Journal of Biomolecular NMR (2005) 33: 15–24
DOI 10.1007/s10858-005-1199-0

Article

An AMBER/DYANA/MOLMOL phosphorylated amino acid library set and incorporation into NMR structure calculations

John W. Craft Jr. & Glen B. Legge*
Department of Biology and Biochemistry, University of Houston, 404 HSC, Houston, TX, 77204-5001, USA

Received 12 April 2005; Accepted 22 June 2005

Key words: AMBER, phosphorylation, phosphoserine, phosphothreonine, phosphotyrosine

Abstract

Protein structure determination using Nuclear Magnetic Resonance (NMR) requires the use of molecular dynamics programs that incorporate both NMR experimental and implicit atomic data. Atomic parameters for each amino acid type are encoded in libraries used by structure calculation programs such as DYANA and AMBER. However, only a few non-standard amino acid library sets are included in these programs or the molecular visualization program MOLMOL. Our laboratory is calculating the phosphorylated and non-phosphorylated states of peptides and proteins using NMR methods. To calculate chemically correct structures, we have extended the available molecular libraries for these programs to include the modified amino acids phosphoserine, phosphothreonine, and phosphotyrosine.

Introduction

Structure calculation by NMR is a balance between the experimental input and the methods used to refine the data. NOEs restraints and other NMR data such as dipolar coupling (Tjandra and Bax, 1997) or *J*-coupling torsional restraints are used in combination with data such as molecular composition, bond lengths, chiralities, and force-field to generate NMR structures (Wüthrich 1986; Güntert 1998; Spronk et al., 2002). Only a few non-standard amino acids and nucleotides library components are publicly available in popular NMR structure calculation programs AMBER (Case et al., 2004), and DYANA (Güntert, et al., 1997), or the molecular visualization program MOLMOL (Koradi et al., 1996). Chemically correct amino acid residue libraries used by these programs are at the heart of structure determination. Covalent modification by phosphorylation is a common mechanism of functional regulation. Structure determination of proteins and peptides that contain phosphorylated residues require a library for each modified residue. However, the libraries for phosphoserine (Sep), phosphothreonine (Thp) and phosphotyrosine (Typ) are not available. This paper describes the basic procedure necessary to generate the parameters for phosphorylated forms of Serine, Threonine, and Tyrosine for each of these programs and the methods employed for subsequent NMR structure calculations.

Methods

The process of compiling a library for each phosphorylated amino acid residue involves generating initial geometry and charge parameters, followed by testing in the computational environment (Figure 1a). A set of atomic (x, y, z) coordinates for each modified amino acid was transformed

*To whom correspondence should be addressed. E-mail: glegge@uh.edu

into the coordinate system reference frame for each program and estimates of the electro-potential environment were represented by partial charges. The complete parameter set was then specified in the appropriate format for each tool's library. A significant number of intermediate scripts and processes also required modification (Table 1). Additionally, the molecular visualization program MOLMOL required an updated library. Once the generation of the parameters was completed, each modified molecular library was tested using NMR data sets (Figure 1b).

Model coordinate generation

The molecular modeling system Insight II (Accelrys, Inc) was used to generate the coordinates of the modified phosphorylated amino acid in step 1 (Figure 1a). Each modified residue was minimized using the gradient descent method in the Discover module of Insight II. All the amino acid residues in DYANA and AMBER libraries follow the convention that the HN, N, C_α, C′, and O atoms lie in a plane. Two torsion angles; one defined by the HN, N, C_α, C′ atoms and the second by O, C′, C_α, N were rotated into the x–y plane defined by N, C_α, and C′. Upon linking the amino acids in a poly peptide chain each program computes its own estimate of ω, ϕ, and ϕ so that the HN, N, C_α, C′, O atoms are no longer artificially constrained to a plane. The parameters developed here follow the same conventions that apply to each program. Once the model was generated in Insight II, the atom nomenclature was changed to comply with the requirements for each program. A phosphate nomenclature of PS, OPA, OPB, and OPC was used to distinguish it from the diester phosphate groups attached to the nucleic acid residues in the AMBER library.

