A Single Residue at the Active Site of CD38 Determines Its NAD Cyclizing and Hydrolyzing Activities*

Richard Graeff‡, Cyrus Munshi‡, Robert Aarhus, Malcolm Johns, and Hon Cheung Lee§

From the Department of Pharmacology, University of Minnesota, Minneapolis, Minnesota 55455

CD38 is a multifunctional enzyme involved in metabolizing two Ca^{2+} messengers, cyclic ADP-ribose (cADPR) and nicotinic acid adenine dinucleotide phosphate (NAADP). When incubated with NAD, CD38 predominantly hydrolyzes it to ADP-ribose (NAD glycohydrolase), but a trace amount of cADPR is also produced through cyclization of the substrate. Site-directed mutagenesis was used to investigate the amino acid important for controlling the hydrolysis and cyclization reactions. CD38 and its mutants were produced in yeast, purified, and characterized by immunoblot. Glu-146 is a conserved residue present in the active site of CD38. Its replacement with Phe greatly enhanced the cyclization activity to a level similar to that of the NAD hydrolysis activity. A series of additional replacements was made at the Glu-146 position including Ala, Asn, Gly, Asp, and Leu. All the mutants exhibited enhanced cyclase activity to various degrees, whereas the hydrolysis activity was inhibited greatly. E146A showed the highest cyclase activity, which was more than 3-fold higher than its hydrolysis activity. All mutants also cyclized nicotinamide guanine dinucleotide to produce cyclic GDP. This activity was enhanced likewise, with E146A showing more than 9-fold higher activity than the wild type. In addition to NAD, CD38 also hydrolyzed cADPR effectively, and this activity was correspondingly depressed in the mutants. When all the mutants were considered, the two cyclase activities and the two hydrolase activities were correlated linearly. The Glu-146 replacements, however, only minimally affected the base-exchange activity that is responsible for synthesizing NAADP. Homology modeling was used to assess possible structural changes at the active site of E146A. These results are consistent with Glu-146 being crucial in controlling specifically and selectively the cyclase and hydrolase activities of CD38.

CD38 was described first as an antigen that is involved in a host of lymphocyte functions including differentiation, proliferation, and apoptosis (reviewed in Refs. 1–3). Its expression has since been found to be widespread among nonhematopoietic tissues as well (4–7). In addition to the antigenic functions, CD38 also possesses a multitude of enzymatic activities (reviewed in Refs. 8 and 9). It catalyzes not only the hydrolysis of NAD and cyclic ADP-ribose (cADPR) to ADP-ribose, but also the cyclization of NAD and its analog, nicotinamide guanine dinucleotide (NGD), to produce cADPR and cyclic GDP-ribose (cGDP), respectively. Additionally, it can use NADP and nicotinic acid as substrates and catalyze a base-exchange reaction to produce nicotinamide adenine dinucleotide phosphate. Both cADPR and nicotinic acid adenine dinucleotide phosphate (NAADP) are Ca^{2+} messengers mediating mobilization of intracellular Ca^{2+} stores in a wide variety of cells from protozoan and plant to human (reviewed in Refs. 10 and 11).

We have previously modeled the structure of CD38 using the crystallographic data of the Aplysia ADP-ribosyl cyclase, a functional and structural homolog (12–14). The model shows a pocket analogous to the active site of the cyclase near the central cleft of CD38 (14, 15). Site-directed mutagenesis of CD38 identifies three critical residues, Glu-226, Trp-125, and Trp-189; all three cluster in the active site pocket. Of the three residues, Glu-226 is most likely the catalytic residue, because its replacement results in the loss of all enzymatic activities. The same residue is also ADP-ribosylated during catalysis, suggesting that a single covalent intermediate is involved (16, 17). The two Trp residues, on the other hand, are likely to be responsible for positioning of the substrate (14, 15). In this study, we use site-directed mutagenesis to investigate the determinants important in controlling the multitude of enzymatic activities of CD38. The results identify another glutamate residue in the active site pocket of CD38 as crucial in determining the NAD cyclizing and hydrolytic activities. Replacement of the residue greatly stimulates the ADP-ribosyl cyclase activity of CD38 while inhibiting its hydrolytic activities.

