Antimalarial Drug Quinacrine Binds to C-Terminal Helix of Cellular Prion Protein

Martin Vogtherr,[†] Susanne Grimme,[†] Bettina Elshorst,[†] Doris M. Jacobs,[†] Klaus Fiebig,[†,‡] Christian Griesinger,[§] and Ralph Zahn*[,#]

Institut für Molekularbiologie und Biophysik, Eidgenössische Technische Hochschule Zürich, CH-8093 Zürich, Switzerland, Institut für Organische Chemie, Universität Frankfurt, Marie-Curie-Strasse 11, 60439 Frankfurt, Germany, and Max Planck Institut für Biophysikalische Chemie, Am Fassberg 11, 37077 Göttingen, Germany

Received May 2, 2003

Abstract: Using NMR spectroscopy we show that the cellular prion protein constitutes a target for binding of various acridine and phenothiazine derivatives. We unambiguously map the quinacrine binding site of recombinant human prion protein to residues Tyr225, Tyr226, and Gln227 of helix α3, which is located near the "protein X" epitope. The millimolar dissociation constant of the complex suggests that in vivo inhibition of prion propagation occurs after 10000-fold concentration of quinacrine within endolysosomes.

Quinacrine has been used in people for more than 60 years for treatment of malaria, before it was rediscovered as a potential drug for treatment of Creutzfeldt−Jakob disease (CJD),[1,2] a neurodegenerative disease leading to spongiform degeneration of the brains of humans and animals.[3] A new "variant" of CJD (vCJD, apparently caused by eating infected beef) has captured public attention and has been the focus of intense research since 1995, when the first victim of vCJD has been reported.[4] Since then, vCJD has killed more than 120 people in Europe and no one knows how many harbor latent vCJD infections from having eaten tainted beef during the height of the "mad cow disease" epidemic in the late 1980s and early 1990s. In 2001, headlines around the world heralded the apparent discovery of Rachel Forber, a young British woman with vCJD who had flown to San Francisco to receive quinacrine as a therapeutic treatment. Although Forber's recovery, like that of Japanese patients treated with quinacrine, was only transient,[5] second-generation drugs based on quinacrine and analogues thereof hold the promise of being more potent for the treatment of CJD.[6]

Recently, the acridine analogues quinacrine and chloroquine and various phenothiazine derivatives have been identified to inhibit PrP^{Sc} formation in cultured cells at micromolar concentrations.[1,2] The reason quinacrine and related compounds have an influence on the conversion of the cellular form of the prion protein, PrP^{C}, into the pathogenic form, PrP^{Sc} (which causes CJD), remains unclear.[2] We have used nuclear magnetic resonance (NMR) spectroscopy to show that PrP^{C} is bound by quinacrine and to map the binding site of quinacrine on uniformly ^{15}N-labeled human prion protein (hPrP) that was recombinantly expressed in bacteria. NMR has served as an excellent technique for mapping ligand−protein interactions by measuring the chemical shift changes of hydrogen and nitrogen resonance frequencies of the protein upon binding of the ligand molecule.[7] In the case of the hPrP, this approach is straightforward because the NMR resonance assignments are available.[8]

We recorded [^{15}N,^{1}H] heteronuclear single quantum coherence spectra of the uniformly ^{15}N-labeled globular domain of hPrP (residues 121−230) at two pH values: pH 4.5, which was used for the NMR structure determination of hPrP,[8] and the more physiological pH 7.0. We found that quinacrine bound at both pH values but with more pronounced chemical shift changes at pH 7.0. This suggests the importance of electrostatic contributions to binding, which is not unexpected considering the positively charged nitrogen heterocycle of quinacrine. Binding could not be disrupted by the addition of 100 mM NaCl to the sample buffer solution, as identified by near-identical chemical shift changes in the presence and absence of salt, indicating that binding is rather specific.

The dissociation constant of the quinacrine−hPrP-(121−230) complex at pH 7.0 was determined to be 4.6 mM, which is 4 orders of magnitude weaker than the EC_{50} value found for PrP^{Sc} inhibition in the cell culture assay.[2] The correlation between cellular activity and the relatively weak binding in the NMR tube might be rationalized by a specific enrichment of this drug in cell organelles. This effect is essential for malaria therapy with chloroquine, which is concentrated within endolysosomes up to 10000-fold relative to the extracellular concentration,[9] resulting in therapeutically obtainable intracellular concentrations in the millimolar range.[10] An interaction between PrP and chloroquine or quinacrine within endolysosomes would be in line with the suggestion that PrP conversion takes place during the endocytic pathway to the lysosomes.[11,12]

We were able to map the binding site of quinacrine within helix α3 of the human prion protein (Figure 1). While the most prominent chemical shift changes occurred for residues Tyr225, Tyr226, and Gln227, nearly all residues of helix α3 and selected residues from other secondary structure elements making contacts with helix α3 showed chemical shift perturbations. The same binding site was identified for a construct comprising the globular domain of the bovine prion protein. It thus seems that the diprotic and hydrophobic quinacrine (Figure 1) specifically binds to the tripeptide 225−227 in human and bovine PrP, resulting in an overall conformational change of helix α3. The upfield-shifted amide proton resonances of Tyr225, Tyr226, and Gln227 (Table 1) are consistent with a close contact to the ring protons of quinacrine, induced by a ring current shift upon binding.