Amino acid rotation

The programs required that the N- C_α unit vector lie parallel to the x axis and thus we used a quaternion to align the minimized model. A quaternion (Bean, 1969) can be defined by a rotation axis and angle and is a 4×1 vector. A quaternion that rotates a system from frame A to frame B can be constructed by:

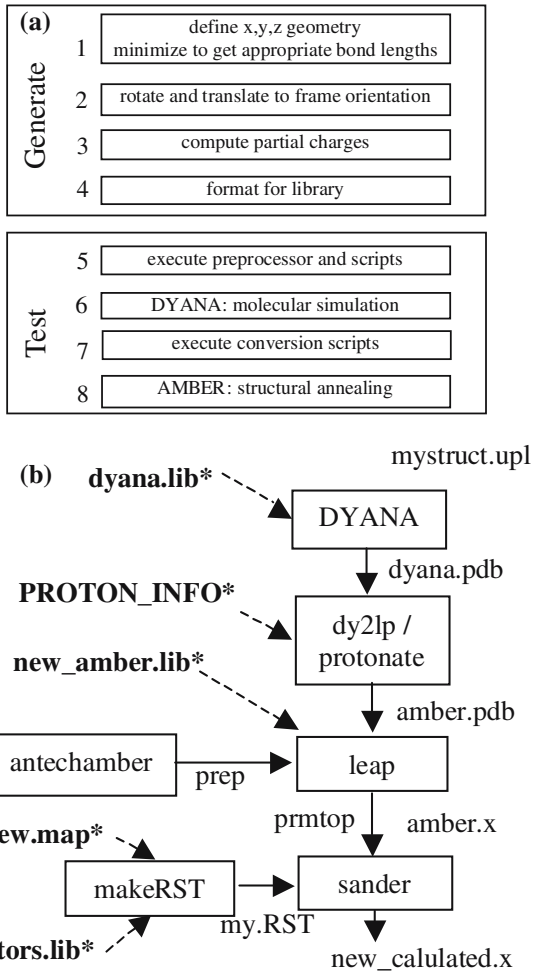

Figure 1. (a) Outline of the steps involved in phospho residue parameter generation and testing. (b) Flow of information from input constant files to output structure files in the DYANA/AMBER calculation process (* indicates input source of modified residue data).

Table 1. List of tools and library files requiring modified amino acid parameter data to enable effective implementation of the phosphorylated amino acids

Tool	Required modified file	Function
DYANA	dyana.lib	modified core library
protonate	PROTON_INFO	preprocessor to AMBER
makeRST	new.map	preprocessor to AMBER
	tors.lib	defines torsions/dihedrals
AMBER	amber_sep.lib	phosphoserine library
	amber_thp.lib	phosphothreonine library
	amber_typ.lib	phosphotyrosine library
MOLMOL	amber94.lib	modified core library

$$q_A^B = \begin{bmatrix} \cos(\theta_q/2) \\ -u_q^r \cdot \sin(\theta_q/2) \end{bmatrix} = \begin{bmatrix} S \\ a \\ b \\ c \end{bmatrix} \quad (1)$$

where the unit vector (u_q) and angle (θ_q) are defined by the cross product between vectors in the two frames. The components of the quaternion are labeled as S for the scalar term and [a, b, c] for the sub-vector term. For example, the rotation of the N- C_α unit vector into the *x*-unit vector can be accomplished by the rotation illustrated in Figure 2. Once the quaternion is defined by u_q and θ_q, then a set of generalized PDB formatted residue coordinates V are rotated using the quaternion operator:

$$V' = q^* V q \quad (2)$$

where q^* is the conjugate of q. The quaternion rotation can also be expanded in classical 3×3 rotation matrix form:

$$\begin{bmatrix} S^2+a^2+b^2+c^2 & 2(cS+ab) & 2(ac-bS) \\ 2(ab-cS) & S^2-a^2+b^2-c^2 & 2(aS+bc) \\ 2(bS+ac) & 2(bc-aS) & S^2-a^2-b^2+c^2 \end{bmatrix} \quad (3)$$

and then the transformation takes the form:

$$V' = [T]V \quad (4)$$

The conventions followed by AMBER define the *y*-axis in an opposite orientation to that of DYANA but rotation quaternions equally apply to the preparation of a library for AMBER. The amine nitrogen by AMBER convention is not at the origin, and therefore an additional translation step was required to place the residue correctly in the AMBER reference frame. We used the matrix