MATERIALS AND METHODS

Expression of the Human CD38 in Yeast—The Pichia expression vector pPICZaA (Invitrogen, Carlsbad, CA) was used, and the construct consisted of cloning the catalytic domain of either the wild-type or mutant human CD38 in frame with the yeast mating α-factor signal sequence as described previously (14, 15, 18). The first 44 residues of CD38, containing the transmembrane and cytoplasmic domains (19), were deleted, and the catalytic domain started at Arg-45. The expressed protein was soluble and was purified from the culture medium in a single step using a cation exchange column (SP5 PW, Waters, Milford, MA; Ref. 18). At least two separate cultures were made from each mutant, and each culture was purified separately. The purified proteins were divided into aliquots and stored at −20 °C. Repeated freeze/thaw cycles caused protein aggregation and decreased enzymatic activities.

Enzyme Assays—The wild-type or mutant CD38 (0.05–2 μg/ml) was incubated (1–40 min) at room temperature with various concentrations of specific substrates, 9 μg/ml bovine serum albumin and 40 mM Tris-HCl (pH 7.8). The reaction was stopped by the addition of SDS (0.05% final concentration). High pressure liquid chromatography (HPLC)

* This work was supported by National Institutes of Health Grants GM60333 and HD17484 (to H. C. L.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
‡ Both authors contributed equally to this work.
§ To whom correspondence should be addressed: Dept. of Pharmacology, 321 Church St. SE, 4-145 Jackson Hall, University of Minnesota, Minneapolis, MN 55455. Tel.: 612-625-7120; Fax: 612-625-0991; E-mail: leehc@tc.umn.edu.it.

† The abbreviations used are: cADPR, cyclic ADP-ribose; NGD, nicotinamide guanine dinucleotide; cGDP, cyclic GDP-ribose; HPLC, high pressure liquid chromatography; NADase, NAD glycohydrolase; NAADP, nicotinic acid adenine dinucleotide.
analyses were performed using an AG MP-1 column (10 × 120 mm) as described previously (15). The NAD glycohydrolase (NADase) and the cADPR hydrolase activities were assayed with various concentrations of NAD (7–160 μM) or cADPR (62.5 μM-1 mM), respectively. The base-exchange reaction was similarly assayed at pH 4.5 in the presence of 50 mM nicotinic acid and various concentrations of NADP (10–160 μM). The GDP-riboosyl cyclase activity was measured with 25 μM NADG and the production of cGDPR was recorded continuously at the emission wavelength of 410 nm (excitation at 300 nm). The rate of cGDPR production was calculated by differentiating the time course of the fluorescence change as previously described (20). In some experiments, GDP-ribose cyclase was also assayed using various [NGD] (10–160 μM), and the product, cGDPR, was measured using HPLC. Enzyme activities of the wild-type and mutant CD38 were measured using at least two different protein preparations. The results shown are mean ± S.E. of at least four determinations. The V_{max} and K_m values were obtained from double-reciprocal plots, and the results shown are mean ± S.E. of at least four determinations. Student’s t test was used to compare kinetic constants of the mutants with the wild type.

### Site-directed Mutagenesis of CD38

The mutations were carried out using the QuickChange site-directed mutagenesis kit obtained from Stratagene as previously described (14, 15, 18). All mutations were verified and confirmed by DNA sequencing.

#### Homology Modeling of CD38

The sequence of human CD38 (19) starting at Pro-53 was aligned first with the sequence of the Aplysia cyclase (21). The “Homology” feature in Insight II (Molecular Simulations, Inc.) was used, together with the crystal coordinates of the cyclase dimer that we have previously determined (14, 22), to generate the homologous structure of the truncated CD38 dimer (14, 15). This structure was then refined using the Powell relaxation routine in X-PLOR as described previously (14, 23). The validity of the resulting model has been substantiated previously (14). Mutation of an individual residue was performed using the program Swiss-PDB Viewer (Glaxo Wellcome Experimental Research, Geneva, Switzerland). After replacing the selected residue with the best rotamer, a 200-cycle energy minimization was applied using the steepest descent algorithm. Only the α-carbons were subjected to a harmonic constraint. The resulting structure of the active site of the wild type and mutants were rendered using the program MolMol (24).

#### RESULTS

We have previously demonstrated the validity and usefulness of the homology model in assessing structural features of CD38 (14). The model reveals that part of the active site pocket of CD38 is formed by a sequence (144TLEDTL), which is conserved totally among three species of CD38 and two species of ADP-ribose cyclases (reviewed in Refs. 8, 9, and 11). In this study, the Glu-146 present in the conserved sequence was targeted for site-directed mutagenesis, and the effects of the mutation on the five enzymatic activities of CD38 were determined. The cyclization reaction was assayed using NAD or NGD as substrate, and the production of cADPR or cGDPR was monitored by HPLC or fluorometrically (20). The exchange of the nicotinamide group of NADP with nicotinic acid was measured as NADP production using HPLC (25). The two hydrolytic reactions, NADase and cADPR hydrolase, were assayed as production of ADP-ribose from NAD and cADPR, respectively.