* To whom correspondence should be addressed. Phone: +41 1 633 2740. Fax: +41 1 633 1073. E-mail: rz@mol.biol.ethz.ch.
[†] Universität Frankfurt.
[‡] Present address: Affinium Pharmaceuticals, 100 University Avenue, Toronto, Ontario M5J 1V6, Canada.
[§] Max Planck Institut für Biophysikalische Chemie.
[#] Eidgenössische Technische Hochschule Zürich.

10.1021/jm034093h CCC: $25.00
Published on Web 07/11/2003

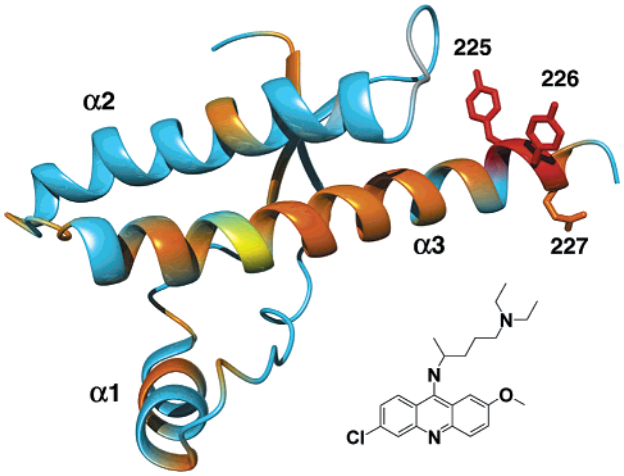

Figure 1. Projection of the chemical shift mapping of the quinacrine−hPrP(121−230) complex onto the NMR structure of hPrP(121−230).[8] Chemical shift variations of residues 125−230 due to quinacrine binding, $\delta\Delta_{av} = [\delta\Delta(^1H^N)^2 + (0.17\delta\Delta(^{15}N))^2]^{1/2}$, are indicated by the following color code for the backbone atoms: blue, $\delta\Delta_{av} \leq 0.005$ ppm; yellow, 0.005 ppm $< \delta\Delta_{av} \leq 0.01$ ppm; orange, 0.01 ppm $< \delta\Delta_{av} \leq 0.025$ ppm; dark-orange, 0.025 ppm $< \delta\Delta_{av} \leq 0.06$ ppm; red, $\delta\Delta_{av} >$ 0.06 ppm. The backbone amide resonances that could not be assigned because of conformational exchange line broadening[8] are indicated in gray. The side chains constituting the quinacrine binding site, i.e., residues Tyr225, Tyr226, and Gln227, are indicated. The structure of quinacrine is shown at the bottom. The NMR measurements were performed at 25 °C on a Bruker DRX600 spectrometer with ^{15}N-labeled samples of 0.25 mM protein solutions containing 5 mM quinacrine, 50 mM sodium phosphate buffer at pH 7.0, 0.05% NaN_3, 5 mM imidazole, 1 mM 3-trimethylsilylpropionic acid, and 10% d_6-dimethyl sulfoxide.

Table 1. Structure−Activity Relationship of Psychopharmacological Substances on PrP^{Sc} Inhibition and PrP^C Binding

compd	EC_{50}[a] (μM)	$\delta\Delta(^1H)$[b] (ppm)	$\delta\Delta(^{15}N)$[b] (ppm)
quinacrine	0.3	−0.027	−0.111
chlorpromazine	2	−0.020	−0.065
chloroquine	4	−0.010	−0.105
acepromazine	5	−0.013	−0.111
promazine	5	−0.010	−0.096
promethazine	8	−0.004	−0.056
imipramine	10	0.0	0.0
carbamazepine	>10	0.0	0.0
haloperidol	>10	0.0	0.0
phenazine	>10	−0.003	0.0

[a] Half-maximal inhibition of PrP^{Sc} formation assayed in ScN2a cells.[2] [b] Chemical shift variations of Tyr225 in the presence of various psychopharmacological substances. The NMR measurements were performed at 25 °C on a Bruker DRX600 spectrometer with ^{15}N-labeled samples of 0.25 mM hPrP(121−230) solutions containing 2 mM of the chemical compounds indicated, 50 mM sodium phosphate buffer at pH 7.0, 0.05% NaN_3, 5 mM imidazole, 1 mM 3-trimethylsilylpropionic acid, and 10% d_6-dimethyl sulfoxide.

