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NMR detection of arginine-ligand interactions in complexes of *Lactobacillus casei* dihydrofolate reductase

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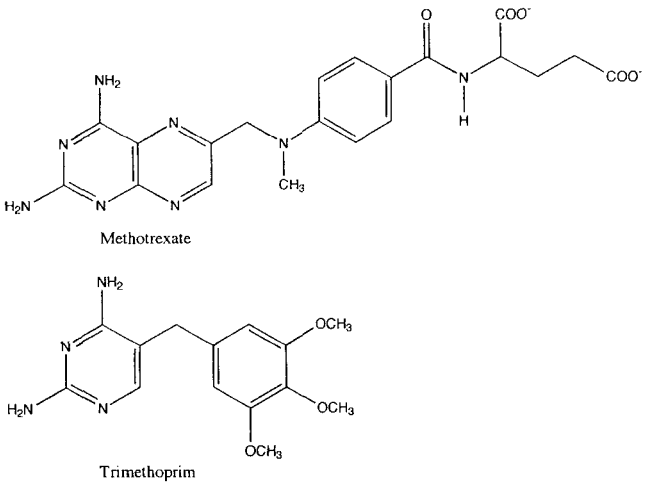
^{1}H-NMR and ^{15}N-NMR signal assignments have been made for the eight arginine residues in *Lactobacillus casei* dihydrofolate reductase in its binary complex with methotrexate and in its ternary complex with methotrexate and NADPH. ^{1}H-NMR chemical shifts for the guanidino groups of two of the arginines (Arg57 and Arg43) were sensitive to different modes of binding of the guanidino groups with charged oxygen atoms of the ligands. In the complexes formed with methotrexate, Arg57 showed four non-equivalent NH$^{\eta}$ proton signals indicating hindered rotation about the N$^{\varepsilon}$-C$^{\zeta}$ and C$^{\zeta}$-N$^{\eta}$ bonds. The NH$^{\eta 12}$ and NH$^{\eta 22}$ protons showed large downfield shifts, which would be expected for a symmetric end-on interaction of these protons with the charged oxygen atoms of a carboxylate group in methotrexate. These effects were not observed for the complex formed with trimethoprim, which does not contain any carboxylate groups. In the complex formed with NADPH present, Arg43 showed a large downfield chemical shift for its NH$^{\varepsilon}$ proton and a retardation of its rate of exchange with water. This pattern of deshielding contrasts with that detected for Arg57 and is that expected for a side-on interaction of the guanidino group protons with charged oxygen atoms of the ribose 2′-phosphate group of NADPH.

Keywords: dihydrofolate reductase; arginine NH$^{\eta}$ proton interactions; NMR.

Dihydrofolate reductase catalyses the reduction of 7,8-dihydrofolate to 5,6,7,8-tetrahydrofolate using NADPH as coenzyme [1]. The enzyme is of considerable pharmacological interest, since it is the target for a number of antifolate drugs such as the antineoplastic agent methotrexate and the antibacterial agent trimethoprim. There have been several X-ray and NMR structural studies aimed at understanding the origins of the specificity of the ligand binding [1–7]. The crystal structure of the *Lactobacillus casei* dihydrofolate reductase · methotrexate · NADPH ternary complex indicates that specific interactions occur between several of the arginine residues and the bound ligands [6, 7]. To obtain more information about such interactions in solution we have examined NMR data from two-dimensional (2D) ^{1}H-^{15}N heteronuclear single quantum coherence (HSQC), three-dimensional (3D) ^{15}N edited NOESY-HSQC and HNHB (three-dimensional NMR experiment for correlating backbone ^{15}N and side-chain $^1H_{\beta}$ in proteins) spectra [8–10] of various binary and ternary dihydrofolate reductase complexes, looking explicitly at the signals arising from the arginine side chain protons. The chemical shifts of these protons should be sensitive to any changes in their local geometry and electronic environment brought about by ligand binding and therefore could act as useful probes of the different modes of ligand binding to arginine residues. An earlier NMR study examining ^{13}C-labelled arginine residues has been reported [11] for *Streptococcus faecium* dihydrofolate reductase, and although this was limited by the lack of specific signal assignments, some classification of the interacting and non-interacting arginine residues was possible. In this present study, the availability of the specific ^{1}H and ^{15}N assignments allows a more detailed consideration of the arginine-ligand interactions.