CD38 and the mutant proteins were produced in transfected yeast. The proteins were purified by cation-exchange chromatography, analyzed by SDS-PAGE, and blotted onto nitrocellulose membranes. The preparations were judged pure because staining of the blots for protein revealed a single band of about 32 kDa in each case. All the mutant proteins were recognized by a monoclonal antibody, IB4, against the wild-type CD38 (data not shown). We have previously raised an antibody against the conserved sequence (144TLEDTL; Ref. 15). When the blot was probed with this antibody, none of the mutant proteins were recognized. The antibody recognized the wild-type CD38 readily, however. These immunoanalyses confirmed that the mutant proteins indeed harbored changes in the conserved sequence, which was verified further by DNA sequencing.

Table I compares the enzymatic parameters of Glu-146 mutants with those of the wild type. CD38 had very high NADase activity, but its cyclase activity was not detectable by HPLC. In striking contrast, E146F, with a Phe substitution, had much depressed NADase activity as compared with the wild type. However, it possessed very high cyclase activity, which was as much as its NADase activity. More dramatic is another mutant, E146A. Its cyclase activity was more than 3-fold higher than its NADase activity. In general, all mutant proteins possessed cyclase activity of various degrees. E146A had the highest activity, and E146L had the lowest. Even for E146L, its cyclase activity of 400 ± 24 nmol/mg/min (V_{max} value) was readily detectable by the HPLC assay. The NADase activities of most mutants are similar to E146A except E146F, which has much higher activity. The concomitant stimulation of the cyclase activity and inhibition of the NADase activity suggest that Glu-146 controls both.

It is more instructive to use the ratio of the cyclase and NADase activities as an index for assessing the stimulation of the cyclase in the mutants. As shown in Table I, the ratio for the wild type was essentially zero because it expressed low cyclase activity but high NADase activity. E146A had the highest ratio; its cyclase activity was more than 3-fold higher than its NADase activity. A common characteristic of the three mutants that had the highest ratios (E146A, E146N, and E146G) is that they all have small and uncharged side chains. The rest of the mutants (E146L, E146F, and E146D) have approximately equal cyclase and NADase activities and a ratio around unity. It thus seems that there is a specific structural requirement of small and uncharged side chain at the 146 position of the CD38 sequence for the enhanced cyclase activity.

The fact that all mutant proteins possessed various enzy-
mative activities indicates that the single-residue replacements did not distort the active site grossly. Clearly they were fully capable of not only binding the substrates but also catalytically converting them to products. The structural changes induced by the substitution were thus subtle. In addition to the Glu-146 mutants, we have produced various substitutions of other residues in the active site of CD38 that included Glu-226, Asp-155, Trp-189, and Trp-125 (14) as well as two Cys residues, Cys-119 and Cys-201 (26). None of these substitutions enhanced the cyclase activity, indicating that Glu-146 is uniquely important in controlling it.

As listed in Table I, substituting Ala for Glu-146 decreased the NADase activity by more than 21-fold. Even E146F, the least inhibited mutant, showed a 6-fold decrease in activity. In contrast, as listed in Table II, the substitutions had less dramatic effects on the base-exchange activity of the same mutants. This is especially the case for E146A, the exchange activity of which was essentially the same as the wild type. The effect of substituting Glu-146 with these selected amino acids on the enzyme activities of CD38 thus is selective for the specific reaction. Two of the mutants (E146D and E146G), however, did show more severe inhibition of the exchange activity.

Although CD38 produces only trace amounts of cADPR from NAD, it does have potent cyclization activity that can be readily shown by using NGD instead of NAD as substrate. The main product in this case is cGDPR (20). Table III compares the GDP-ribosyl cyclase activity of the wild-type CD38 with its Glu-146 mutants. E146A and E146F, the two mutants that had the highest ADP-ribosyl cyclase activity, also had the highest GDP-ribosyl cyclase activity, which was 2- to 3-fold higher than that of the wild type. The rest of the mutants had GDP-ribosyl cyclase activities similar to the wild type.

