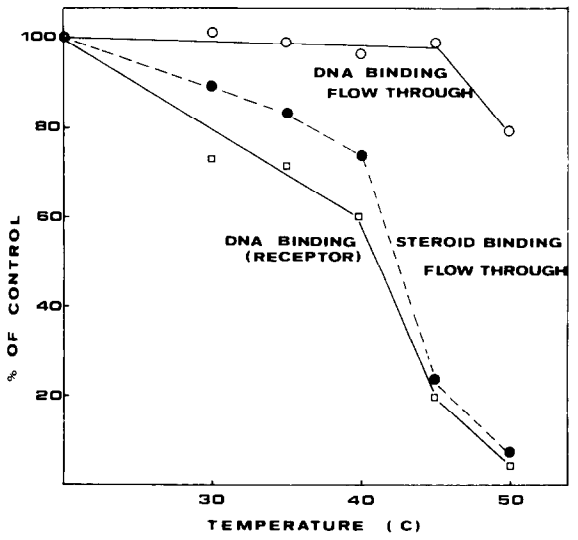

Fig.2. Influence of temperature on steroid binding and DNA binding activity of the glucocorticoid receptor and other hepatic proteins. The flowthrough fraction of a phosphocellulose column (○), and the purified glucocorticoid receptor (□——□) were heated at the indicated temperature for 10 min, chilled, and their DNA-binding activity tested with ^{32}P-labelled λ-DNA (spec. act. 3.5 × 10^7 cpm/μg). The values are expressed as % of control samples that were kept in ice. Aliquots of the flowthrough reaction were also used to investigate the steroid-binding activity remaining after incubation at the indicated temperature, using the charcoal technique [6]. The values are given as % of the unheated controls (●).

receptor was thermolabile (fig.2). These findings suggest, therefore, that the DNA-binding activity of hepatic cytosol proteins other than the receptor is also similarly inhibited by pyridoxal 5′-phosphate.

To test the generality of this finding, and to clear the question whether sequence specific binding is also affected by pyridoxal phosphate we studied the influence of this drug on the binding of the *lac*-repressor of *E. coli* to operator and non-operator DNA (fig. 1b). The specificity of the binding reaction was confirmed by the inhibitory influence of the inducer IPTC (not shown). As in the case of the hepatic proteins there is an inhibition of DNA binding at mM levels of pyridoxal phosphate. Moreover, there is no marked difference between the sensitivy to the drug of sequence specific binding to operator DNA and non-specific binding to λ-DNA.

4. Discussion

In [8] the activated glucocorticoid receptor of rat liver bound to immobilized DNA of several sources without evident sequence specificity. We now confirm those observations obtained with crude cytosol preparations, that binding of steroid receptors to DNA-cellulose can be inhibited by pyridoxal 5′-phosphate [1–5]. In addition, they show that the inhibition of DNA-binding activity by pretreatment with pyridoxal phosphate is a rather general phenomenon common to several DNA-binding proteins including an heterogeneous population of hepatic proteins and the *lac*-repressor of *E. coli*. It is probable that this inhibition results from the interaction of the 4-aldehyde function of pyridoxal phosphate with lysine residues in the active site, although this specific question was not addressed in these experiments. Moreover, not only the unspecific DNA binding as studied with the hepatic proteins but also the sequence specific binding of the *lac*-repressor to the *lac*-operator is inhibited by preincubation with pyridoxal phosphate. The electrostatic interaction of the amino groups of proteins and the phosphates of DNA, thus, seems to contribute markedly to the specific binding of regulatory proteins to DNA. It is of course possible that the effect of pyridoxal phosphate is indirect, and mediated through allosteric interactions with sites other than the DNA-binding site; further studies on the nature of the inhibition are required to clarify this point.

The use of pyridoxal 5′-phosphate to elute the glucocorticoid receptor from DNA-cellulose columns, as has been recommended [9, 10], may be less specific than originally thought. Our findings do not exclude the possibility that pyridoxal phosphate acts as a modulator of the nuclear binding of the glucocorticoid receptor [3], but show that this function, if actually operative, is more general and not restricted to steroid hormone receptors.

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