We also observed a binding between hPrP(121−230) and other tricyclic compounds, i.e., chloroquine and the phenothiazine derivatives acepromazine, chlorpromazine, and promazine. For these, there is a close relationship between the ^1H chemical shift perturbations of Tyr225, as monitored by NMR spectroscopy, and the PrP^{Sc} inhibition activity in cultured cells (Table 1). Substances that were identified in the cell culture assay to be "inactive" showed no effect in the NMR spectrum. The fact that significant chemical shift changes for residues Tyr226 and Gln227 were exclusively observed for quinacrine is consistent with the approximately 10-fold higher EC_{50} value of quinacrine versus the other psychopharmacological substances with antiprion activity (Table 1) and may indicate specific interactions between PrP and quinacrine not present for the other compounds.

The identification of helix α3 as the binding site of quinacrine in recombinant PrP is intriguing because helix α3 has been identified as an intermolecular interaction site of "protein X", a hypothetical factor that supposedly participates in the disease-related conformational transformation of PrP in vivo,[13] e.g., by promoting the unfolding of PrP^C into a conversion competent folding intermediate.[3,14] Furthermore, monoclonal[15] and recombinant[16] antibodies raised against the C-terminus of helix α3 have been shown to specifically inhibit the generation of new PrP^{Sc} in ScN2a cells, indicating that helix α3 of PrP^C represents an interesting target for prion therapeutics. In conclusion, from our structural data we propose that quinacrine and related molecules decrease efficiency of prion propagation by competing with protein X for PrP^C binding.

References

(1) Doh-Ura, K.; Iwaki, T.; Caughey, B. Lysosomotropic agents and cysteine protease inhibitors inhibit scrapie-associated prion protein accumulation. *J. Virol.* **2000**, *74*, 4894−4897.
(2) Korth, C.; May, B. C. H.; Cohen, F. E.; Prusiner, S. B. Acridine and phenothiazine derivatives as pharmacotherapeutics for prion disease. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 9836−9841.
(3) Prusiner, S. B. Prions. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 13363−13383.
(4) Will, R. G.; Ironside, J. W.; Zeidler, M.; Cousens, S. N.; Estibeiro, K.; et al. A new variant of Creutzfeldt−Jakob disease in the UK. *Lancet* **1996**, *347*, 921−925.
(5) Follette, P. New perspectives for prion therapeutics meeting: Prion disease treatment's early promise unravels. *Science* **2003**, *299*, 191−192.
(6) May, B. C. H.; Fafarman, A. T.; Hong, S. B.; Rogers, M.; Deady, L. W.; et al. Potent inhibition of scrapie prion replication in cultured cells by bis-acridines. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 3416−3421.
(7) Shuker, S. B.; Hajduk, P. J.; Meadows, R. P.; Fesik, S. W. Discovering high-affinity ligands for proteins: SAR by NMR. *Science* **1996**, *274*, 1531−1534.
(8) Zahn, R.; Liu, A.; Lührs, T.; Riek, R.; von Schroetter, C.; et al. NMR solution structure of the human prion protein. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 145−150.
(9) O'Neill, P. M.; Bray, P. G.; Hawley, S. R.; Ward, S. A.; Park, B. K. 4-Aminoquinolines−Past, present, and future: A chemical perspective. *Pharmacol. Ther.* **1998**, *77*, 29−58.
(10) French, J. K.; Hurst, N. P.; Odonnell, M. L.; Betts, W. H. Uptake of chloroquine and hydroxychloroquine by human-blood leukocytes in vitro−relation to cellular concentrations during antirheumatic therapy. *Ann. Rheum. Dis.* **1987**, *46*, 42−45.
(11) Caughey, B.; Raymond, G. J.; Ernst, D.; Race, R. E. N-terminal truncation of the scrapie-associated form of PrP by lysosomal protease(s): implications regarding the site of conversion of PrP to the protease-resistant state. *J. Virol.* **1991**, *65*, 6597−6603.
(12) Borchelt, D. R.; Taraboulos, A.; Prusiner, S. B. Evidence for synthesis of scrapie prion proteins in the endocytic pathway. *J. Biol. Chem.* **1992**, *267*, 16188−16199.
(13) Telling, G. C.; Scott, M.; Hsiao, K. K.; Foster, D.; Yang, S. L.; et al. Transmission of Creutzfeldt−Jakob disease from humans to transgenic mice expressing chimeric human−mouse prion protein. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 9936−9940.
(14) Zahn, R. Prion propagation and molecular chaperones. *Q. Rev. Biophys.* **1999**, *32*, 309−370.
(15) Horiuchi, M.; Caughey, B. Specific binding of normal prion protein to the scrapie form via a localized domain initiates its conversion to the protease-resistant state. *EMBO J.* **1999**, *18*, 3193−3203.
(16) Peretz, D.; Williamson, R. A.; Kaneko, K.; Vergara, J.; Leclerc, E.; et al. Antibodies inhibit prion propagation and clear cell cultures of prion infectivity. *Nature* **2001**, *412*, 739−743.

JM034093H