Cyclization of NGD occurs at the N-7 position of the guanine ring instead of the N-1 position of the adenine ring of cADPR (27) and requires a rotation of the guanine ring around an anti-orientation. The similar enhancement of these two very dissimilar cyclization reactions suggests that the residue Glu-146 is important in controlling both. This is illustrated in Fig. 1A by plotting one activity against the other, and an approximately linear relation is revealed with a regression coefficient of 0.77.

Of the five enzymatic activities measured, the GDP-ribosyl cyclase of the mutants was the only one that had $K_m$ values significantly higher than those of the wild type, suggesting a reduction of substrate affinity by the substitutions. In addition to comparing the maximal cyclase activity at high substrate concentration ($V_{max}$ values), Table III also lists $V_{max}/K_m$ ratios of the wild type and mutants. The Michaelis-Menten model predicts that at low substrate concentrations, the enzyme activity is proportional to the substrate concentration, and the

---

**Table I**

| Mutant | $V_{max}$ (nmol/mg/min) | $K_m$ ($\mu$M) |
|--------|--------------------------|----------------|
| WT     | 50,000 ± 4,400            | 20 ± 2         |
| E146A  | 33,700 ± 5,800            | 47 ± 5         |
| E146F  | 27,900 ± 5,900            | 82 ± 18        |
| E146L  | 11,100 ± 550              | 113 ± 15       |
| E146G  | 8,500 ± 900               | 36 ± 3         |
| E146D  | 2,200 ± 260               | 8 ± 1          |

---

**Table II**

| Mutant | $V_{max}$ (nmol/mg/min) | $K_m$ (S.E. for 4–6 determinations) |
|--------|-------------------------|-------------------------------------|
| WT     | 28,000 ± 1,300          | 4 ± 0                               |
| E146A  | 92,900 ± 3,500          | 53 ± 5,7                      |
| E146F  | 76,000 ± 11,600         | 125 ± 18,9                     |
| E146L  | 36,000 ± 1,400          | 91 ± 27                        |
| E146N  | 32,400 ± 3,200          | 211 ± 34,6                     |
| E146D  | 18,900 ± 3,500          | 36 ± 36,1                      |
| E146G  | 11,800 ± 3,500          | 78 ± 56,3                      |

---

**Table III**

| Mutant | $V_{max}$ (nmol/mg/min) | $K_m$ (S.E. for 4–6 determinations) | $V_{max}/K_m$ |
|--------|-------------------------|-------------------------------------|---------------|
| WT     | 26,800 ± 1,300          | 4 ± 0                               | 6,700         |
| E146A  | 92,900 ± 3,500          | 53 ± 5,7                      | 1,772         |
| E146F  | 76,000 ± 11,600         | 125 ± 18,9                     | 608           |
| E146L  | 36,000 ± 1,400          | 91 ± 27                        | 396           |
| E146N  | 32,400 ± 3,200          | 211 ± 34,6                     | 154           |
| E146D  | 18,900 ± 3,500          | 36 ± 36,1                      | 525           |
| E146G  | 11,800 ± 3,500          | 78 ± 56,3                      | 151           |

---

2 R. Graeff, C. Munshi, R. Aarhus, M. Johns, and H. C. Lee, unpublished data.
proportionality constant is the $V_{\text{max}}/K_m$ ratio. The ratios for all the mutants were less than those for the wild type, which can be attributed to their higher $K_m$ values. Thus, substitution of Glu-146 inhibited the GDP-ribosyl cyclase activity of all the mutants when assayed at low substrate concentrations, further indicating that Glu-146 is important in controlling the enzyme activity.

Another major reaction catalyzed by CD38 is the hydrolysis of cADPR to ADP-ribose, which is responsible for terminating the signaling function of the Ca$^{2+}$ messenger (reviewed in Ref. 28). In fact, CD38 is the only enzyme reported so far that is capable of hydrolyzing cADPR. Table IV compares the cADPR hydrolysis reaction of the mutants with that of the wild type.

Similar to the NADase, the substitutions produced various degrees of inhibition of the cADPR hydrolase activity. Indeed, the two activities seem to be correlated. This correlation is shown in Fig. 1B, in which the cADPR hydrolase activity of each mutant is plotted against its NADase activity. A roughly linear relationship with a regression coefficient of 0.68 is observed. Summarizing, substitution of Glu-146 with amino acids such as Ala and Phe inhibit the two hydrolase activities of CD38 while stimulating the $V_{\text{max}}$ of the two cyclase reactions. The base-exchange activity, on the other hand, seems minimally affected.