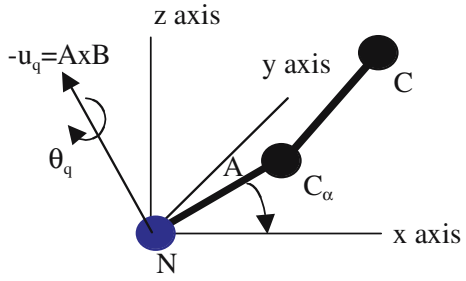

Figure 2. The quaternion rotation as defined by the rotation unit vector u_q and the rotation angle θ_q.

tool MATLAB (The MathWorks Inc.) to implement a toolbox for the quaternion math utilities and a toolbox for the algorithm used to convert a general set of PDB coordinates into a DYANA (pdb2dyana.m) or an AMBER (pdb2amber.m) compliant library atom coordinate set.

Library implementation for DYANA

The ECEPP/2 force field (Momany et al., 1975; Némethy et al., 1983) defines the conventions of the standard geometry for DYANA. The AMBER force field (Cornell et al., 1995) defines the conventions of the covalent geometry used in DYANA. The nomenclature, atom types, dihedral angle definitions, standard geometry, and the covalent connectivities are encoded in the library input file, dyana.lib. This standard library, dyana.lib, was extended to include the three new amino acid residues and included the implementation of pseudo atoms for non- stereospecifically assigned atom pairs.

Data processing in AMBER

The correct atomic nomenclature for each program is essential. AMBER requires a mapping of Distance Geometry (DG) pseudostructure nomenclature to full IUPAC conventions (Markley et al., 1998). This mapping is in a file, new.map, and requires that the new residues be added. It is important to specify the type of each atom correctly. For example, the hydroxyl oxygen (OH) on Serine changes type to OS to characterize it as an ester oxygen. Equally, the oxygens on the phosphate group have type O2 when they participate in the resonance equivalence in the unprotonated state.

Incorporation of the new residue information into the AMBER programs is outlined in Figure 1b. A script (dy2lp) converts DYANA formatted to AMBER formatted PDB files and requires the program protonate so that the appropriate hydrogen atoms are added to the protein. This program had its own input called PROTON_INFO. The script adds protons and changes atom and residue names to suit AMBER nomenclature while pseudoatoms are deleted and the PDB file is renumbered.

LEaP is the primary program to either create a new molecular system in AMBER, or modify

Table 2. The AMBER geometries and partial charges for phosphoserine, phosphothreonine, and phosphotyrosine used in the preparation of the modified library

