FOR THE RECORD

Mapping the surface of *Escherichia coli* peptide deformylase by NMR with organic solvents

DOUGLAS W. BYERLY,¹ CRAIG A. MCELROY,¹ AND MARK P. FOSTER^{1,2}

¹Ohio State Biochemistry Program, The Ohio State University, Columbus, Ohio 43210, USA

²Biophysics Program and Protein Research Group, Department of Biochemistry, The Ohio State University, Columbus, Ohio 43210, USA

(RECEIVED January 24, 2002; FINAL REVISION April 4, 2002; ACCEPTED April 15, 2002)

Abstract

Identifying potential ligand binding sites on a protein surface is an important first step for targeted structure-based drug discovery. While performing control experiments with *Escherichia coli* peptide deformylase (PDF), we noted that the organic solvents used to solubilize some ligands perturbed many of the same resonances in PDF as the small molecule inhibitors. To further explore this observation, we recorded ¹⁵N HSQC spectra of *E. coli* peptide deformylase (PDF) in the presence of trace quantities of several simple organic solvents (acetone, DMSO, ethanol, isopropanol) and identified their sites of interaction from local perturbation of amide chemical shifts. Analysis of the protein surface structure revealed that the ligand-induced shift perturbations map to the active site and one additional surface pocket. The correlation between sites of solvent and inhibitor binding highlights the utility of organic solvents to rapidly and effectively validate and characterize binding sites on proteins prior to designing a drug discovery screen. Further, the solvent-induced perturbations have implications for the use of organic solvents to dissolve candidate ligands in NMR-based screens.

Keywords: Surface pockets; chemical shift perturbation; organic solvent; HSQC; peptide deformylase

Supplemental material: See www.proteinscience.org.

Knowledge of the location and characteristics of potential binding sites on proteins is essential for targeted ligand design. Simple uncharged organic solvent molecules have recently been shown to be useful for probing the reactivity of binding sites on proteins. For instance, crystallization of elastase in the presence of acetonitrile results in the solvent populating known inhibitor binding sites (Allen et al. 1996), while solvent–protein NOEs have been used to similarly locate solvent molecules bound to cavities in lysozyme and

FKBP (Liepinsh and Otting 1997; Dalvit et al. 1999). Further, systematic screening approaches (Shuker et al. 1996; Fejzo et al. 1999) can detect weak-binding ligands and provide genuine lead compounds for drug discovery. Once binding sites have been identified, the myriad of computational (Miranker and Karplus 1991; Eisen et al. 1994; Rosenfeld et al. 1995; Brady and Stouten 2000) and experimental (Erlanson et al. 2000; Wei et al. 2000; Clements et al. 2001) techniques for optimizing the affinity of ligands can be applied.

In an ongoing project to characterize ligand-induced changes in enzyme structure and dynamics, we noted in two-dimensional ¹⁵N HSQC spectra of *E. coli* peptide deformylase (PDF) (Meinzel et al. 1993; Rajagopalan et al. 1997b), that many of the amide resonances perturbed by the candidate ligands were also perturbed by the organic solvents used to solubilize the ligands. To further explore this

Reprint requests to: Mark P. Foster, Ohio State Biochemistry Program, Biophysics Program and Protein Research Group, Department of Biochemistry, The Ohio State University, Columbus, OH 43210, USA; e-mail: foster.281@osu.edu; fax: (614) 292-6773.

Abbreviations: PDF, *E. coli* peptide deformylase; Fo-Met, formyl-methionine; HSQC, 2D ¹⁵N-edited ¹H spectrum; DMSO, dimethyl sulfoxide.

Article and publication are at <http://www.proteinscience.org/cgi/doi/10.1110/ps.0203402>.

observation, we recorded ^{15}N HSQC spectra in the presence of small amounts of various organic solvents (2.5–5% v/v acetone, DMSO, ethanol, and isopropanol) to identify their sites of interaction on PDF.

