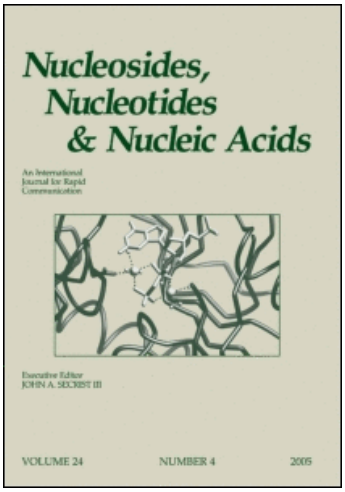


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Down-Regulation of H Tau 40 Protein Expression by Minor Groove Binders

Leonhard Kittler[a]; Leo Wollweber[a]
[a] Department of Single-Cell and Single Molecule Techniques, Institute of Molecular Biotechnology, Jena, Germany

To cite this Article Kittler, Leonhard and Wollweber, Leo(2000) 'Down-Regulation of H Tau 40 Protein Expression by Minor Groove Binders', Nucleosides, Nucleotides and Nucleic Acids, 19: 8, 1241 — 1248
To link to this Article: DOI: 10.1080/15257770008033047
URL: http://dx.doi.org/10.1080/15257770008033047

NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS, 19(8), 1241-1248 (2000)

DOWN-REGULATION OF H TAU 40 PROTEIN EXPRESSION BY MINOR GROOVE BINDERS

Leonhard Kittler* and Leo Wollweber
Institute of Molecular Biotechnology, Department of Single-Cell and Single Molecule Techniques, Beutenbergstraße 11 D 07745 Jena, Germany

ABSTRACT. The DNA minor groove binders SN6999, SN6570, and SN6113, structurally related to netropsin and distamycin, were investigated for sequence –specific interactions with the 154 base pair cDNA fragment of the human tau 40 protein, involved in pathology of Alzheimer's disease. Footprinting results indicated that both the former compounds displayed a pronounced AT-preference, while the latter SN-derivative bound to DNA in a non-sequence specific manner. The influence of these ligands on the protein synthesis was analysed using monoclonal antibodies against h tau protein. Both sequence –specific binders markedly impeded protein synthesis. The non-specific binder, however, did not affect protein biosynthesis.

INTRODUCTION

Alzheimer's disease (AD) is the most common neurodegenerative disease which affects about twenty million people world-wide[1]. AD is clinically characterised by a progressive loss of memory and other cognitive functions resulting in a profound dementia. At present, the aetiology and the pathogenesis of the disease is not fully established, but probably involves genetic, environmental and metabolic factors [2,3]. The major form is sporadic (~ 90%) characterised by a late onset (behind 65 years), while the familiar related form (~ 10%) has an early onset (age about 40) [4,5]. Changes on chromosomes 14, 19, 21 have been found to be associated with mutations of an amyloid precursor protein responsible for generation of "senile plaques" as a hallmark of the AD brains [6,7]. On the other hand, unbalanced phosphorylation/dephosphorylation processes at tau proteins are suspected to be related to paired helical filament formation as the main indication for sporadic neurofibrillary degeneration in AD [8,9].

Selective down-regulation of the gene for tau protein might be considered as a potential approach to the therapy of AD. Along this line, interactions of minor groove binders seem to

provide a promising approach for investigation [11,12]. The currently achievable specificity by ligand interaction encompasses on average five to ten base pairs [12]. This may be sufficient to affect the binding of several enzymes involved in DNA metabolism. DNA and RNA polymerases [13], topoisomerases [14] and restriction endonucleases [15] have already been described as being inhibited as a consequence of binding of sequence-reading ligands to DNA.

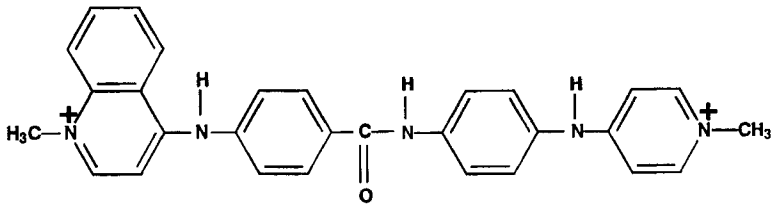
Based on enzymatic DNA-footprinting technique, we have analysed here the binding specificity of several minor groove binders (structures shown in Fig.1) to the AT-rich 154 base pair *Hind*III-*Rsa*I restriction fragment of the cDNA of h tau 40 protein. By means of immunological methods, the influence of ligands in dependence on their binding modes on the protein synthesis rate has been followed.