Atom	Type	Charge	X-pos	Y-pos	Z-pos
Atom parameters for phosphoserine					
N	N	−0.4937	3.326	1.548	0.000
H	H	0.3018	3.909	0.724	0.000
CA	CT	−0.238	3.970	2.846	0.000
HA	H1	0.0937	3.714	3.371	−0.955
CB	CT	0.0782	3.430	3.763	1.158
HB2	H1	−0.0602	2.337	3.915	1.080
HB3	H1	−0.0602	3.564	3.280	2.147
OG	OS	−0.5593	4.061	5.065	1.175
PS	P	1.4	3.683	6.196	2.269
OPA	O2	−0.85	3.983	5.613	3.654
OPB	O2	−0.85	4.509	7.455	2.037
OPC	O2	−0.85	2.190	6.493	2.178
C	C	0.6731	5.486	2.705	0.000
O	O	−0.5854	6.009	1.593	0.000
Atom parameters for phosphothreonine					
N	N	−0.4937	3.326	1.548	0.000
H	H	0.3018	3.909	0.724	0.000
CA	CT	−0.174	3.970	2.846	0.000
HA	H1	0.0164	3.760	3.360	−0.969
CB	CT	0.1531	3.346	3.825	1.079
HB	H1	−0.0909	2.271	3.950	0.835
CG2	CT	−0.1617	3.474	3.291	2.517
HG21	HC	0.0496	3.007	3.986	3.236
HG22	HC	0.0496	2.978	2.310	2.628
HG23	HC	0.0496	4.533	3.179	2.805
OG1	OS	−0.6375	4.007	5.123	1.061
PS	P	1.4	3.640	6.382	2.025
OPA	O2	−0.85	2.187	6.819	1.816
OPB	O2	−0.85	4.562	7.560	1.696
OPC	O2	−0.85	3.843	6.009	3.498
C	C	0.6731	5.486	2.705	0.000
O	O	−0.5854	6.009	1.593	0.000
Atom parameters for phosphotyrosine					
N	N	−0.4937	3.326	1.548	0.000
H	H	0.3018	3.909	0.724	0.000
CA	CT	−0.001	3.970	2.846	0.000
HA	H1	0.0556	3.723	3.401	−0.938
CB	CT	0.0211	3.425	3.672	1.206
HB2	HC	0.0225	3.559	3.093	2.141
HB3	HC	0.0225	2.324	3.759	1.113
CG	CA	−0.0017	3.991	5.091	1.381
CD1	CA	−0.1849	3.555	5.899	2.438
HD1	HA	0.1195	2.816	5.544	3.140
CE1	CA	−0.268	4.052	7.192	2.583
HE1	HA	0.21	3.704	7.836	3.378
CZ	CA	−0.338	4.973	7.697	1.666
CE2	CA	−0.268	5.418	6.892	0.619
HE2	HA	0.21	6.122	7.302	−0.090

Table 2. (Continued)

Atom	Type	Charge	X-pos	Y-pos	Z-pos
CD2	CA	−0.1849	4.925	5.597	0.471
HD2	HA	0.1195	5.263	5.000	−0.363
OS	OS	−0.28	5.495	8.962	1.831
PS	P	1.4	6.562	9.439	0.693
OPA	O2	−0.85	7.061	10.856	0.997
OPB	O2	−0.85	5.907	9.408	−0.692
OPC	O2	−0.85	7.754	8.481	0.702
C	C	0.6731	5.486	2.705	0.000
O	O	−0.5854	6.009	1.593	0.000

existing molecules. It allows for the modification of atoms and their relationships to other parts in the system using a set of standard commands. LEaP produces the essential input files for the AMBER simulation program, sander. We adopted a strategy of implementing the phosphorylated residues directly in AMBER's core library format so that amber_sep.lib, amber_thp.lib, and amber_typ.lib (new_amber.lib in Figure 1b) contained the information read by LEaP. LEaP synthesizes default force field data together with the newly generated phosphorylated amino acid libraries to provide the parameters for energy terms such as angles, dihedrals, impropers, and non-bonded interactions. The libraries apply for molecular simulations using either in vacuo (Cornell et al., 1995), or generalized Born (Nina et al., 1999; Onufriev et al., 2002) solvent model configurations by configuring the IGB flag in LEaP as discussed in the AMBER8 manual (Case et al., 2004).

The protocols to determine NMR structures in our laboratory require generating initial structures in DYANA and refining with AMBER, and are discussed in detail in Duggan et al. (2001) (Figure 1a). The polypeptide project in our lab has developed a set of structures with NMR data to be reported elsewhere (Legge et al., manuscript in preparation), and was used as the sample system for the evaluation of the libraries in AMBER version 8. The use of generalized Born is preferred in our production simulations because of improved results (Tsui and Case, 2001; Xia et al., 2002), however, in vacuo computations were also evaluated. The system was first minimized using 5000 steps. The annealing was completed using a target temperature of 1000 K after an initial 4 ps ramp to 400 K. Cooling was applied from 8 ps to the end of the 20 ps run.

Charge calculation in AMBER

The AMBER force field requires an estimation of the electro-potential charges, which are represented by partial charges located at the atom centers. In our approach, parameterization of phosphate group was based on characterizing the delocalized charge estimates and resulting partial double bond character from Insight II.