Scheme 1. Structures of methotrexate and trimethoprim ligands.



MATERIALS AND METHODS

^{15}N-labelled *L. casei* dihydrofolate reductase was expressed in *Escherichia coli* cells grown on a minimal medium and iso-

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Abbreviations. 2D, two dimensional; 3D, three dimensional; GARP, computer-optimised wide-band decoupling technique; HNHB, three-dimensional NMR experiment for correlating backbone ^{15}N and side-chain $^1H_{\beta}$ in proteins; HSQC, heteronuclear single quantum coherence.

Enzyme. Dihydrofolate reductase (EC 1.5.1.3).

Table 1. The ^{1}H-NMR and ^{15}N-NMR chemical shifts of arginine residues in ligand complexes of *L. casei* dihydrofolate reductase. The ^{1}H-NMR chemical shifts at 281 K are referenced to 2,2-dimethyl-2-silapentane-5-sulphonate (after measuring from dioxane) and the ^{15}N-NMR chemical shifts are referenced to liquid NH_3 calibrated using the γ ratios method [22]. ^{1}H errors (±0.02 ppm), ^{15}N errors (±0.1 ppm) except where stated otherwise.

Residue	Chemical shift of							
	dihydrofolate reductase · methotrexate				dihydrofolate reductase · methotrexate · NADPH			
	$^{15}N^{\varepsilon}$	$^1H^{\varepsilon}$	$^{15}N^{\eta}$	$^1H^{\eta}$	$^{15}N^{\varepsilon}$	$^1H^{\varepsilon}$	$^{15}N^{\eta}$	$^1H^{\eta}$
	ppm							
R9	85.1	7.33	70 ± 1.5 70 ± 1.5	6.47 6.94	85.6	7.36	70 ± 1.5 70 ± 1.5	6.53 6.90
R31	85.7	7.39	70 ± 1.5 70 ± 1.5	6.57 6.87	85.1	7.29	70 ± 1.5 70 ± 1.5	6.54 6.84
R43	85.1	8.32	70 ± 1.5	6.86	84.4	9.23	70 ± 1.5 (70 ± 1.5	6.79, 6.65 6.65, 7.12)[a]
R44	84.0	7.29	70 ± 1.5	6.48	84.1	7.12	70 ± 1.5	6.61
R52	81.4	6.69		[b]	81.9	6.73		[b]
R57	79.9	5.66	75.0 79.2	6.89, 9.33 6.77, 10.17	80.2	5.62	75.2 78.6	6.87, 9.33 6.55, 10.0
R117	85.4	8.52	[c] 70 ± 1.5	5.61 6.75	86.1	8.54	[c] 70 ± 1.5	5.21 6.74
R142	83.1	7.44	70.3 70 ± 1.5	6.23 6.99	83.1	7.44	70 ± 1.5 70 ± 1.5	6.23 6.96

[a] These measurements were made at 308 K where an additional broad signal for NH^{η} proton (at 7.12 ppm) with an NOE connection to NH^{ε} was detected.

[b] H^{ε} and H^{η} protons are possibly degenerate: no NOE connections observed from NH^{ε}.

[c] Signal not detected in HSQC spectrum.

lated and purified as described previously [12–14]. Trimethoprim, methotrexate, and NADPH were obtained from Sigma. All other chemicals used were analytical grade.

The NMR experiments were performed on 0.6-ml samples of the binary complexes dihydrofolate reductase · methotrexate and dihydrofolate reductase · trimethoprim and the ternary complex dihydrofolate reductase · methotrexate · NADPH. The samples were 4 mM in protein complex in 90% H_2O/10% D_2O containing 50 mM potassium phosphate and either 50 mM KCl (methotrexate and methotrexate · NADPH) or 500 mM KCl (trimethoprim), pH* 6.5 (the pH* values are meter readings, unadjusted for deuterium isotope effects).

The NMR experiments were performed at 281 K on a Varian Unity spectrometer operating at 14.1 T. All the NMR experiments used the Watergate technique for water suppression [15] and all employed the GARP (computer-optimised wide-band decoupling technique) sequence [16] for ^{15}N decoupling during the detection period. Quadrature detection in all indirectly detected dimensions was achieved using the method of States et al. [17].

