J. Enzyme Inhibition, 1992, Vol. 5, pp. 331–338
Reprints available directly from the publisher
Photocopying permitted by license only

SHORT COMMUNICATION

THE CONFORMATIONAL ANALYSIS OF SERINE PROTEASE INHIBITORS AND ITS APPLICATIONS FOR DRUG DESIGN

K.S. WIBLEY and D.J. BARLOW

Department of Pharmacy, Kings's College London, Manresa Road, Chelsea, London, SW3 6LX

(Received April 24, 1991)

KEY WORDS: Serine protease inhibitors, conformational analysis

INTRODUCTION

Human serine protease enzymes are involved in a wide range of physiological processes including the complement system, blood coagulation and cytolysis.[1,2] Their catalytic activities are normally regulated by endogenous inhibitors,[3] and imbalances in the levels of these can sometimes lead to tissue destruction associated with certain disease states.[4] For example, an inherited deficiency[5] in, or smoking induced structural modification[6] of the inhibitor α_1-antitrypsin (α_1-antiproteinase), can lead to the uncontrolled activity of the enzyme human leukocyte elastase (HLE). In the lung, this results in the destruction of connective tissue, and leads to pulmonary emphysema.[7,8] Similar malfunctions in other serine protease systems are considered to initiate or contribute to inflammatory conditions such as pancreatitis, rheumatoid arthritis, asthma and adult respiratory distress syndrome.[9,10].

There is considerable interest, therefore, in the development of serine protease inhibitors as therapeutic agents. The inhibitors that are currently available, however, are generally unsuitable for use as pharmaceuticals. The various classes of synthetic peptidyl inhibitors like the chloromethyl ketones, aldehydes, and boronic acids[11,12] are all conveniently small, but only rarely exhibit the necessary selectivity to avoid side-effects and toxicity. Conversely, the naturally occurring protein inhibitors such as eglin-c and α_1-antiproteinase, are large and much more selective, but are relatively unstable and potentially immunogenic, and consequently are difficult to formulate and deliver.[13]

In the present work (the preliminary results of which have been reported earlier[14]) the aim has been to find a suitable compromise between inhibitor size and selectivity, to obtain a small but selective peptidyl inhibitor of HLE, for the treatment of emphysema. The design specification for the inhibitor has been obtained by extending and generalizing the earlier analyses of the crystal structures of serine protease inhibitors,[15,16] looking at the conformations of their surface regions that are responsible for their inhibitory activity.

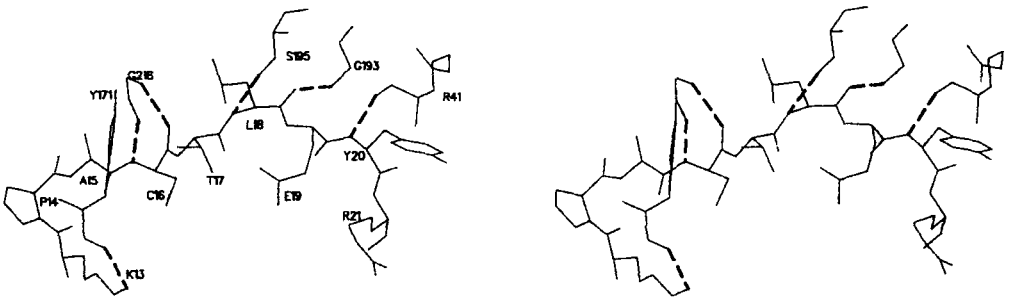

FIGURE 1 A stereo-view of the inhibitory loop of TOM complexed with *Streptomyces griseus* proteinase B[24]. The inhibitor residues form a continuous loop whereas the enzyme structure is discontinuous. Intermolecular hydrogen bonds are shown by broken lines. Residues Cys-16, Thr-17, Leu-18, Glu-19, Tyr-20, and Arg-21 of TOM are designated P_3, P_2, P_1, $P_{1'}$, $P_{2'}$, and $P_{3'}$, respectively, according to the nomenclature of Schecter and Berger.[34] The peptide bond between residues P_1 and $P_{1'}$ is the scissile bond in a substrate.

MATERIALS AND METHODS

The 15 crystal structures for the serine protease inhibitors considered in the analyses (listed in Table I) were obtained from the Brookhaven protein data bank[17] using the SERC SEQNET computer at Daresbury.

The structures were analysed on a U-Microcomputer's pUMa transputer graphics system using the molecular modeling program DRUID.[31] Least squares fitting of the inhibitor structures was carried out using the routine MATFIT.[32] The solvent accessibilities of the inhibitors and enzyme-inhibitor complexes were calculated according to the method of Lee and Richards,[32] using a solvent probe radius of 1.4 Å.

