ESTROGEN RECEPTOR CAN DISTINGUISH AMONG VARIOUS HALODEOXYURIDINE-SUBSTITUTED DNAs

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1. Introduction

Although the interplay between steroid hormone receptors and the genome has been extensively studied [1–6], it is not yet known how the receptor protein interacts with double-stranded helical DNA. To examine the specificity and the dynamics of DNA–estrogen receptor (ER) interactions, we have initiated a study to determine how specific changes in the DNA will affect receptor binding. We have found that ER binds preferentially to AT-rich DNA [7] and also to DNA substituted with bromodeoxyuridine (BrdUrd) [8]. Numerous studies, in a wide spectrum of cell systems, have examined various changes in gene expression which follow BrdUrd-substitution in the DNA [9,10]. Relatively few experiments, however, have focused on the possible mechanism(s) by which this defined replacement (of the 5-methyl group in thymine by a bromide atom) might cause subtle electronic or steric effects in DNA which, in turn, might alter gene expression in the eukaryotic cell [8–12]. One such mechanism involves altered binding of regulatory proteins to BrdUrd-substituted DNA [8–12]. One approach to the question of how the bromine atom causes its particular effects, is to ask if other halogen atoms in the same position cause the same effects [12]. Therefore, we have incorporated 5-chloro-, 5-bromo- and 5-iodo-deoxyuridine into DNA in equivalent molar amounts and determined the effect of these substitutions on the ER–DNA interaction. We report here, an increased binding of ER to halodeoxyuridine-substituted DNA in the following order of preference at the 5 position of thymine: iodo > bromo > chloro > methyl. Our evidence is based on the kinetics of ER transfer from soluble DNA to DNA-cellulose. These transfer experiments provide a simple and sensitive assay to monitor the effect of DNA alterations on receptor binding [8].

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2. Materials and methods

2.1. *Receptor transfer assay*

Estrogen receptor was prepared from rabbit uterus [7,8]. The estrogen receptor transfer assay was conducted as in [8].

2.2. *Preparation and analysis of DNA*

Unsubstituted soluble DNA was prepared from the 3460 Syrian hamster cell line [8,13]. Soluble DNA, in which all thymidines of one strand were replaced by chlorodeoxyuridine (CldUrd), bromodeoxyuridine (BrdUrd) or iododeoxyuridine (IdUrd), were also prepared from 3460 cells in the following way: DNA was isolated from unsynchronized cultures, grown for 24 h (1.3 generations) in the presence of 100 μM hypoxanthine, 0.4 μM aminopterin and 10 μM halodeoxyuridine [13]. The three DNAs were subjected to preparative CsCl density gradients from which a major band of partly-substituted DNA was collected. The densities of these DNAs were determined by subsequent analytical CsCl gradients [14] which showed that, in each case, 50% (±2%) of the thymidines were substituted with CldUrd, BrdUrd or IdUrd. Sub-

stituted DNA prepared by this method are 'heavy–light', one strand is fully substituted with halodeoxyuridine and the other strand is unsubstituted. The following formula was used to derive % substitution levels from observed density in CsCl:

$$\% \text{ Substitution} = \frac{\rho_{sub} - \rho_{unsub}}{(\%T/100\,M}$$

For 3460 cells, $\%T = 29.8\%$ and $\rho_{unsub} = 1.6998$. For CldUrd-, BrdUrd- and IdUrd-substituted DNA, M is equal to 0.0879, 0.1730 and 0.2419, respectively [14]. These M values were empirically established from a series of DNAs whose substitution levels were independently determined by chromatography [13,14].

3. Results and discussion

Briefly, [^{3}H] ER was allowed to bind to soluble DNA (either unsubstituted or halodeoxyuridine-substituted) and later an excess of DNA-cellulose was added; the rate of ER transfer onto DNA-cellulose was followed by measuring the appearance of radioactivity in the DNA-cellulose pellet [8]. The rate of transfer is a sensitive measure of the stability of the initial ER–DNA complex; more stable complexes dissociate more slowly [8,15]. The transfer experiments depicted in fig.1 illustrate several points:

(i) Each transfer reaction is characteristic of a first order process;

(ii) ER transfers more slowly from each halodeoxyuridine-substituted DNA than it does from unsubstituted DNA;

(iii) The rates of transfer from each DNA are different in the following order of decreasing rate: unsubstituted > chloro- > bromo- > iododeoxyuridine-substituted.

Thus, the replacement of the 5-methyl of thymine by a halogen atom clearly stabilizes the ER–DNA interaction, iodine substitution being the most effective stabilizer.