The 2D HSQC sequence used was essentially that proposed by Mori et al. [18]. These experiments were performed using acquisition times of 15–50 ms and 128 ms in the ^{15}N and ^{1}H dimensions respectively. The data were processed with zero-filling in both dimensions using Varian software (VNMR, version 5.1).

The 3D NOESY-HSQC experiments were carried out using a Watergate version of the original sequence of Marion et al. [9]. One experiment was carried out with a mixing time of 50 ms and one with a mixing time of 100 ms. Acquisition times were 7.6 ms and 10.5 ms in the ^{15}N dimension, 12.8 ms and 15.0 ms in the indirect ^{1}H dimension, and 51 ms and 64 ms in the real-time ^{1}H dimension.

The sequence used for the HNHB experiment was essentially a Watergate version of the constant-time method of Archer et al. [10]. The delay in the sequence that allows long-range ^{15}N-^{1}H couplings to evolve was set to 38 ms [10]. This experiment was carried out with acquisition times of 16.8, 15.3 and 71 ms in the ^{15}N, indirect ^{1}H, and direct ^{1}H dimensions, respectively.

The data from 3D experiments were transformed and displayed using software written in-house. The ^{15}N dimension of the data set with the shortest acquisition time was extended by 50% using linear prediction methods [19]. The real part of each final data matrix had a size of 128 (^{15}N) ×512 (indirect ^{1}H) ×1024 (direct ^{1}H) points.

RESULTS

Arginine NH^{ε} assignments. The ^{1}H-NMR assignments for most resonances in the spectra of the binary dihydrofolate reductase · methotrexate and dihydrofolate reductase · trimethoprim complexes have been published [14, 20, 21]. For the dihydrofolate reductase · methotrexate complex, these included the partial assignments for six of the eight arginine residues [20]. From a detailed analysis of the 3D ^{13}C-edited correlation spectra [20] in combination with the ^{13}C-edited HMQC-NOESY spectrum (data not shown), the side-chain CH assignments of the previously unassigned arginine residues have now been obtained. The NH^{ε} proton assignments were then made from examination of the low temperature (281 K) 3D ^{15}N/^{1}H NOESY-HSQC spectrum where the NH^{ε} protons in all eight arginine residues could be connected to the assigned side-chain CH protons in the same residues (see Table 1). The signal from the NH^{ε} proton of Arg43 is shifted 0.91 ppm downfield by the addition of NADPH to the dihydrofolate reductase · methotrexate complex. In the 3D

