JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

Subscriber access provided by American Chemical Society

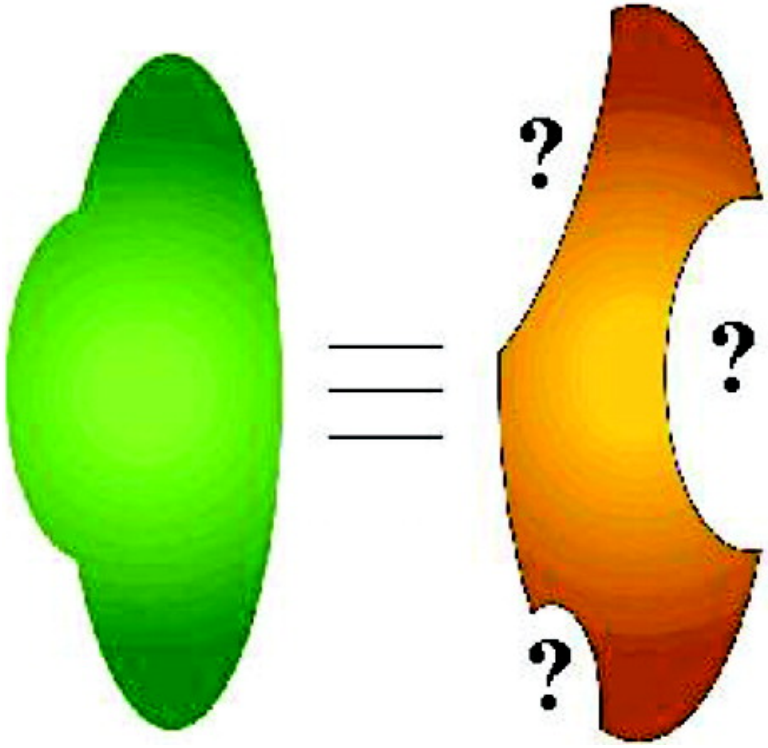
Communication

Fast Mapping of Protein–Protein Interfaces by NMR Spectroscopy

Michael L. Reese, and Volker Dtsch

J. Am. Chem. Soc., **2003**, 125 (47), 14250-14251• DOI: 10.1021/ja037640x • Publication Date (Web): 04 November 2003

Downloaded from http://pubs.acs.org on March 30, 2009



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 4 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

ACS Publications
High quality. High impact.

Journal of the American Chemical Society is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

Subscriber access provided by American Chemical Society

View the Full Text HTML

ACS Publications
High quality. High impact.

Journal of the American Chemical Society is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

J|A|C|S
COMMUNICATIONS

Published on Web 11/04/2003

Fast Mapping of Protein−Protein Interfaces by NMR Spectroscopy

Michael L. Reese† and Volker Dötsch*,‡,§

Graduate Group in Biophysics, and Departments of Pharmaceutical Chemistry and Cellular & Molecular Pharmacology, University of California San Francisco, San Francisco, California 94143

Received July 30, 2003; E-mail: vdoetsch@em.uni-frankfurt.de

One of the advantages of NMR spectroscopy is its ability to efficiently map interfaces.[1,2] This is based on the fact that the chemical shift of a particular nucleus is a very sensitive function of changes in its chemical environment. A prerequisite for using this chemical shift mapping method, however, is that the assignment of the nuclei showing chemical shift changes be known. While these assignments are a necessary byproduct of structural determination by NMR spectroscopy, they are unavailable for protein structures determined by X-ray crystallography. The development of a method that allows one to map the interface of protein complexes, without knowledge of the chemical shift assignments, would clearly be very useful and could open an avenue toward building structures of complexes in a very efficient way. Here, we report such a method and demonstrate its feasibility on the complex of the PDZ domain of neuronal nitric oxide synthase (nNOS) and the second PDZ domain of PSD-95.[3−6]