RESULTS AND DISCUSSION

Computer graphics inspection of the structures of the serine protease enzyme-inhibitor complexes reveals that there is only one consistent area of interaction between each of the various enzymes and their inhibitors. This involves the active site of each of the enzymes and a 5–6 residue segment of a surface loop present on each of the inhibitors (see Figure 1).

A comparison of the inhibitory loops' ϕ, ψ torsion angles (Figure 2) shows that they have essentially the same main chain conformation. This main chain conformation is not only conserved between the different inhibitors in their complexed states, but also between the uncomplexed and complexed inhibitors (see Figure 3). The manner in which this inhibitory loop conformation is achieved varies from inhibitor to inhibitor. Many of them have a disulphide link at position P_3 anchoring the inhibitory loop to the rest of the inhibitor molecule (see Table I). However BPTI has its disulphide at postion P_2 and PCI-1 has an additional disulphide at $P_{2'}$. CI-2 and eglin-c have no disulphides connecting their inhibitory loop to the rest of their structure, but appear to be linked at positions P_2 and $P_{1'}$ via polar interactions with the guanidyl groups of two arginine residues projecting from the main body of the inhibitors.[26,28]

The fact that the conserved main chain conformation is independent of both the inhibitor family, the source of the inhibitor and the loop sequence (see Table I) is

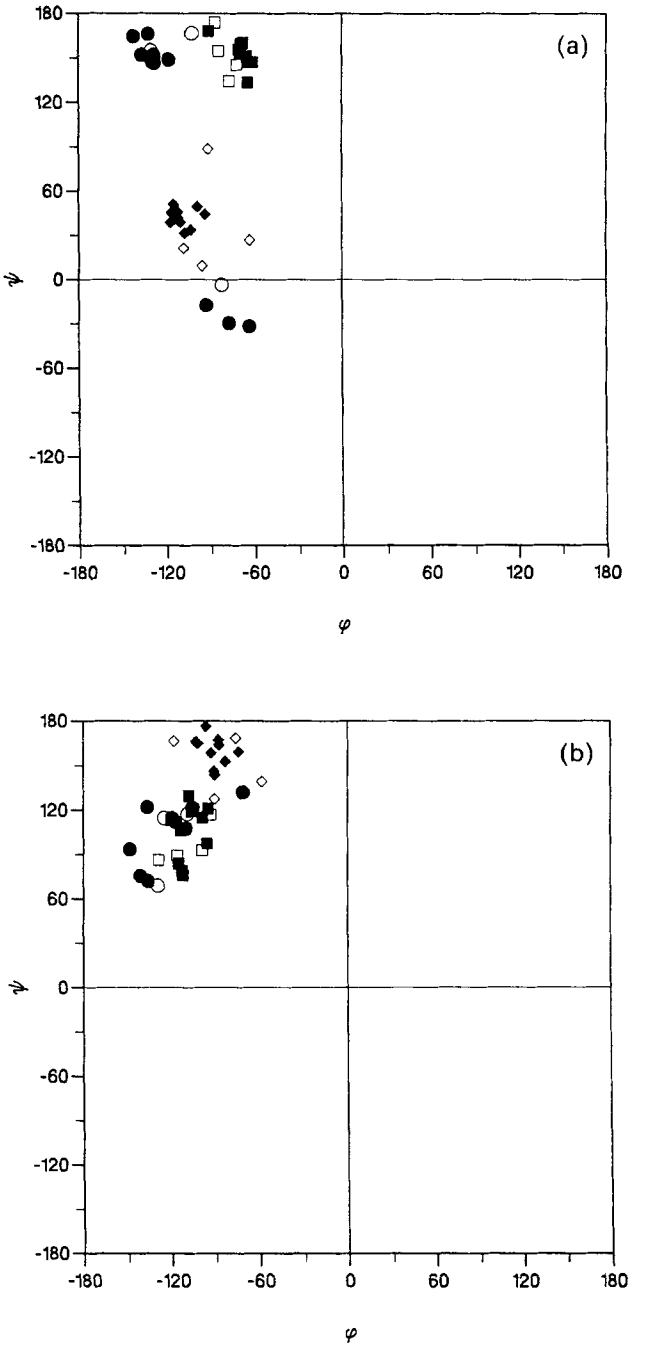

FIGURE 2 The (ϕ, ψ) main chain torsion angles for inhibitor residues (a) P_3 to P_1 and (b) $P_{1'}$ to $P_{3'}$ in both their free (unfilled symbols) and complexed (filled symbols) states. P_1 and $P_{1'}$ (○, ●), P_2 and $P_{2'}$ (□, ■), and P_3 and $P_{3'}$ (◇, ◆).