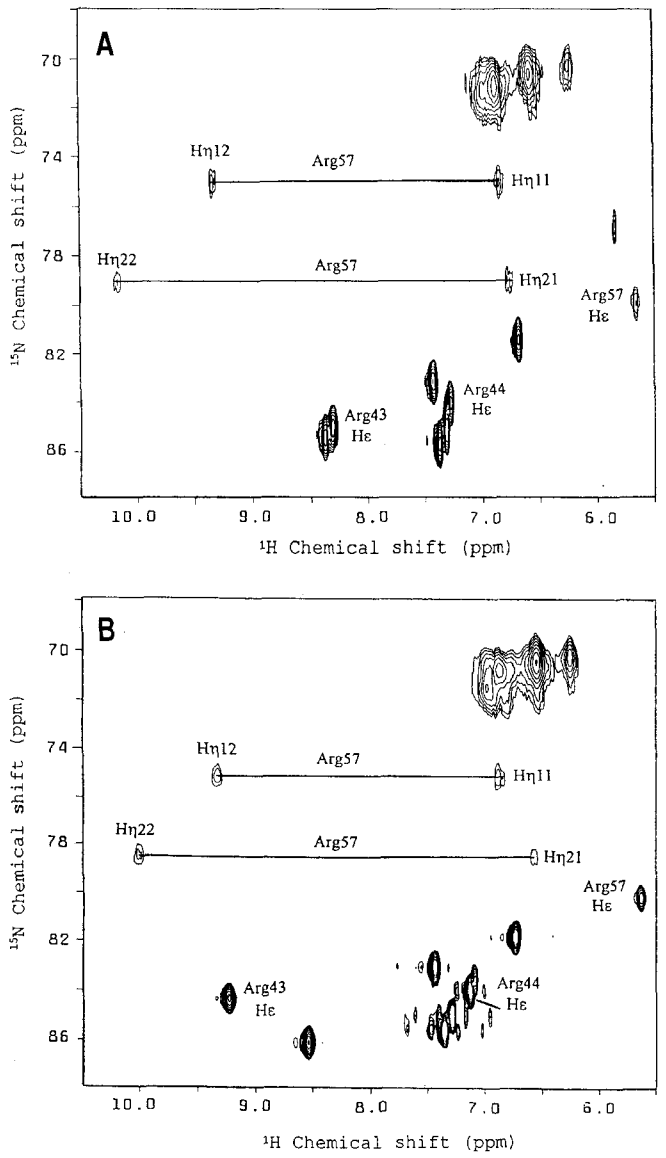

Fig. 1. Part of the ^{1}H/^{15}N HSQC spectrum at 600 MHz (281 K) recorded for complexes of *L. casei* dihydrofolate reductase with (A) methotrexate and (B) methotrexate and NADPH. Both complexes were formed with one equivalent of each ligand (4 mM at pH* 6.5).

NOESY-HSQC spectrum of the dihydrofolate reductase · methotrexate · NADPH complex at 281 K, the Arg43 NH$^{\varepsilon}$ proton showed no exchange cross-peak to the water signal whereas such a cross-peak was detected in the corresponding spectrum of the dihydrofolate reductase · methotrexate complex. This indicates that the addition of NADPH retards the proton exchange rate of the Arg43 NH$^{\varepsilon}$ proton with water.

Arginine NH$^{\eta}$ assignments. The HSQC spectrum of the dihydrofolate reductase · methotrexate complex (Fig. 1A) revealed a set of four signals from two NH_2 groups with ^{15}N chemical shifts at 79.2 ppm and 75.0 ppm. Chemical shift comparisons with data from earlier NMR studies on arginine residues allowed these signals to be assigned to nuclei from arginine guanidino NH_2 groups [23–25]. The observation of NOEs between these NH$^{\eta}$ protons in the 3D NOESY-HSQC spectra showed that these two groups belong to the same arginine residue. The signals were assigned to the guanidino group of Arg57 on the basis of an NOE between one of the NH$^{\eta}$ protons and the previously assigned NH$^{\varepsilon}$ of Arg57. This also allowed the stereospecific assignment of the protons of one of the NH_2 groups (H$^{\eta 11}$, 6.89 ppm; H$^{\eta 12}$, 9.33 ppm) (Fig. 2). The fact that the four guanidino H$^{\eta}$ nuclei and two N$^{\eta}$ nuclei of Arg57 have different chemical shifts indicates that there is hindered rotation about both the N$^{\varepsilon}$-C$^{\zeta}$ and C$^{\zeta}$-N$^{\eta}$ bonds of Arg57 in this complex. It can be seen in Fig. 1B that a similar pattern of chemical shifts is observed for the Arg57 NH$^{\eta}$ protons in the ternary complex dihydrofolate reductase · methotrexate · NADPH indicating that there is hindered rotation in this complex as well.

In the HNHB experiment on the dihydrofolate reductase · methotrexate · NADPH complex at 308 K, a cross-peak between the Arg43 ^{15}NH$^{\varepsilon}$ and a signal at 6.65 ppm was detected and the latter could thus be firmly assigned as an NH$^{\eta}$ signal. In the 3D NOESY-HSQC spectrum recorded at 308 K, the Arg43 NH$^{\varepsilon}$ proton shows two NOE cross-peaks to signals at frequencies assignable to NH$^{\eta}$ protons, one at 6.65 ppm as expected from the HNHB results and a broad signal at 7.12 ppm. This broad signal was not observed in the 3D NOESY-HSQC spectrum recorded at 281 K and its appearance at 308 K is consistent with the signal arising from coalescence of signals from a pair of exchanging guanidino NH_2 protons which are in intermediate exchange at the lower temperature. The results indicate that there is hindered rotation about at least one of the C$^{\zeta}$-N$^{\eta}$ bonds and that none of the observed NH$^{\eta}$ protons have been appreciably deshielded by the presence of NADPH. The ^{1}H and ^{15}N assignments for the guanidino group nuclei for the arginine residues are listed in Table 1.