To map the interface between two proteins, without the knowledge of the assignment, we have used a combinatorial approach. The method is based on preparing several protein samples, each one selectively ^{15}N labeled with one particular amino acid.[7,8] These samples are then used to form a complex with its unlabeled binding partner. By comparing [^{15}N,^{1}H]-HSQC spectra of the selectively labeled free protein and its complex, the number of shifted amino acids of a certain type can be identified. The combined results from several differently labeled samples allow one to define the minimum number of a certain type of amino acid located in the interface. Comparison of this list with the known structure of the protein is then used to identify the interface (Figure 1). This approach is further divided into two phases. First, we identify amino acid types with low abundance in the protein's sequence. The combination of data from experiments with several of these rare residues allows us to identify the location of the binding site. Second, we use more common residues as probes to characterize the extent and shape of the interface.

In the case of the nNOS PDZ domain, we have used the five lysines, four phenylalanines, two histidines, and one tyrosine as initial probes to identify the site of the interface. Figure 2 shows characteristic [^{15}N,^{1}H]-HSQC spectra for a titration experiment. As the ^{15}N-lysine labeled nNOS PDZ was titrated with unlabeled PSD-95 PDZ2, two changes in the spectrum occurred. One peak disappeared while another peak appeared, as is characteristic of a complex in slow exchange. Two other peaks exhibited severe spectral broadening, as is characteristic of a complex in intermediate exchange. Finally, that the single shifted peak was not broadened shows that two distinct interfaces exist. To distinguish these two interfaces, we will refer to the binding site exhibiting slow exchange as "primary" and the site exhibiting intermediate exchange as "secondary".

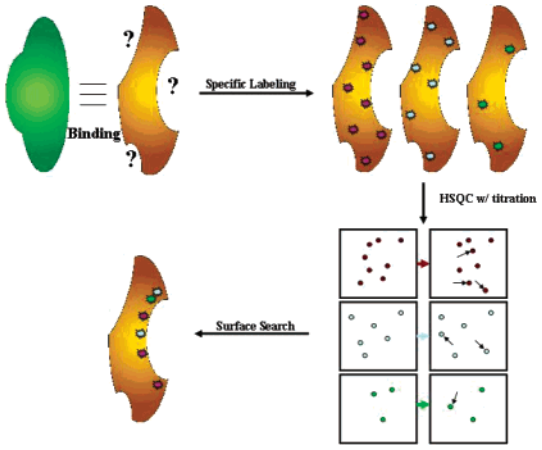


Figure 1. Schematic representation of the method to map the interface between two proteins described in the text.

In the nNOS, two pairs of lysines lie in the canonical PDZ domain, separated by one or four amino acids, and a single lysine lies in the β-hairpin finger. This constellation makes the lysine in the β-finger the most likely candidate for the primary binding interface because it is likely that more than one significant shift would result should any of the other lysines be involved.

To further investigate the interface, we labeled the nNOS with ^{15}N-tyrosine, histidine, or phenylalanine (see Supporting Information). The single tyrosine, located in the canonical peptide-binding site, did not shift. Instead, it showed severe line broadening at higher PSD-95 concentrations. The experiment with the histidine-labeled sample revealed that one of the two histidines shifted and neither histidine peak broadened. The phenylalanine-labeled sample showed two shifted peaks. We also observed significant line broadening of another phenylalanine peak at higher PSD-95 concentrations. These results predict that the primary binding site contains at least one lysine, one histidine, and two phenylalanines, but does not contain the single tyrosine. Thus, comparison with the structure of the nNOS[3,5] (Figure 2D) suggests that the primary binding site includes the β-finger. The second binding site contains two lysines, one tyrosine, no histidines, and one phenylalanine. This constellation exists in the canonical peptide-binding site of the domain.