By mapping the sites of chemical shift perturbation onto the crystal structure of PDF (Chan et al. 1997; Becker et al. 1998), we found a strong correlation between sites perturbed by the solvents and the inhibitors. This correlation illustrates that valuable insights into the reactivity and location of ligand binding sites can be readily obtained by solvent-induced shift perturbations prior to performing systematic small molecule screens (Shuker et al. 1996; Fejzo et al. 1999; Moy et al. 2001), or de novo structure determinations (Allen et al. 1996; Liepinsh and Otting 1997; Dalvit et al. 1999). This work extends findings from computational methods such as MCSS (Miranker and Karplus 1991), and crystallographic screening methods like MSCS (Allen et al. 1996), which have shown that binding sites can be characterized by screening with solvent molecules. Further, it serves as a reminder that the use of organic solvents to deliver candidate ligands can interfere with the detection of important weak ligand interactions.

Results and Discussion

Sites of solvent interaction were identified by chemical shift changes in ^{15}N -edited HSQC spectra through the course of a solvent titration; the effect of solvent on 135 of the 141 nonproline residues could thus be monitored without the need for de novo resonance assignments. Solvent-induced shift perturbations ranged from zero to a maximum of 0.27 ppm for ^1H and 0.93 for ^{15}N resonances (both in 5% isopropanol); a representative spectrum of PDF free compared with that in 5% ethanol is shown in Figure 1a. The identity of the residues whose resonances were perturbed and the degree of shift perturbation varied between the solvents, indicating that the probes interact in different ways with the protein, yielding unique insights into the characteristics of the binding sites. For instance, while the probes induce similar perturbations within the active site, isopropanol shifted additional resonances within a β -strand flanking the substrate binding site (residues 85–90) and the loop comprised of residues 62–68 (supplementary material).

The weighted-average shift perturbations $\Delta_{\text{av}}(\text{HN})$ (Grzesiek et al. 1996; Garrett et al. 1997; Foster et al. 1998) induced by ethanol are mapped onto the structure of PDF in Figure 1b, and in Figure 1c those induced by the high-affinity inhibitor, actinonin (scaled down threefold) (Chen et al. 2000) (unpublished data). All of the probes induced significant shift perturbations or line broadening of amide resonances of residues in the substrate-binding site of PDF; chemical shift perturbations were observed for many of these same residues (e.g., Ile⁸⁶, Gly⁸⁹, Glu¹³³) for PDF in the presence of actinonin and the substrate analogs Fo-Met

and Met-Ala-Ser (Meinzel et al. 1996). In addition to the substrate-binding pocket, solvent-induced perturbations were consistently observed for some residues (Leu⁷⁸, Val¹⁰⁰, and Arg¹⁰²) forming the second largest surface cavity located on the opposite face of the protein. This cavity, comprised by residues 76–83, 100–103, 131, 134–135, and 145, was also identified by analysis of the protein surface with the computer programs PASS and CAST (Liang et al. 1998; Brady and Stouten 2000).

The chemical shift changes are interpreted to reflect a preferential interaction of the solvent probes with the perturbed sites. Each probe used in the experiments has the capacity to accept a hydrogen bond, favoring an interaction with the backbone amides whose shifts were being monitored, plus an additional small aliphatic/hydrophobic region. Although many amides in PDF are available for hydrogen bonding to solvent molecules, only a fraction of those exhibited measurable shift changes. The absence of global changes in the spectrum and the correlation between the observed solvent-induced changes and those induced by substrates and specific inhibitors suggest that the shift perturbations identify sites of preferential solvent–protein interaction, rather than partial solvent-induced denaturation.

A final implication of these observations regards the use of organic solvents in NMR-based screening methods that have been gaining popularity (Shuker et al. 1996; Fejzo et al. 1999). Because of limited compound solubility, concentrated DMSO solutions of the candidate ligands are typically added to the target protein solution (~400 μL), to a final ligand concentration of ~1 mM. In practice, particularly when screening mixtures, this can result in substantial concentrations of the organic solvent in the solution (i.e., $\geq 1\%$ or in excess of 0.14 M), as previously noted (Fejzo et al. 1999). Because dissociation constants for solvent–protein interaction can overlap this range (Liepinsh and Otting 1997; Dalvit et al. 1999), the presence of organic solvents in a protein solution can both compete with a candidate ligand for a binding site, and obscure the results of the screen.

The simple experiments described here have shown that by acquiring ^{15}N -edited HSQC spectra in the presence of trace amounts of organic solvents, the reactivity of the protein surface can be rapidly probed without the need for conducting more time-consuming NMR experiments or carrying out a complete structure determination. By identifying both potential reactive surfaces and specific residues in or near ligand binding sites, the approach would be particularly valuable for guiding the design of targeted chemical screens (Erlanson et al. 2000) or site-directed mutagenesis experiments.