MATERIALS AND METHODS

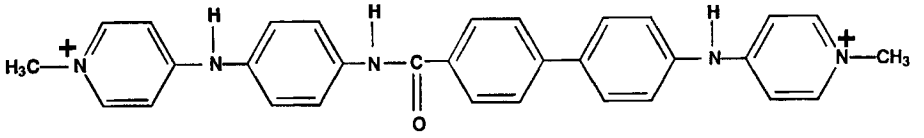
Minor groove binders and labelling of the DNA restriction fragment: DNA minor groove binders employed are compiled in Fig.1. The cDNA clone of h tau 40 protein in the expression plasmid pRK172 was used. For footprinting the 154mer *Hind*III-*Rsa*I restriction fragment has been prepared (Fig.2). It was labelled at the 3'-end of the *Hind*III site with [α-32P]dATP using Klenow fragment. The radioactively labelled fragment was purified by running on a non-denaturing 6% polyacrylamide gel. After autoradiography, the gel regions containing the band was excised and extracted with an aqueous solution containing 500 mM ammonium acetate and 10 mM magnesium acetate. The purified DNA was precipitated with sodium acetate-ethanol, washed twice with 70% ethanol and resuspended in 10 mM NaCl, 10 mM Tris-HCl buffer (pH 7.9).

Footprinting and gel electrophoresis: Radioactively labelled DNA fragments (2μl) were incubated with 4 μl of ligands at room temperature for 30 min, then digested with 2 μl DNaseI (final concentration 0.01 U/ml) in DNase buffer (20 mM NaCl, 2 mM $MgCl_2$, 2 mM $MnCl_2$). After 4 min, the reaction was stopped by adding 3 μl of 80% formamide containing 0.1% bromophenol blue, 0.1% xylene cyanol and 10 mM EDTA. Samples were heated at 90°C for 3 min and chilled in ice to electrophoresis.

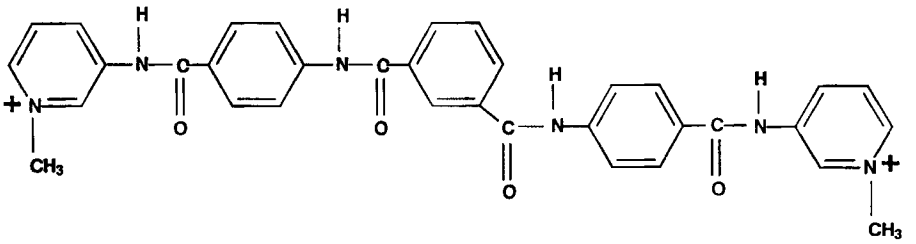
The reaction products of the enzymatic digestion were loaded onto an 8% polyacrylamide gel (0.3 mm thick) containing 8% urea and Tris-borate-EDTA buffer (pH 8.3). After 2 h of electrophoresis at 1500 V, the gels were treated with 10% acetic acid for 15 min, dried at 80°C and subjected to autoradiography at -70°C with an intensifying screen. The chemical identities of digestion products were assigned by co-electrophoresis of sequencing standards



SN 6999



SN 6570



SN 6113

FIG. 1: Chemical structures of the DNA minor groove binders investigated

generated by treatment of the DNA fragment with dimethylsulfate and ethyl chloroformate, followed by piperidine-induced cleavage at the guanine and adenine sites, respectively.

Expression of the h tau 40 protein: The recombinant plasmid pRK172 [16] was transformed into E.coli BL21 (DE3) cells. The E.coli cells were grown in LB medium containing ampicillin (100μg/ml) up to an optical density of 0.6 – 0.7 at 600 nm. Isopropyl-β-D-thiogalacto-pyranoside (IPGT) to a final concentration of 0.4 mM and various minor groove binders (SN6570, SN6999 or SN6113) to final concentrations of 100, 10, 1 and 0.1 μg/ml were added. After shaking for 2h at 37 °C the cells were collected by centrifugation.