Several proteins (e.g., the *lac* repressor, the catabolite gene activator protein, estrogen receptor and H1 histones) are now known to have a markedly enhanced affinity for halodeoxyuridine-substituted DNA [8,11,12,16,17]. Of the 4 proteins listed, the first 3 can distinguish among chloro-, bromo- and iododeoxyuridine-substitution [8,12]; H1 histones, however, apparently cannot – at least when the level of substitution is low to moderate (0–50% of thymines replaced) [17]. Although the molecular mechanism(s) by which halodeoxyuridine-substitution alters DNA–protein interactions is not known, several possibilities might be considered. The halogen atoms themselves may be the primary determinants of enhanced receptor binding via some direct contact between the receptor protein and the halogen atoms (located in the major groove of DNA). Alternatively (or in addition), the halogen atoms may induce or promote

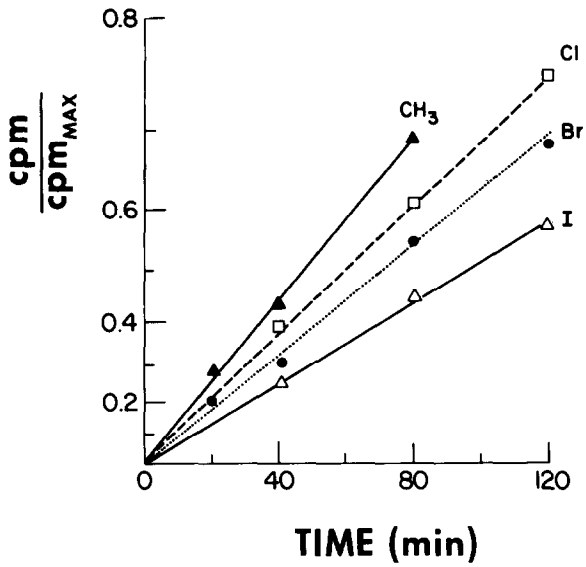

Fig.1. Rate of transfer of [^{3}H]ER from soluble DNA to DNA-cellulose. [^{3}H]ER in 200 μl buffer A (50 mM KCl, 20 mM Tris–HCl (pH 7.5), 1 mM EDTA and 1 mM β-mercaptoethanol) was added to 5 μg soluble DNA (pre-treated with S-1 nuclease to remove any single-stranded tails) in 800 μl same buffer and incubated at 25°C for 40 min at which time, 1 ml DNA-cellulose slurry (containing 450 μg DNA) in buffer A was added. The time-dependent transfer of [^{3}H]ER onto DNA-cellulose was then monitored by removing duplicate 150 μl aliquots at the indicated times and assaying them as in [8]. The maximum amount of [^{3}H]ER which could be transferred to DNA-cellulose (cpm_{max}) was determined by a parallel reaction from which the soluble DNA was omitted [8]. The soluble DNAs used were unsubstituted (▲) or substituted to 50% with chlorodeoxyuridine (□), bromodeoxyuridine (●) or iododeoxyuridine (△).

some change in the DNA and this changed form may be the actual determinant of enhanced receptor binding. On the basis of current evidence, therefore, we cannot yet conclude that the ER binds to the major groove of DNA where the halogen atoms are situated. Our caution in this regard is well illustrated by the recent finding [18] that actinomycin D, which binds to DNA via the minor groove [19,20], also has an enhanced affinity for halodeoxyuridine-substituted DNA. Chlorine, bromine and iodine, being increasingly hydrophobic in the order listed, may stabilize or promote direct contacts with apolar protein groups in the major groove [12]. The halogen atoms, in this order, are also decreasingly electronegative and may, therefore, induce a variety of significant polarization effects [21]. Halogen substitution is known to cause enhanced base stacking and this has been attributed in large part to polarization effects [21]. If certain DNA-binding proteins intercalate (or partially insert) some aromatic amino acid residues between base pairs upon binding, tighter binding may result from factors (e.g., halogen substitution) which enhance the interaction of bases with aromatic ring systems [21,22]. It is possible that the halodeoxyuridine-substituted DNA series may more readily undergo conformational fluctuations (e.g., bending, kinking or superhelical twisting) which may be required for protein binding [22]. Also, DNA is enveloped by a thin shell of ordered water molecules; disruption of this structured water is an essential step in the binding process. Therefore, factors which tend to destabilize this structured water may also facilitate binding of proteins to DNA.

Some DNA-binding proteins exhibit a low to moderate affinity for DNA in general, but a high affinity for certain specific DNA sequences [23,24]; however, no sequence-specific, DNA-binding sites have yet been demonstrated for steroid hormone receptors [2]. The present study, in fact, is part of a systematic search for possible specificity determinants which may be operative in the binding of these receptors to chromatin. Our results show that certain well-defined alterations in DNA cause an enhanced binding of estrogen receptor to the altered DNA. This approach, i.e., introducing replacements into DNA, has become increasingly useful in the analysis of specificity, mechanism and topographical relationships of DNA-binding proteins [12,25,26].

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