Materials and methods

Uniformly ^{15}N PDF (1–147) (Rajagopalan et al. 1997a) was obtained by overexpression in *E. coli* BL21(DE3) cells grown in M9

Fig. 1. (A) Overlay of the 2D $^1\text{H}/^{15}\text{N}$ HSQC spectra of PDF in the absence (black contours) and presence (red contours) of 5% ethanol. The local shift perturbations are indicative of preferential local interactions with the solvent probes. Similar spectra were recorded with acetone, DMSO, and isopropanol. Cartoon diagrams of PDF (1–147) with the magnitude of the solvent-induced (B) or actinonin-induced (C) backbone amide shift perturbations, $\Delta_{\text{av}}(\text{HN})$, mapped onto the radius of the α -carbons (radius = $\Delta_{\text{av}}(\text{HN}) \times 15$ for ethanol and $\Delta_{\text{av}}(\text{HN}) \times 5$ for actinonin). The catalytic metal is light blue, and the metal ligands (Cys⁹⁰, His¹³², and His¹³⁶) are indicated in green.

minimal media supplemented with 50 $\mu\text{g}/\text{L}$ carbenicillin, 100 μM ZnCl_2 , and 10 mL Eagle basal vitamin mix (Life Technologies) at 37°C, with 1 g/L $^{15}\text{NH}_4\text{Cl}$ (Martek). The protein was then purified by a two-step process that involved purification by Q Sepharose (Pharmacia), and gel filtration (Toso Haas G2000SW, 21.5 mm $\times$ 30 cm) chromatographies, followed by concentration and buffer exchange by ultrafiltration (Centriprep-10, Amicon). Sample purity was assayed by SDS-PAGE and electrospray mass spectrometry to be >95%.

NMR spectroscopy

The purified protein sample (0.6 mM) was exchanged into NMR buffer (20 mM $\text{d}_11\text{-Tris}$, pH 7.2 at 25°C (Cambridge isotopes), 10% D_2O , 0.02% NaN_3). Two-dimensional ^{15}N HSQC spectra

were acquired prior to and after addition of 25 μL each of acetone, DMSO, ethanol, and isopropanol (in two 12.5- μL increments) to 475 μL of protein solution (2.5, 5% v/v).

The NMR data were recorded on a Bruker DRX-600 spectrometer at 318 K. Amide proton and nitrogen assignments for free PDF were obtained from the BioMagResBank (Accession No. 4089) (Meinzel et al. 1996; Dardel et al. 1998); resonance assignments of the PDF/actinonin complex will be published elsewhere (unpublished data). The NMR data were processed using NMRPipe (Delaglio et al. 1995) and analyzed with NMRVIEW (Johnson and Blevins 1994) and PIPP (Garrett et al. 1991).

Chemical shift mapping

Ligand–protein interactions were monitored by identifying perturbations in the ^{15}N HSQC spectra. To determine the per-residue

chemical shift perturbation upon binding and account for differences in spectral widths between ^{15}N and ^1H resonances (Farmer et al. 1996), weighted average chemical shift differences, $\Delta_{\text{av}}(\text{HN})$, were calculated for the backbone amide ^1H and ^{15}N resonances, using the equation:

$$\Delta_{\text{av}}(\text{NH}) = [(\Delta H^2 + (\Delta N/5)^2)/2]^{1/2}$$

where ΔH and ΔN are the differences between free and bound chemical shifts (Grzesiek et al. 1996; Garrett et al. 1997; Foster et al. 1998). The weighted average chemical shift differences were mapped to the PDF crystal structure (1BS7) (Becker et al. 1998) using MOLMOL (Koradi et al. 1996), with C^α radii rendered at 15 and 5 times the corresponding $\Delta_{\text{av}}(\text{HN})$ for ethanol and actinonin, respectively.

Electronic supplemental material

1. Two-dimensional HSQC spectra of PDF overlaid with spectra acquired in the absence and presence of actinonin, acetone, isopropanol, or DMSO (byerly_esm_f1.eps).
2. A graph of the per-residue $\Delta_{\text{av}}(\text{HN})$ in each solvent (byerly_esm_f2.eps).
3. Cartoon diagrams of the shift perturbations induced by acetone, DMSO, isopropanol mapped to the structure of PDF, plus a figure indicating the sites of the two surface pockets identified by PASS computer analysis (byerly_esm_f3.jpg).