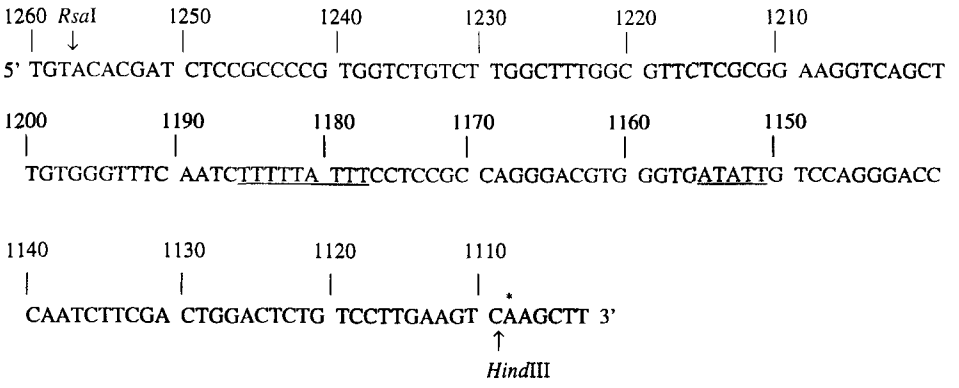


FIG. 2: Nucleotide sequence of cDNA of the *HindIII-RsaI* restriction fragment of h tau protein.

Underlined AT-rich sequences indicate location of footprints. Asterisk and arrows mean position of radioactive labelling and cleavage sites of restriction endonucleases, respectively.

SDS-PAGE and immunoblot analysis: The pellet from 1ml culture was lysed in 50µl SDS electrophoresis sample buffer according to Laemmli [17] by a 30 s sonication using a device GM70 (Brandelin, Berlin, Germany) at 50% power. The lysed probes were diluted with sample buffer and 3µl of each probe were run on 10% SDS-PAGE gels. Following electrophoresis the gels were blotted onto 0.2 µm nitrocellulose membranes (Bio-Rad, München, Germany). Non-specific protein binding sites were blocked by incubation with 2% non-fatt-free milk in Dulbecco's PBS, (Biochrom, Berlin, Germany) containing 0.05% Tween20 (PBS-T).The blots were then successively incubated with an anti- tau mouse monoclonal antibody (1:1000 dilution in PBS-T containing 2% bovine serum albumin, Sigma, Deisenhofen, Germany) and a horse-radish peroxidase-conjugated anti-mouse Ig antibody (1:2000). The h tau 40 band was visualized by using 3, 3'-diaminobenzidine/H_2O_2.

RESULTS AND DISCUSSION

The footprint analyses for three minor groove binders (Fig. 1) at the 154 base pair restriction fragment of the cDNA of the tau 40 protein (Fig. 2) indicate that the derivatives SN6570 and SN 6999 are capable of binding sequence-specifically to DNA. Ligand binding sites were located at positions 1152-1156 (5'-ATATT-3') and 1177-1185 (5'-TTTTTATTT-3') emphasising the AT-binding preference of both minor groove binders,