DISCUSSION

Interactions with Arg57. From considerations of the crystal structure of dihydrofolate reductase · methotrexate · NADPH, Bolin et al. [6] inferred the presence of hydrogen bonding interactions between the guanidino group of Arg57 and the α-carboxylate of the glutamate moiety of methotrexate. The NMR results (Table 1 and Fig. 1) provide clear evidence that this interaction is also present in solution: the interactions cause the hindered rotation in the guanidino group leading to separate signals being detected for the four non-equivalent NH_2 protons with two of the protons showing large downfield chemical shifts.

The only way in which a carboxylate group can interact with the NH_2 groups of Arg57 to perturb the shielding of the NH$^{\eta 12}$ and one of the NH$^{\eta 2}$ protons is for the carboxylate oxygen atoms to form hydrogen bonds with the centrally situated hydrogens, NH$^{\eta 12}$ and NH$^{\eta 22}$, described as type 1 by Lancelot et al. [26] and shown in Fig. 2A. In an analysis of arginine-aspartate interactions by Mitchell et al. [27], such an end-on symmetric structure was found to be one of the most favoured orientations for intermolecular interactions between arginine guanidino NH_2 and carboxylate groups. In this model, the NH$^{\eta 12}$ and NH$^{\eta 22}$ protons interact with the carboxylate oxygen atoms. The large downfield shift of Arg57 NH$^{\eta 12}$ (9.33 ppm) compared to other Arg NH$^{\eta}$ signals is fully consistent with this model and it is also reasonable to assign the other downfield shifted signal at 10.17 ppm to the NH$^{\eta 22}$ proton.

Yamazaki et al. [24] have reported similar results for a complex of the SH2 domain and a phosphotyrosyl peptide where they suggested that the hindered rotation resulted from the interactions between the arginine guanidino NH_2 groups and a phosphate group. In this case, the NH$^{\eta 12}$ and NH$^{\eta 22}$ protons formed hydrogen bonds with the phosphate oxygen atoms.

Further support for the interaction between Arg57 and a carboxylate group in the glutamate moiety of methotrexate comes

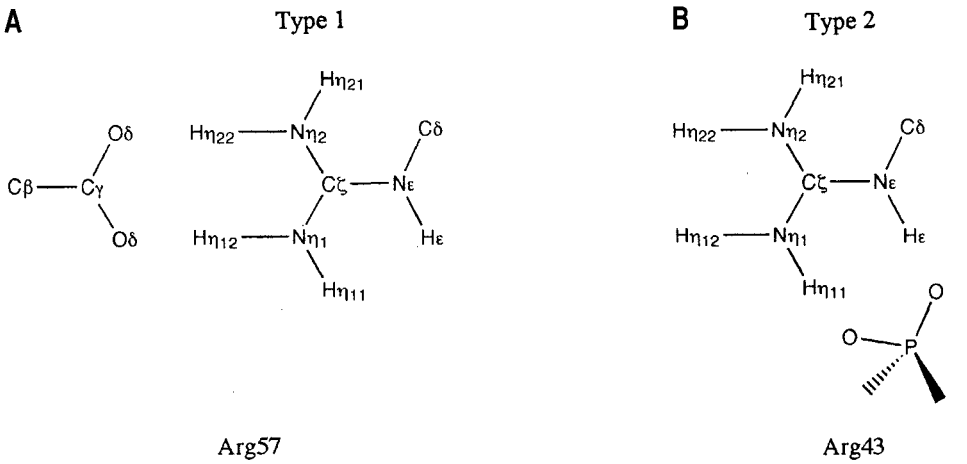

Fig. 2. Structures of arginine residues (A) in a type 1 end-on symmetrical interaction with a carboxylate group, as found in the interaction of Arg57 with the glutamate α-carboxylate of methotrexate, and (B) in a type 2 side-on interaction with a phosphate group, as found in the interaction of Arg43 with the 2′-phosphate group of NADPH.

from examining the spectra of the trimethoprim and trimetrexate complexes with dihydrofolate reductase (data not shown). The unusual Arg57 NH^{η} signals present in the HSQC spectrum of the dihydrofolate reductase · methotrexate complex are not observed in the spectra of binary complexes formed with these ligands. These compounds lack the carboxylate groups of methotrexate (Scheme 1) and are unable to interact with the Arg57 residue.