To further map the extent of the interface, we prepared samples of nNOS individually ^{15}N-labeled with leucine, valine, or isoleucine, which are more abundant in the nNOS sequence. In the titration experiments with these samples, we found four valine peaks that shifted, two isoleucine peaks, and one leucine peak. One leucine, one isoleucine, and one valine are located in the β-finger in the region between His 106 and Lys 118 and would, therefore, be expected to show differences in chemical shift. Isoleucines that are spatially close to the β-finger are found in strand 1 (Ile 16) or strand

† Graduate Group in Biophysics.
‡ Departments of Pharmaceutical Chemistry and Cellular & Molecular Pharmacology.
§ Present address: Institute for Biophysical Chemistry, University of Frankfurt, 60439 Frankfurt, Germany.

10.1021/ja037640x CCC: $25.00 **© 2003 American Chemical Society**

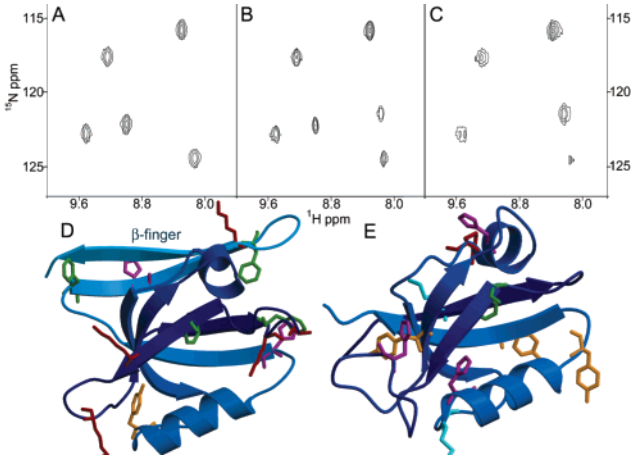


Figure 2. (A) shows the [^{15}N,^{1}H]-HSQC spectrum of the free lysine-labeled PDZ domain from nNOS with five peaks, corresponding to the five lysines. (B) shows the spectrum of a sample in which 50% of the nNOS PDZ domain molecules are in complex with the PDZ2 domain from PSD-95. (C) shows a spectrum of a 1:1 complex of both PDZ domains. (D) shows the surface distribution of those amino acids on the nNOS PDZ domain that were used as initial probes in the determination of the interface with PSD-95 PDZ2. His are magenta, Lys are red, Phe are green, and Tyr are gold. (E) shows the surface distribution of those amino acids on the PSD-95 PDZ2 that were used as initial probes in the determination of the interface with nNOS PDZ. Arg are red, His are magenta, Met are cyan, Phe are green, and Tyr are gold. In summary, 2 His, 2 Tyr, 1 Phe, 1 Met, and no Arg were seen to be involved in the primary interface, and 1 His, 1 Tyr, 1 Arg, and neither Phe nor Met were involved in the secondary interface. All spectra were measured with a standard Watergate HSQC sequence on a 500 MHz Bruker Avance instrument equipped with a cryoprobe. For each spectrum, 512 complex points were measured in the acquisition and 50 complex points in the indirect dimension with 16 scans per increment at 25 °C. The labeled protein was at a concentration of 0.3 mM in a 10 mM Hepes buffer, pH 7.0.

4 (Ile 58). This arrangement provides two possibilities for the extended interface, including the β-finger and either strand 1 or strand 4. Leu 57, however, precedes Ile 58. As only one significant shift is observed in the leucine-labeled spectrum, and this leucine is likely the one located in the β-finger, these data suggest that strand 1 is part of the interface. This result is confirmed by the data from the valine-labeled nNOS. While strand 4 and its vicinity are devoid of valines, four are present in and around strand 1. These combined results predict that PSD-95 PDZ2 binds the upper part of the β-finger and strand 1.

Such interpretation is consistent with the crystal structure of the α-syntrophin/nNOS complex.[3] nNOS residues in direct contact with syntrophin include 1 His, 1 Leu, 2 Ile, 1 Phe, and 3 Val. The additional 1 Lys and 1 Val are not in direct contact with syntrophin, but lie on the backside of the β-finger, and are affected due to rearrangements in packing around the interface. It is therefore important to note, as with all chemical shift perturbation studies, that the region of perturbation will be slightly larger than the region of direct interaction.