Acknowledgments

We gratefully acknowledge D. Pei and R. Rajagopalan for the PDF expression plasmid, purification protocol, and a substrate analog inhibitor; Z. Yuan (Versicor, Inc.) for a sample of actinonin; M. Chan (OSU) for coordinates and helpful discussions; and C. Cottrell (OSU) for help with NMR instrumentation. This work was supported by a grant to M.F. from the American Heart Association.

The publication costs of this article were defrayed in part by payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 USC section 1734 solely to indicate this fact.

References

- Allen, K.N., Bellamacina, C.R., Ding, X., Jeffery, C.J., Mattos, C., Petsko, G.A., and Ringe, D. 1996. An experimental approach to mapping the binding surfaces of crystalline proteins. *J. Phys. Chem.* **100**: 2605–2611.
- Becker, A., Schlichting, I., Kabsch, W., Schultz, S., and Wagner, A.F. 1998. Structure of peptide deformylase and identification of the substrate binding site. *J. Biol. Chem.* **273**: 11413–11416.
- Brady, G.P., Jr. and Stouten, P.F. 2000. Fast prediction and visualization of protein binding pockets with PASS. *J. Comput. Aided Mol. Des.* **14**: 383–401.
- Chan, M.K., Gong, W., Rajagopalan, P.T., Hao, B., Tsai, C.M., and Pei, D. 1997. Crystal structure of the *Escherichia coli* peptide deformylase. *Biochemistry* **36**: 13904–13909.
- Chen, D.Z., Patel, D.V., Hackbarth, C.J., Wang, W., Dreyer, G., Young, D.C., Margolis, P.S., Wu, C., Ni, Z.J., Trias, J., White, R.J., and Yuan, Z. 2000. Actinonin, a naturally occurring antibacterial agent, is a potent deformylase inhibitor. *Biochemistry* **39**: 1256–1262.
- Clements, J.M., Beckett, R.P., Brown, A., Catlin, G., Lobell, M., Palan, S., Thomas, W., Whittaker, M., Wood, S., Salama, S., Baker, P.J., Rodgers, H.F., Barynin, V., Rice, D.W., and Hunter, M.G. 2001. Antibiotic activity and characterization of BB-3497, a novel peptide deformylase inhibitor. *Antimicrob. Agents Chemother.* **45**: 563–570.
- Dalvit, C., Floersheim, P., Zurini, M., and Widmer, A. 1999. Use of organic solvents and small molecules for locating binding sites on proteins in solutions. *J. Biomol. NMR* **14**: 23–32.
- Dardel, F., Ragusa, S., Lazennec, C., Blanquet, S., and Meinnel, T. 1998. Solution structure of nickel-peptide deformylase. *J. Mol. Biol.* **280**: 501–513.
- Delaglio, F., Grzesiek, S., Vuister, G.W., Zhu, G., Pfeifer, J., and Bax, A. 1995. NMRPipe: A multidimensional spectral processing system based on UNIX pipes. *J. Biomol. NMR* **6**: 277–293.
- Eisen, M.B., Wiley, D.C., Karplus, M., and Hubbard, R.E. 1994. HOOK: A program for finding novel molecular architectures that satisfy the chemical and steric requirements of a macromolecule binding site. *Proteins* **19**: 199–221.
- Erlanson, D.A., Braisted, A.C., Raphael, D.R., Randal, M., Stroud, R.M., Gordon, E.M., and Wells, J.A. 2000. Site-directed ligand discovery. *Proc. Natl. Acad. Sci. USA* **97**: 9367–9372.
- Farmer, B.T., 2nd, Constantine, K.L., Goldfarb, V., Friedrichs, M.S., Wittekind, M., Yanchunas, J., Jr., Robertson, J.G., and Mueller, L. 1996. Localizing the NADP+ binding site on the MurB enzyme by NMR. *Nat. Struct. Biol.* **3**: 995–997.
- Fejzo, J., Lepre, C.A., Peng, J.W., Bemis, G.W., Ajay, Murcko, M.A., and Moore, J.M. 1999. The SHAPES strategy: An NMR-based approach for lead generation in drug discovery. *Chem. Biol.* **6**: 755–769.