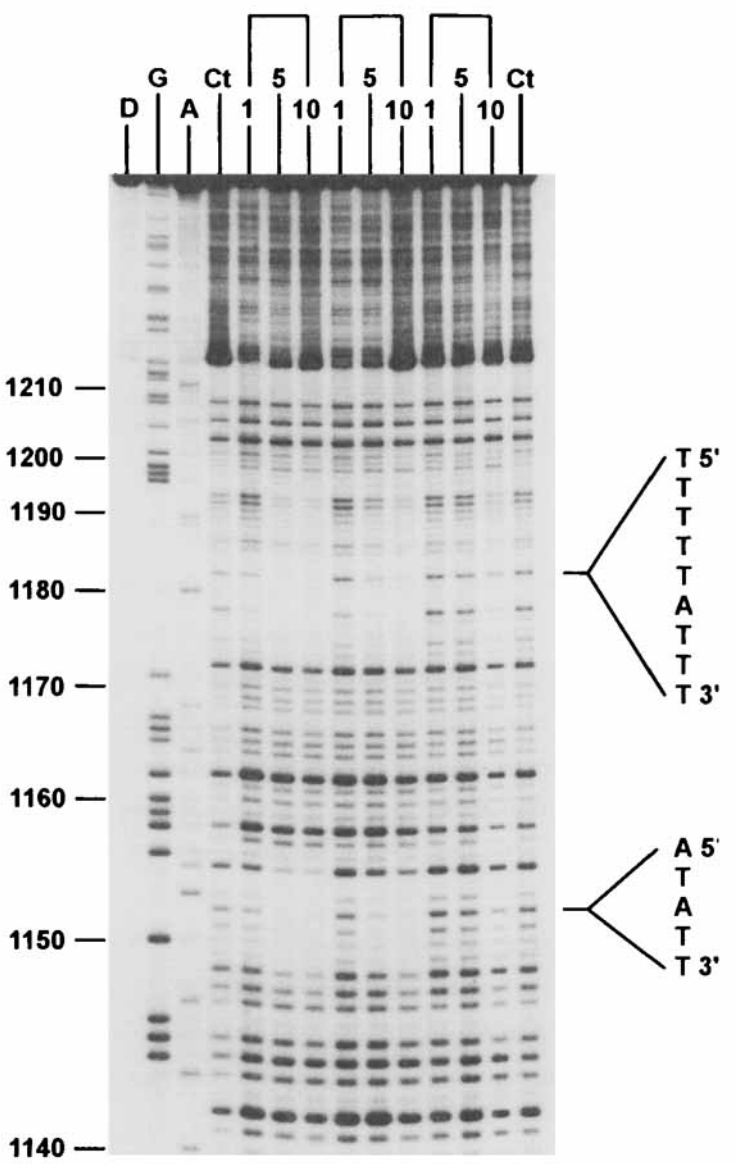


FIG. 3: DNaseI footprinting of compounds SN6999, SN6570 and SN6113 on the *HindIII-RsaI* 154 base pair fragment of the cDNA of h tau 40 protein. Numbers at the top show the ligand concentration (μM). Numbers at the left side indicate bands with reference to sequence shown in Fig.2. Sequences at the right side denote sites of ligand interaction.

D, uncleaved DNA; G and A, dimethylsulfphate-piperidine and ethyl chloroformate-piperidine makers for guanine and adenine, respectively. Ct, control DNA (DNaseI digestion pattern without ligand).

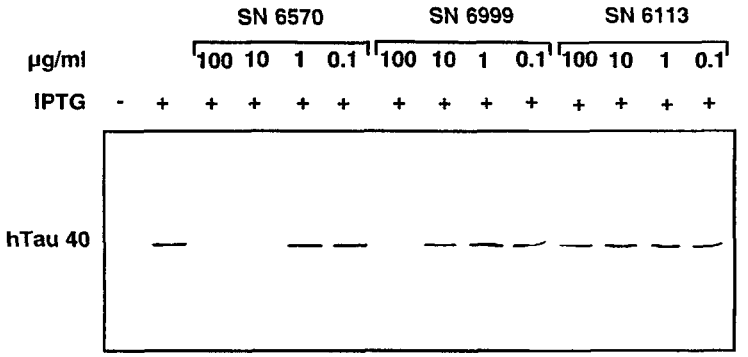


FIG.4: Immunoblot analysis of the h tau 40 protein expression.
Details see Material and Methods.

while the compound SN6113 binds non-sequence specifically (Fig. 3). These results confirmed well to data based on circular dichroism studies [18] and NMR- measurements [19]. Minor variations of the ligand structure may cause changes in base pair preference. The sequence-specific binding of these ligands to DNA is mostly influenced by variations in the length of spacer groups between the aromatic rings as well as by the position of the positively charged nitrogen atoms in both terminal rings [13]. Recently published molecular modelling studies indicate that for selective binding in the minor groove of DNA helix the curvature of ligands is decisive [20].

The inhibitory effect of sequence-reading minor groove binders on enzymes which are involved in DNA metabolism has been frequently described [12-15, 21, 22]. This prompted us to investigate the influence of sequence-specific DNA-binders versus non-specific ones on the biosynthesis rate of h tau 40 protein which is suspected to be involved in the pathogenesis of AD.