For each residue, an estimate of the sidechain charge overall distribution was made based on similarity with negatively charged amino acids in the AMBER library. The charge estimates for phosphoserine and phosphothreonine were chosen so that the overall charge of −2 was preserved, while the alpha and beta localized charge sum largely matched those in the aspartic acid library component. The residual charge required to maintain the −2 total charge on the phosphotyrosine residue was averaged over the resonance ring carbons.

Results and discussion

Structure calculations

The results of the dynamic simulations verified that the phosphorylated residues did not present any numerical errors in bond lengths, bond angles, and stereochemistry. The phosphorus atom remained as a tetrahedral center as expected, and the bonds lengths of 1.63 Å (O—P ester linkage) and 1.53 Å (P—O partial double bond) were maintained throughout both the torsion angle dynamics (DYANA) and the simulated annealing (AMBER) test runs.

Table 3. The AMBER geometries represented in internal coordinates for phosphoserine, phosphothreonine, and phosphotyrosine used in the preparation of the modified library

Atom type	Atom number	Bond lengths	Atom number	Bonds angles	Atom number	Dihedrals
Internal coordinates for phosphoserine						
14						
N	1					
H	1	1.026				
C	1	1.493	2	109.6		
H	3	1.107	1	106.4	2	115.9
C	3	1.557	1	110.2	2	232.4
H	5	1.106	3	112.4	1	301
H	5	1.109	3	112.0	1	57.7
O	5	1.447	3	111.1	1	180
P	8	1.618	5	122.3	3	180
O	9	1.532	8	107.4	5	59.7
O	9	1.524	8	110.4	5	180
O	9	1.525	8	108.9	5	302.2
C	3	1.558	1	113.8	2	0.1
O	13	1.236	3	124.0	1	359.9
Internal coordinates for phosphothreonine						
17						
N	1					
H	1	1.024				
C	1	1.493	2	108.8		
H	3	1.103	1	107.0	2	113.4
C	3	1.569	1	110.7	2	227.4
H	5	1.109	3	108.3	1	299.8
C	5	1.539	3	113.6	1	61
Hg	7	1.104	5	110.8	3	180
Hg	7	1.105	5	111.4	3	299.8
Hg	7	1.103	5	111.0	3	60.7
O	5	1.457	3	109.8	1	180
P	11	1.628	5	125.4	3	180
O	12	1.532	11	110.7	5	299.9
O	12	1.532	11	109.4	5	180
O	12	1.533	11	110.6	5	60.8
C	3	1.566	1	109.8	2	0.1
O	16	1.428	3	118.4	1	360
Internal coordinates for phosphotyrosine						
24						
N	1					
H	1	1.026				
C	1	1.478	2	109.8		
H	3	1.108	1	108.9	2	116.5
C	3	1.551	1	107.6	2	234.7
H	5	1.108	3	110.1	1	54.7
H	5	1.108	3	109.4	1	301.7
C	5	1.538	3	115.6	1	180
C	8	1.4	5	120.3	3	180
H	9	1.079	8	121.0	5	359.7

Table 3. (Continued)

Atom type	Atom number	Bond lengths	Atom number	Bonds angles	Atom number	Dihedrals
C	9	1.393	8	120.2	5	181.1
H	11	1.081	9	121.0	8	178.3
C	11	1.394	9	120.2	8	0.9
C	13	1.394	11	119.7	9	358.6
H	14	1.08	13	118.8	11	179.2
C	14	1.394	13	120.2	11	1.3
H	16	1.08	14	119.0	13	178.3
O	13	1.378	11	120.3	9	181.9
P	18	1.631	13	115.6	11	180
O	19	1.533	18	110.2	13	180
O	19	1.532	18	110.2	13	58.8
O	19	1.529	18	108.8	13	299.4
C	3	1.541	1	108.7	2	0
O	23	1.234	3	123.5	1	0

Library conventions

The library generation process identified the DYANA convention for HN—N—C_α—C—O backbone atoms to be coplanar after variable distances in the C_α—C bond length in some members of the computed structure ensemble caused subsequent high energies in AMBER. The initially observed errant bond distances were eliminated once the library was corrected by conforming with that convention.