The NMR spectrum of the dihydrofolate reductase · methotrexate · NADPH ternary complex showed similar characteristic chemical shifts to those in the binary complex for the Arg57 guanidino NH_2 protons again indicating the presence of the same interactions between Arg57 and the bound methotrexate in the ternary complex.

Arg43 and Arg44 interactions. In the spectrum of the dihydrofolate reductase · methotrexate · NADPH complex (Fig. 1 B), the chemical shift of the NH^{ε} proton of Arg43 is shifted 0.91 ppm downfield from its value in the spectrum of the binary dihydrofolate reductase · methotrexate complex. From the crystal structure, Filman and coworkers [7] proposed that the NH^{ε} and NH^{η} protons of Arg43 make hydrogen bonds to the adenine ribose 2′-phosphate of the bound NADPH (Fig. 2 B). This orientation, described by Lancelot et al. [26] as type 2, is a side-on interaction which lies in one of the energy-minimum regions and is favoured by intermolecular interactions [27]. The difference in chemical shifts of the NH^{ε} proton of Arg43 between the binary and ternary complexes (0.91 ppm) and the decrease in its rate of proton exchange with water strongly supports the idea of an interaction between the NH^{ε} proton and one of the oxygens of the ribose 2′-phosphate group in a side-on interaction with the phosphate group. However, the absence of an appreciable downfield chemical shift for any of the Arg43 NH^{η} protons on addition of NADPH means that there is no direct evidence for the involvement of an NH^{η} proton in the interaction.

The X-ray data [6, 7] indicated that the O atom of the adenine ribose 3′-OH group interacts with one of the NH^{η} protons of Arg44. However, no low-field-shifted NH^{η} signals could be detected for this residue.

Arg117 interactions. Arg117 is the only other arginine residue which shows H^{η} atoms with a large chemical shift difference, which suggests that its guanidino group is involved in a hydrogen bonding interaction. From a consideration of the crystal structure [6, 7], the most likely candidate for an interaction with Arg117 is Glu156. Direct NOEs between Arg117 and Glu156 would not be expected because of the relative orientations of their side chains. However, other detected NOEs involving nearby residues (such as that between Arg117 NH^{ε} and Trp158 $H^{\delta 1}$) indicate that the crystal and solution structures are similar in this region of the protein.

Conclusions. We have shown that the guanidino group NH proton chemical shifts of two arginine residues in *L. casei* dihydrofolate reductase (Arg57 and Arg43) are sensitive to distinct modes of binding with charged oxygen atoms on the ligands methotrexate and NADPH (Fig. 2). The ligand-induced differences in chemical shifts of the NH^{ε} and NH^{η} protons and the observations of the presence or absence of hindered rotation in the NH_2 fragments of their guanidino groups allows us to monitor interactions between arginine residues and specific groups on ligands binding to dihydrofolate reductase. This could provide a useful approach for monitoring dihydrofolate reductase interactions with substrate analogues containing carboxylate or phosphate groups. Typical examples are the previously studied trimethoprim analogues having side chains containing carboxylate groups designed to interact specifically with conserved arginine residues in dihydrofolate reductase [28–30].

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REFERENCES

1. Blakley, R. L. (1985) Dihydrofolate reductase, in *Folates and pterins* (Blakley, R. L. & Benkovic, S. J. K., eds) vol. 1, pp. 191–253, J. Wiley, New York.
2. Matthews, D. A., Bolin, J. T., Burridge, J. M., Filman, D. J., Volz, K. W., Kaufman, B. T., Beddell, C. R., Champness, J. N., Stammers, D. K. & Kraut, J. (1985) Refined crystal structures of *Escherichia coli* and chicken liver dihydrofolate reductase containing bound trimethoprim, *J. Biol. Chem. 260*, 381–391.
3. Freisheim, J. H. & Matthews, D. A. (1984) The comparative biochemistry of dihydrofolate reductase, in *Folate antagonists as therapeutic agents* (Sirotnak, F. M., Burchill, J. J., Ensminger, W. D. & Montgomery, J. A., eds) vol. 1, pp. 69–131, Academic Press Inc., New York.