**DISCUSSION**

Results described in this study identify Glu-146 as uniquely important in controlling both the cyclase and hydrolase activities of CD38. Glu-146 is a special residue. Not only is it conserved among several species of mammalian CD38 and *Aplysia* ADP-ribosyl cyclase (reviewed in Refs. 9 and 28), it is also located in the active site of CD38 as revealed by homology modeling (14). This is illustrated in Fig. 2A, which shows the three-dimensional surfaces of the active site of the wild-type CD38. Glu-146 is located directly opposite Glu-226, which is likely the catalytic residue because its substitution with even conservative residues abolishes all enzymatic activities of CD38 (14). The distance between the two residues is 8.1 Å, which is about the width of a molecule of cADPR (29).

The homology model can be used also to assess the effect of substitution of Glu-146 on the structure of the active site. The six mutants produced in this study can be divided into two groups, those with a high cyclase/NADase ratio and those with a ratio near unity. The ratio of the cyclase and NADase activities can be viewed as normalization, minimizing the nonspecific effects of the substitution. Thus, effects such as changes in substrate affinity that affect both enzyme activities equally would be corrected by the procedure. It is thus a better measure of the cyclase activation than the specific activity alone.

**FIG. 2. Models of the active sites of CD38 and its mutant E146A.** The homology models were constructed based on the crystallographic coordinates of the *Aplysia* ADP-ribosyl cyclase and energy-minimized as described under “Materials and Methods.” The van der Waals contact surfaces of the sites are shown for the wild-type CD38 (A), E146A (B), and E146F (C). The critical residue Glu-146 (E146) and its replacements, Ala-146 (A146) and Phe-146 (F146), as well as the catalytic residue, Glu-226 (E226), are shown in space-filled rendering. The region tinted yellow in the active site of E146A shows the most structural change as compared with that of the wild type. Oxygen is red, carbon is green, nitrogen is blue, and hydrogen is yellow.

E146A, a representative of the group that had a high cyclase/ NADase ratio, was analyzed first. After replacing Glu-146 with Ala, energy minimization was performed with harmonic constraints placed only on the $\alpha$-carbons. The resulting structure is shown in Fig. 2B. The side chain of alanine is smaller than glutamate. The distance between Ala-146 and Glu-226 is thus larger at 9.8 Å, making the opening of the active site pocket slightly wider. In addition, the region around the alanine (tinted yellow) appears more restricted as compared with the wild type. The net effect of these changes could be to allow the substrate to bind deeper into the pocket, occluding it and thereby restricting access of water into the active site. This could lead to concomitant enhancement of the cyclization reaction and depression of the hydrolysis reaction as we have previously suggested (8, 11, 27). According to our proposed mechanism of catalysis, binding of NAD to the active site is followed by the release of nicotinamide and the formation of an activated intermediate. Recent results indicate that the intermediate is likely to be covalent, because the catalytic residue Glu-226 is shown to be ADP-ribosylated during catalysis (17). Intramolecular attack of the intermediate by N-1 of the adenine would result in cyclization and the formation of cADPR from NAD. On the other hand, nucleophilic attack of the intermediate by water would instead lead to hydrolysis and the formation of ADP-ribose from NAD. In the wild type, the highly hydrophilic Glu-146 allows ready access of water to the inter-
mediate, and thus the NADase reaction is dominant. Replacement with a small and uncharged residue would lead to a more restrictive active site and reduce water access. This would increase the chance of intramolecular attack and stimulate cyclization as is observed in this study. Similar to E146A, E146G and E146N both have relatively small and uncharged side chains. Indeed, both mutants have a cyclase/NADase ratio similar to that of E146A, and the analysis described above is equally applicable as well.

Fig. 2C shows the energy-minimized model of the active site of E146F, a representative of the group of mutants having a lower cyclase/NADase ratio. The hydrophobic nature of the phenyl group tends to reduce water access to the active site and thus promote cyclization. The hydrophobic effect, however, is counterbalanced to a certain extent by the bulkiness of the side chain, which reduces the size of the mouth of the active pocket. The distance between Glu-226 and Phe-146 is now only 7.5 Å, smaller than the width of a molecule of cADPR. This would require the substrate to tilt and insert sideways into the active site instead. The less-than-perfect fit could result in leakage of water into the site, accounting for the higher hydrolase activity of E146F than E146A. Water access as an important determinant of the cyclase activity represents a working hypothesis that is consistent with some of the structure-function features of the mutants described in this study. More quantitative understanding of the exact mechanism of the catalysis of CD38, however, must await crystallographic determination of the active site with a substrate bound.