The complete set after transformation into the correct frame and orientation is listed in Table 2 as *x*, *y*, *z* coordinates or in Table 3 as internal coordinate target values. A comparison between the ball and stick representations of the final phosphorylated amino acid residues added to the libraries and the unphosphorylated amino acid structures are shown in Figure 3.

Evaluation of partial charges

We computed the individual charge distribution down the sidechain between serine, aspartic and glutamic acid and phosphoserine (Figure 4) to ensure the phosphoserine charge distribution was similar to the acidic residues. The method of reducing the positive charge averages at the alpha and beta station provides an estimate of the sidechain charge distribution, and is preferable to placing a charged phosphate group on the end of a serine. This is because the serine localized charge

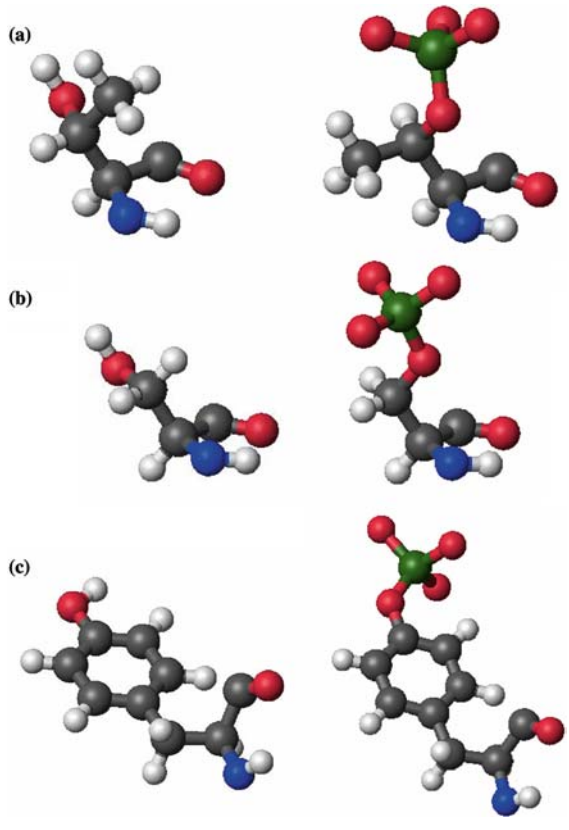

Figure 3. (a) Comparison of threonine and phosphothreonine (b) serine and phosphoserine and (c) tyrosine and phosphotyrosine residues in the AMBER library as shown by ball and stick figures with atoms colored by CPK representations. Note that the back bone atoms are planar as required by both DYANA and AMBER library convention. Figure was produced by MOLMOL.