4. Feeney, J. (1990) NMR studies of interactions of ligands with dihydrofolate reductase, *Biochem. Pharmacol. 40*, 141–152.
5. Roberts, G. C. K. (1990) NMR and mutagenesis studies of dihydrofolate reductase, in *Chemistry and biology of pteridines 1989. Pteridines and folic acid derivatives* (Curtius, H. C., Ghisla, S. & Blau, N., eds) pp. 681–693, de Gruyter, Berlin.
6. Bolin, J. T., Filman, D. J., Matthews, D. A., Hamlin, R. C. & Kraut, J. (1982) Crystal structures of *E. coli* and *L. casei* dihydrofolate reductase refined to 1.7 Å resolution. I. General features and binding of methotrexate, *J. Biol. Chem. 257*, 13650–13662.
7. Filman, D. J., Bolin, J. T., Matthews, D. A. & Kraut, J. (1982) Crystal structures of *Escherichia coli* and *Lactobacillus casei* dihydrofolate reductase refined at 1.7 Å resolution: II. Environment of bound NADPH and implications for catalysis, *J. Biol. Chem. 257*, 13663–13672.
8. Bodenhausen, G. & Ruben, D. J. (1980) Natural abundance nitrogen-15 NMR by enhanced heteronuclear spectroscopy, *Chem. Phys. Lett. 69*, 185–188.
9. Marion, D., Driscoll, P. C., Kay, L. E., Wingfield, P. T., Bax, A., Gronenborn, A. M. & Clore, G. M. (1989) Overcoming the overlap problem in the assignment of ^{1}H NMR spectra of larger proteins by use of three-dimensional heteronuclear ^{1}H-^{15}N Hartmann-Hahn-multiple quantum coherence and nuclear Overhauser-multiple quantum coherence spectroscopy: application to interleukin 1β, *Biochemistry 28*, 6150–6156.
10. Archer, S. J., Ikura, M., Torchia, D. A. & Bax, A. (1991) An alternative 3D NMR technique for correlating backbone ^{15}N with side-chain Hβ resonances in larger proteins, *J. Magn. Reson. 95*, 636–641.
11. Cocco, L., Blakley, R. L., Walker, T. I., London, R. E. & Matwiyoff, N. A. (1978) Nuclear magnetic resonance studies on bacterial dihydrofolate reductase containing [*guanidino*-^{13}C] arginine, *Biochemistry 17*, 4285–4290.
12. Andrews, J., Clore, G. M., Davies, R. W., Gronenborn, A. M., Gronenborn, B., Kalderon, D., Papadopoulos, P. C., Schafer, S., Sims, P. F. G. & Stancombe, R. (1985) Nucleotide sequence of the dihydrofolate reductase gene of methotrexate-resistant *Lactobacillus casei*, *Gene (Amst.) 35*, 217–222.
13. Dann, J. G., Ostler, G., Bjur, R. A., King, R. W., Scudder, P., Turner, P. C., Roberts, G. C. K., Burgen, A. S. V. & Harding, N. G. L. (1976) Large scale purification and characterisation of dihydrofolate reductase from a methotrexate-resistant strain of *Lactobacillus casei*, *Biochem. J. 157*, 559–571.
14. Carr, M. D., Birdsall, B., Frenkiel, T. A., Bauer, C. J., Jimenez-Barbero, J., McCormick, J. E., Feeney, J. & Roberts, G. C. K. (1991) Dihydrofolate reductase: sequential resonance assignments using 2D and 3D NMR and secondary structure determination in solution, *Biochemistry 30*, 6330–6341.
15. Sklenar, V., Piotto, M., Leppik, R. & Sauder, V. (1993) Gradient-tailored water suppression for ^{1}H-^{15}N HSQC experiments optimized to retain full sensitivity, *J. Magn. Reson. 102*, 241–245.
16. Shaka, A. J., Barker, P. B. & Freeman, R. (1985) Computer-optimized decoupling scheme for wideband applications and low-level operation, *J. Magn. Reson. 64*, 547–552.
17. States, D. J., Haberkorn, R. A. & Ruben, D. J. (1982) A two-dimensional nuclear Overhauser experiment with pure absorption phase in four quadrants, *J. Magn. Reson. 48*, 286–292.