In a similar way, we mapped the interface on the PSD-95 PDZ2 domain (Figure 2E). We identified the canonical peptide-binding site as the primary interface and the C-terminal tail of the construct as the secondary binding site. That nNOS can interact with PSD-95 through its β-finger while maintaining its competency to bind carboxy-termini with its canonical PDZ-domain has been previously demonstrated.[4]

The mapping method described here is a very efficient tool for identifying interfaces if the NMR assignment is not known and is in principle also amenable to automated computer-based search algorithms. Initial tests with a simple surface-scanning script have indeed identified the correct interface. To provide final proof that the identified surface is indeed the interface, one can use a double-selective labeling method to unambiguously identify a specific amino acid of the interface.[9] Alternatively, a mutagenesis approach can be used.

One prerequisite of the method is that the amino acid type selective labeling is specific and cross-labeling of other amino acid types can be suppressed. Amino acids that are at the end of biosynthetic pathways (Lys, Arg, His) can be added directly to the minimal medium without any further precautions. For other amino acids, we have, in the past, used auxotrophic strains (DL39) that suppress any cross-labeling.[8] These, however, also reduce the yield of expressed protein. Alternatively, cross-labeling can be suppressed in BL21(DE3) bacteria by adding unlabeled amino acids. For the current study, we have added unlabeled Val and Ile for labeling Leu, unlabeled Leu and Val for labeling Ile, und unlabeled Phe for labeling Tyr (and vice versa). Only for Val could cross-labeling not be suppressed, requiring expression in the DL39 strain.

An additional prerequisite is that the binding event should affect the immediate binding site without inducing major conformational changes. Such changes would lead to chemical shift differences outside of the interface. However, if conformational changes occur, the method proposed here can be combined with the saturation transfer method proposed by Takahashi et al.[1] This would require the use of ^{15}N-labeled and deuterated amino acids, which, however, are only recently becoming available.

Acknowledgment. Financial support was provided by NIH (Grants GM-56531 and GM66039). M.L.R. was supported by an NIH training grant (GM08284).

Supporting Information Available: Results of the titration experiments (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Takahashi, H.; Nakanishi, T.; Kami, K.; Arata, Y.; Shimada, I. *Nat. Struct. Biol.* **2000**, *7*, 220−223.
(2) Foster, M. P.; Wuttke, D. S.; Clemens, K. R.; Jahnke, W.; Radhakrishnan, I.; Tennant, L.; Reymond, M.; Chung, J.; Wright, P. E. *J. Biomol. NMR* **1998**, *12*, 51−71.
(3) Hillier, B. J.; Christopherson, K. S.; Prehoda, K. E.; Bredt, D. S.; Lim, W. A. *Science* **1999**, *284*, 812−815.
(4) Harris, B. Z.; Hillier, B. J.; Lim, W. A. *Biochemistry* **2001**, *40*, 5921−5930.
(5) Tochio, H.; Zhang, Q.; Mandal, P.; Li, M.; Zhang, M. *Nat. Struct. Biol.* **1999**, *6*, 417−421.
(6) Wang, P.; Zhang, Q.; Tochio, H.; Fan, J. S.; Zhang, M. *Eur. J. Biochem.* **2000**, *267*, 3116−3122.
(7) McIntosh, L. P.; Dahlquist, F. W. *Q. Rev. Biophys.* **1990**, *23*, 1−38.
(8) Ou, H. D.; Lai, H. C.; Serber, Z.; Dötsch, V. *J. Biomol. NMR* **2001**, *21*, 269−273.
(9) Weigelt, J.; Dongen, M. v.; Uppenberg, J.; Schultz, J.; Wikstrom, M. *J. Am. Chem. Soc.* **2002**, *124*, 2446−2447.

JA037640X