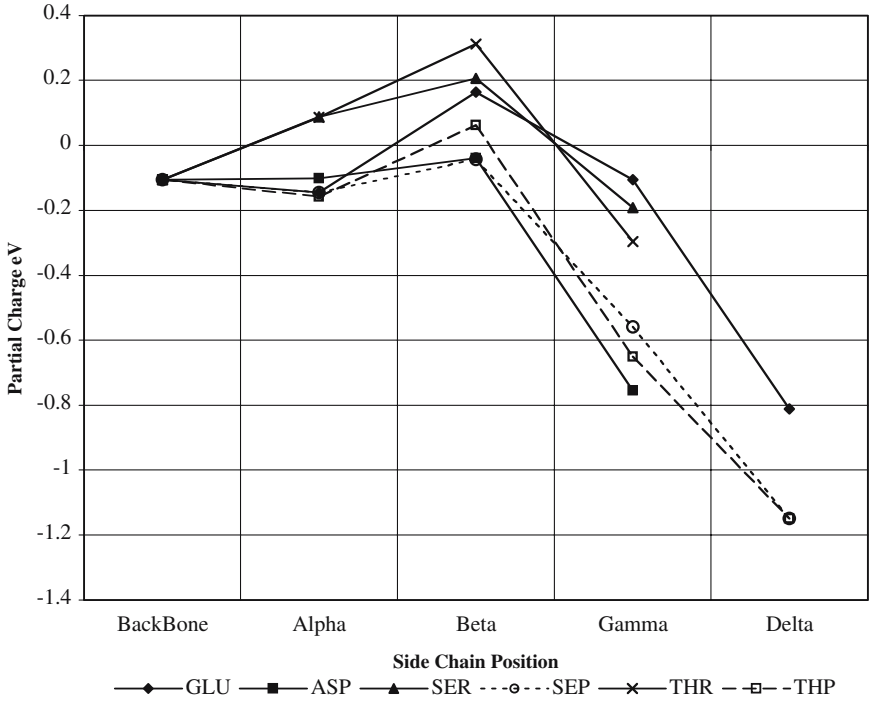

Figure 4. Localized charge sum distribution (eV) of glutamic acid (GLU), aspartic acid (ASP), serine (SER), and threonine (THR) compared with the modified amino acid, phosphoserine (SEP) and phosphothreonine (THP).

sum at the alpha (0.09) and beta (0.21) stations are positive and not consistent with the large −2 charge that is present on the phosphate moiety.

Insight II's estimate of the delocalized charge on the phosphate group yielded a partial charge of 1.4 on the phosphorus atom and −0.85 on each oxygen atom (Table 2). This was in contrast to the phenyl moiety of dianionic phenyl phosphate used by Feng et al. (1997) in which they chose a different mix of double, partial, and single bonds in the phosphate group. In addition we did not use the existing estimates of phosphate group charges and bond distances for the nucleic acid residues in the AMBER libraries as these phosphate groups are optimized for the formation of the diester.

We have also used a central multipole expansion (Leach, 2001) to compare the effect of the −2 charge in the phosphorylated residues with the electrostatic properties of the unphosphorylated and acidic residues in the AMBER library. The central multipole expansion approach is based upon the electric moments of multipoles. They are represented by q (charge), μ (dipole), and Θ (quadrupole). The key definitions are:

$$\mu = \sum q_i r_i \tag{5}$$

$$\Theta = \begin{bmatrix} \sum q_i x_i^2 & \sum q_i x_i y_i & \sum q_i x_i z_i \\ \sum q_i y_i x_i & \sum q_i y_i^2 & \sum q_i y_i z_i \\ \sum q_i z_i x_i & \sum q_i z_i y_i & \sum q_i z_i^2 \end{bmatrix} \tag{6}$$

where r_i, x_i, y_i, and z_i are the radius and position of each atom in the molecule and q_i is the partial charge associated with that atom.

The dipoles were computed for a number of AMBER library residues (Table 4), and the residues fall into families based on their overall charge distribution and dihedrals (χ_i). As expected neutral charged amino acids have small dipole moments, while the polar forms have significant dipole contribution. A comparison of the dipole magnitude from neutral serine (0.7) to its phosphorylated −2 charged form (9.0) indicates the difference in the calculated dipole is of an order of magnitude greater. Comparison between charged aspartic acid (3.4) and glutamic acid (5.2) indicates a larger dipole for the Glu anionic oxygen, which is further out along the side chain. The phospho-

Table 4. Residue dipoles compared between non-polar and standard polar to the phosphorylated residues

Residue	X	Y	Z	Magnitude	Charge
SER	0.49	−0.38	−0.33	0.7	0
THR	0.55	−0.33	−0.30	0.7	0
TYR	0.81	−0.08	0.25	0.9	0
ASP	−3.15	0.41	1.13	3.4	−1
GLU	−3.81	1.75	3.05	5.2	−1
SEP	−7.28	3.00	4.36	9.0	−2
THP	−7.67	3.34	3.70	9.1	−2
TYP	−15.10	0.88	1.29	15.2	−2

threonine dipole (9.1) falls between phosphoserine and the longest phosphorylated residue, phosphotyrosine (15.2).