- Foster, M.P., Wuttke, D.S., Clemens, K.R., Jahnke, W., Radhakrishnan, I., Tennant, L., Reymond, M., Chung, J., and Wright, P.E. 1998. Chemical shift as a probe of molecular interfaces: NMR studies of DNA binding by the three amino-terminal zinc finger domains from transcription factor IIIA. *J. Biomol. NMR* **12**: 51–71.
- Garrett, D.S., Powers, R., Gronenborn, A.M., and Clore, G.M. 1991. A common sense approach to peak picking in two-, three-, and four-dimensional spectra using automatic computer analysis of contour diagrams. *J. Magn. Reson.* **95**: 214–220.
- Garrett, D.S., Seok, Y.J., Peterkofsky, A., Clore, G.M., and Gronenborn, A.M. 1997. Identification by NMR of the binding surface for the histidine-containing phosphocarrier protein HPr on the N-terminal domain of enzyme I of the *Escherichia coli* phosphotransferase system. *Biochemistry* **36**: 4393–4398.
- Grzesiek, S., Bax, A., Clore, G.M., Gronenborn, A.M., Hu, J.S., Kaufman, J., Palmer, I., Stahl, S.J., and Wingfield, P.T. 1996. The solution structure of HIV-1 Nef reveals an unexpected fold and permits delineation of the binding surface for the SH3 domain of Hck tyrosine protein kinase. *Nat. Struct. Biol.* **3**: 340–345.
- Johnson, B.A. and Blevins, R.A. 1994. NMRView: A computer program for the visualization and analysis of NMR data. *J. Biomol. NMR* **4**: 603–614.
- Koradi, R., Billeter, M., and Wuthrich, K. 1996. MOLMOL: A program for display and analysis of macromolecular structures. *J. Mol. Graph.* **14**: 51–55, 29–32.
- Liang, J., Edelsbrunner, H., and Woodward, C. 1998. Anatomy of protein pockets and cavities: Measurement of binding site geometry and implications for ligand design. *Protein Sci.* **7**: 1884–1897.
- Liepinsh, E. and Otting, G. 1997. Organic solvents identify specific ligand binding sites on protein surfaces. *Nat. Biotechnol.* **15**: 264–268.
- Meinnel, T., Blanquet, S., and Dardel, F. 1996. A new subclass of the zinc metalloproteases superfamily revealed by the solution structure of peptide deformylase. *J. Mol. Biol.* **262**: 375–386.
- Meinnel, T., Mechulam, Y., and Blanquet, S. 1993. Methionine as translation start signal: A review of the enzymes of the pathway in *Escherichia coli*. *Biochimie* **75**: 1061–1075.
- Miranker, A. and Karplus, M. 1991. Functionality maps of binding sites: A multiple copy simultaneous search method. *Proteins* **11**: 29–34.
- Moy, F.J., Haraki, K., Mobilio, D., Walker, G., Powers, R., Tabei, K., Tong, H., and Siegel, M.M. 2001. MS/NMR: A structure-based approach for discovering protein ligands and for drug design by coupling size exclusion chromatography, mass spectrometry, and nuclear magnetic resonance spectroscopy. *Anal. Chem.* **73**: 571–581.
- Rajagopalan, P.T., Datta, A., and Pei, D. 1997a. Purification, characterization, and inhibition of peptide deformylase from *Escherichia coli*. *Biochemistry* **36**: 13910–13918.
- Rajagopalan, P.T.R., Yu, X.C., and Pei, D. 1997b. Peptide deformylase: A new type of mononuclear iron protein. *J. Am. Chem. Soc.* **119**: 12418–12419.
- Rosenfeld, R., Vajda, S., and DeLisi, C. 1995. Flexible docking and design. *Annu. Rev. Biophys. Biomol. Struct.* **24**: 677–700.
- Shuker, S.B., Hajduk, P.J., Meadows, R.P., and Fesik, S.W. 1996. Discovering high-affinity ligands for proteins: SAR by NMR. *Science* **274**: 1531–1534.
- Wei, Y., Yi, T., Huntington, K.M., Chaudhury, C., and Pei, D. 2000. Identification of a potent peptide deformylase inhibitor from a rationally designed combinatorial library. *J. Comb. Chem.* **2**: 650–657.