Acknowledgment—We thank Fabio Malavasi (Università di Torino, Italy) for generously providing the IB4 monoclonal antibody.

REFERENCES

1. Malavasi, F., Funaro, A., Roggero, S., Horenstein, A., Calosso, L., and Mehta, K. (1994) Immunol. Today 15, 95–97
2. Mehta, K., Shahid, U., and Malavasi, F. (1996) FASEB J. 10, 1408–1417
3. Ferrero, E., and Malavasi, F. (1999) J. Leukocyte Biol. 65, 151–161
4. Koguma, T., Takasawa, S., Toledo, A., Karasawa, T., Furuya, Y., Yonekura, H., and Okamoto, H. (1994) Biochim. Biophys. Acta 1223, 160–162
5. Yamada, M., Mizuguchi, M., Otsuka, N., Ikeda, K., and Takahashi, H. (1997) Brain Res. 756, 52–60
6. Fernandez, J. E., Deaglio, S., Donati, D., Srohoda-Beusan, I., Corno, F., Aranega, A., Forni, M., Falini, B., and Malavasi, F. (1998) J. Biol. Regul. Homeost. Agents 12, 81–91
7. Khou, K. M., and Chang, C. F. (1999) Brain Res. 821, 17–25
8. Lee, H. C. (1999) Biol. Chem. Hoppe-Seyler 380, 785–793
9. Lee, H. C., Munshi, C., and Graeff, R. (1999) Mol. Cell. Biochem. 193, 89–98
10. Lee, H. C. (2000) J. Membr. Biol. 173, 1–8
11. Lee, H. C. (1997) Physiol. Res. 77, 1133–1164
12. Lee, H. C., and Aarhus, R. (1991) Cell Regul. 2, 203–209
13. States, D. J., Walseth, T. F., and Lee, H. C. (1992) Trends Biochem. Sci. 17, 495
14. Munshi, C., Aarhus, R., Graeff, R., Walseth, T. F., Levitt, D., and Lee, H. C. (2000) J. Biol. Chem. 275, 21566–21571
15. Munshi, C., Thiel, D. J., Mathews, I. I., Aarhus, R., Walseth, T. F., and Lee, H. C. (1999) J. Biol. Chem. 274, 30770–30777
16. Sauve, A. A., Munshi, C., Lee, H. C., and Schramm, V. L. (1998) Biochemistry 37, 13239–13249
17. Sauve, A. A., Deng, H. T., Angeletti, R. H., and Schramm, V. L. (2000) J. Am. Chem. Soc. 122, 7855–7859
18. Munshi, C. B., Fryxell, K. B., Lee, H. C., and Branton, W. D. (1997) Methods Enzymol. 280, 318–330
19. Jackson, D. G., and Bell, J. I. (1990) J. Immunol. 144, 2811–2815
20. Graeff, R. M., Walseth, T. F., Fryxell, K., Branton, W. D., and Lee, H. C. (1994) J. Biol. Chem. 269, 30260–30267
21. Glick, D. L., Hellmich, M. R., Beushausen, S., Tempst, P., Bayley, H., and Strumwasser, F. (1991) Cell Regul. 2, 211–218
22. Prasad, G. S., McRee, D. E., Stura, E. A., Levitt, D. G., Lee, H. C., and Stout, C. D. (1996) Nat. Struct. Biol. 3, 957–964
23. Brunger, A. T. (1992) X-PLOR Version 3.1, A System for X-ray Crystallography and NMR, Yale University Press, New Haven, CT
24. Koradi, R., Billeter, M., and Wuthrich, K. (1996) J. Mol. Biol. 260, 30327–30333
25. Aarhus, R., Graeff, R. M., Dickey, D. M., Walseth, T. F., and Lee, H. C. (1995) J. Biol. Chem. 270, 30327–30333
26. Tohgo, A., Takasawa, S., Noguchi, N., Koguma, T., Furuya, Y., Yonekura, H., and Okamoto, H. (1994) J. Biol. Chem. 269, 28555–28557
27. Graeff, R. M., Walseth, T. F., Hill, H. K., and Lee, H. C. (1996) Biochemistry 35, 379–386
28. Lee, H. C. (2000) Chem. Immunol. 75, 39–59
29. Lee, H. C., Aarhus, R., and Levitt, D. (1994) Nat. Struct. Biol. 1, 143–144