To determine the effect of DNA minor groove binders on the h tau 40 protein expression in E.coli, we measured levels of those protein by immunoblot analysis after two hours incubation with IPTG in presence of the derivatives SN6570, SN6999, and SN 6113, respectively. From Fig.4 it is obvious that addition of the non-specific DNA-binder SN6113 up to 100µg/ml in the growth medium has no measurable influence on h tau 40 protein expression. In contrast, under comparable conditions the sequence-reading ligands SN6570 and SN6999 display a pronounced suppression of the h tau 40 protein biosynthesis. The present findings with minor groove binding ligands represent an important step towards the current goal in molecular medicine of sequence-selective regulation of gene expression.

Prelimenary experiments to see whether in vitro transcription of h tau 40 gene from plasmids can be blocked by ligands investigated here have yielded encouraging results, although much more work will need to be done before selective action directed at those genes can be evidenced in whole genomic DNA.

Acknowledgements

The generous gifts of the SN-compounds and the recombinant plasmid pRK172 by Dr's. B.C. Baguley, Auckland (New Zealand) and M. Goedert, Cambridge (UK.), respectively, are highly appreciated. We are grateful to B. Härtl, S. Hoffmann, B. Lanick and M. Wollmann for excellent technical assistance.

REFERENCES

[1] Maringa, M. Science, **1995 269**, 917-918.

[2] Harman, D. Age, **1995 18**, 97-119.

[3] Davis, J.N.; Hunnicutt, E.J.; Chisholm, J.C. Molec. Med. Today, **1995** 240-247.

[4] Goedert, M. Arzneim. Forsch./Drug Res, **1995 45**, 403-409.

[5] Robakis, N.K. J. Neuropath. and Exptl. Neurol, **1995** 165-175.

[6] Schellenberg, G.D. Proc. Natl. Acad. Sci. USA, **1995 92**, 8552-8559.

[7] Dewji, N.N.; Singer, S.J. Science, **1996 271**, 159-160.

[8] Delacourte, A. Biomed. and Pharmacother., **1994 48**, 287-295.

[9] Drewes, G., Lichtenberg-Kraag, B., Döring, F., Mandelkow, E.M., Biernat, J., Goris, J., Dorée, M., and Mandelkow, E. EMBO-J., **1992 11**, 1593-1597.

[10] Hunter, T. Cell, **1995 80**, 225-236.

[11] Waring, M.J.; Bailly, C. J. Molec. Recog., **1994 7**, 109-122.

[12] Kittler, L.; Baguley, B.C.; Löber, G.; Waring M.J. J.Molec.Recog., **1999 12,** 121-130.

[13] Kittler, L.; Wähnert, U.; Baguley, B.C.; Bailly, C.; Waring, M.J.; Löber, G. Anti- Cancer Drug Design **1996 11**, 101-115.

[14] Bell,A.; Kittler,L.; Löber,G.; Zimmer,Ch. J.Molec.Recog., **1997 10,** 245-255.

[15] Kittler, L.; Bell, A; Baguley, B.C.; Löber, G. Biol.Chem., **1998 379** 519-525.

[16] Goedert, M.; Jakes,R. EMBO J., **1990 13,** 4225-4230.

[17] Laemmli, U. K. Nature, **1970 227**, 680-685.

[18] Luck, G.; Störl, J.; Baguley, B.; Zimmer, Ch. J. Biomolec. Struct. & Dyn., **1992 10,** 551-564.

[19] Chen, S.M.; Leupin, W.; Rance, M.; Chazin, W. J. Biochemistry, **1992 31**, 4406-4413.

[20] Slickers, P; Hillebrand, M.; Kittler, L.; Löber, G.; Sühnel, J. Anti-Cancer Drug Design, **1998 13**, 463-488.

[21] Beerman, T.A.; Woyanowski, J.M.; Sigmund, R.D.; Gawron, L.S.;, Rao, K.E.; Lown, J.M. Biochim. Biophys. Acta, **1991 1090**, 52-60.

[22] Burckhardt, G.; Förtsch, I.; Simon, H.; Birch-Hirschfeld, E.; Kittler, L.; Schütz, H; Shama, S.K.; Lown, J.W. J.Biomolec. Struct. & Dyn., in press.