18. Mori, S., Abeygunawardana, C., O'Neil Johnson, M. & Van Zijl, P. C. M. (1995) Improved sensitivity of HSQC spectra of exchanging protons at short interscan delays using a new fast HSQC (FHSQC) detection scheme that avoids water saturation, *J. Magn. Reson. 108*, 94–98.
19. Zhu, G. & Bax, A. (1990) Improved linear prediction for truncated signals of known phase, *J. Magn. Reson. 90*, 405–410.
20. Soteriou, A., Carr, M. D., Frenkiel, T. A., McCormick, J. E., Bauer, C. J., Sali, D., Birdsall, B. & Feeney, J. (1993) 3D ^{13}C/^{1}H NMR-based assignments for side-chain resonances of *Lactobacillus casei* dihydrofolate reductase: evidence for similarities between the solution and crystal structures of the enzyme, *J. Biomol. NMR 3*, 535–546.
21. Martorell, G., Gradwell, M. J., Birdsall, B., Bauer, C. J., Frenkiel, T. A., Cheung, H. T. A., Polshakov, V. I., Kuyper, L. & Feeney, J. (1994) Solution structure of bound trimethoprim in its complex with *Lactobacillus casei* dihydrofolate reductase, *Biochemistry 33*, 12416–12426.
22. Live, D. H., Davis, D. G., Agosta, W. C. & Cowburn, D. (1984) Observation of 1000-fold enhancement of ^{15}N NMR via proton-detected multiquantum coherences: studies of large peptides, *J. Am. Chem. Soc. 106*, 1939–1941.
23. Glushka, J., Barany, F. & Cowburn, D. (1989) Observation of arginyl-deoxyoligonucleotide interactions in Taq 1 endonuclease by detection of specific ^{1}H signals from 140 kDa [$N^{\eta 1}$, $N^{\eta 2}$, ^{15}N] Taq 1/oligomer complexes, *Biochem. Biophys. Res. Commun. 164*, 88–93.
24. Yamazaki, T., Pascal, S. M., Singer, A. U., Forman-Kay, J. D. & Kay, L. E. (1995) NMR pulse schemes for the sequence-specific assignment of arginine guanidino ^{15}N and ^{1}H chemical shifts in proteins, *J. Am. Chem. Soc. 117*, 3556–3564.
25. Pascal, S. M., Yamazaki, T., Singer, A. U., Kay, L. E. & Forman-Kay, J. D. (1995) Structural and dynamic characterization of the phosphotyrosine binding region of a Src homology 2 domain-phosphopeptide complex by NMR relaxation, proton exchange, and chemical shift approaches, *Biochemistry 34*, 11353–11362.
26. Lancelot, G., Mayer, R. & Helene, C. (1979) Conformational study of the dipeptide arginylglutamic acid and of its complex with nucleic bases, *J. Am. Chem. Soc. 101*, 1569–1576.
27. Mitchell, J. B. O., Thornton, J. M., Singh, J. & Price, S. L. (1992) Towards an understanding of the arginine-aspartate interaction, *J. Mol. Biol. 226*, 251–262.
28. Kuyper, L. F., Roth, B., Baccanari, D. P., Ferone, R., Beddell, C. R., Champness, J. N., Stammers, D. K., Dann, J. G., Norrington, F. E. A., Baker, D. J. & Goodford, P. J. (1982) Receptor-based design of dihydrofolate reductase inhibitors: comparison of crystallographically determined enzyme binding with enzyme affinity in a series of carboxy-substituted trimethoprim analogues, *J. Med. Chem. 25*, 1120–1122.
29. Birdsall, B., Feeney, J., Pascual, C., Roberts, G. C. K., Kompis, I., Then, R. L., Muller, K. & Kroehn, A. (1984) A ^{1}H study of the interactions and conformations of rationally designed brodimoprim analogues in complexes with *Lactobacillus casei* dihydrofolate reductase, *J. Med. Chem. 23*, 1672–1676.
30. Morgan, W. D., Birdsall, B., Polshakov, V. I., Sali, D., Kompis, I. & Feeney, J. (1995) Solution structure of a brodimoprim analogue in its complex with *Lactobacillus casei* dihydrofolate reductase, *Biochemistry 34*, 11690–11702.