Conclusions

Modified amino acid libraries are required to enable chemically correct NMR structures to be calculated by molecular dynamics and simulated annealing. The ongoing refinement of phosphorylated protein and peptide structures prompted our lab to compute the complete library parameters and associated tools and scripts for phosphorylated amino acids. These parameters were developed by generating the initial (x, y, z) atom coordinates of each phosphorylated residues and orienting them into the correct frame for each tool used, and associating with them the partial charge of that atom. We present an empirical method for partial equilibration of orbital electronegativity by a localized charge sum comparison. The MATLAB scripts are available to the user community to orient other modified amino acid residues following the conventions of DYANA and AMBER. The three modified amino acids developed here were subsequently tested in NMR calculations in our lab until a complete toolkit was achieved.

Acknowledgements

We thank Dr. Ravi Ampapathi for providing numerous suggestions on using Insight II and examining the NMR spectra. We also thank Mehmet Sen and Tam Trinh for testing and critiquing the scripts and processes developed throughout the project, and Andrea Creath for proofing the manuscript. This work was supported by grants from the National Institutes of Health (PO1 HL016411) and the Welch Foundation (E-1580). Scripts, libraries, and readme files are available on the web at: http://www.bchs.uh.edu/~glegge/phospho_lib/index.html

References

Bean, W.C. (1969) NASA MSC Internal Note #69-FM-237.

Case, D.A., Darden, T.A., Cheatham, T.E., Simmerling, C.L., III, Wang, J., Duke, R.E., Luo, R., Merz, K.M., Wang, B., Pearlman, D.A., Crowley, M., Brozell, S., Tsui, V., Gohlke, H., Mongan, J., Hornak, V., Cui, G., Beroza, P., Schafmeister, C., Caldwell, J.W., Ross, W.S. and Kollman, P.A. (2004) AMBER 8, University of California, San Francisco.

Cornell, W.D., Cieplak, P., Bayly, C.I., Gould, I.R., Merz, K.M. Jr., Ferguson, D.M., Spellmeyer, D.C., Fox, T., Caldwell, J.W. and Kollman, P.A. (1995) *J. Am. Chem. Soc.* **117**, 5179–5197.

Duggan, B.M., Legge, G.B., Dyson, H.J. and Wright, P.E. (2001) *J. Biomol. NMR* **19**, 321–329.

Feng, M.-H., Philippopoulos, M., MacKerell, A. Jr. and Lim, C. (1996) *J. Am. Chem. Soc.* **118**, 11265–11277.

Güntert, P., Mumenthaler, C. and Wüthrich, K. (1997) *J. Mol. Biol.* **273**, 283–298.

Güntert, P. (1998) *Q. Rev. Biophys.* **31**, 145–237.

Koradi, R., Billeter, M. and Wüthrich, K. (1996) *J. Mol. Graph.* **14,** 51–55, 29–32.

Leach, A.R. (2001) *Molecular Modeling; Principles and Applications* Harlow, Prentice-Hall.

Markley, J.L., Bax, A., Arata, Y., Hilbers, C.W., Kaptein, R., Sykes, B.D., Wright, P.E. and Wüthrich, K. (1998) *J. Biomol. NMR* **12**, 1–23.

Momany, F.A., McGuire, R.F., Burgess, A.W. and Scheraga, H.A. (1975) *J. Phys. Chem.* **79**, 2361–2381.

Némethy, G., Pottle, M.S. and Scheraga, H.A. (1983) *J. Phys. Chem.* **87**, 1883–1887.

Nina, M., Im, W. and Roux, B. (1999) *Biophys. Chem.* **78**, 89–96.

Onufriev, A., Case, D.A. and Bashford, D. (2002) *J. Comp. Chem.* **23**, 1297–1304.

Spronk, C.A., Linge, J.P., Hilbers, C.W. and Vuister, G.W. (2002) *J. Biomol. NMR* **22**, 281–289.
Tjandra, N. and Bax, A. (1997) *Science* **278**, 1111–1114.
Tsui, V. and Case, D.A. (2001) *Biopolymers* **56**, 275–291.
Wuthrich, K. (1986) *NMR of Proteins and Nucleic Acids* Wiley, New York.
Xia, B., Tsui, V., Case, D.A., Dyson, H.J. and Wright, P.E. (2002) *J. Biomol. NMR* **22**, 317–331.