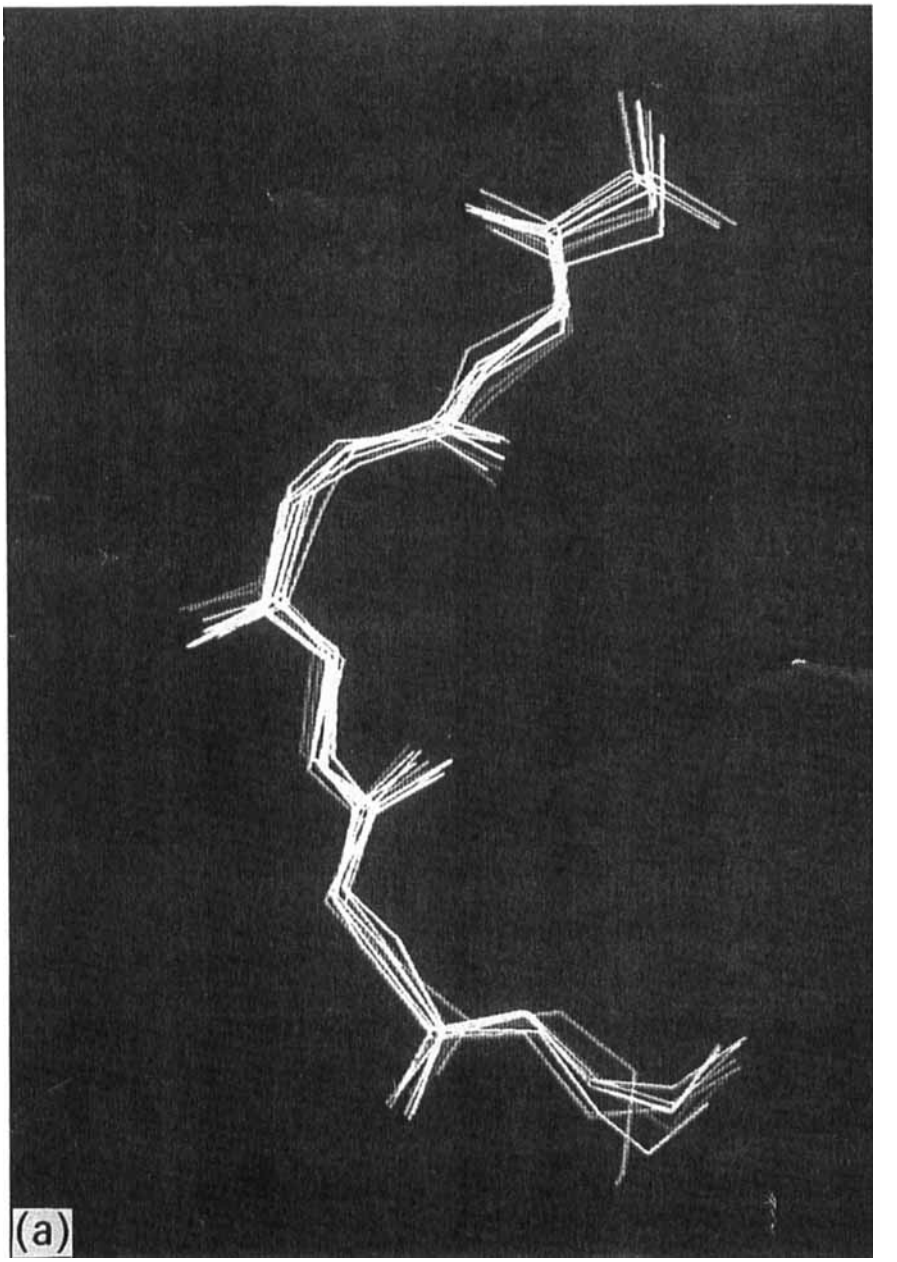

FIGURE 3 (a) The main chain of residues P_3 to $P_{3'}$ from complexed forms of the inhibitors, superimposed by least squares fitting. BPTI (red), TOM (green), PSTI (magenta), CI-2 (yellow), Eglin-C (cyan), PCI-1 (blue). (For codes see Table I). (b) The main chains of the inhibitory loops P_3 to $P_{3'}$ in the free inhibitors (green) superimposed onto the corresponding residues of the complexed inhibitors (red). (See colour plate at rear).

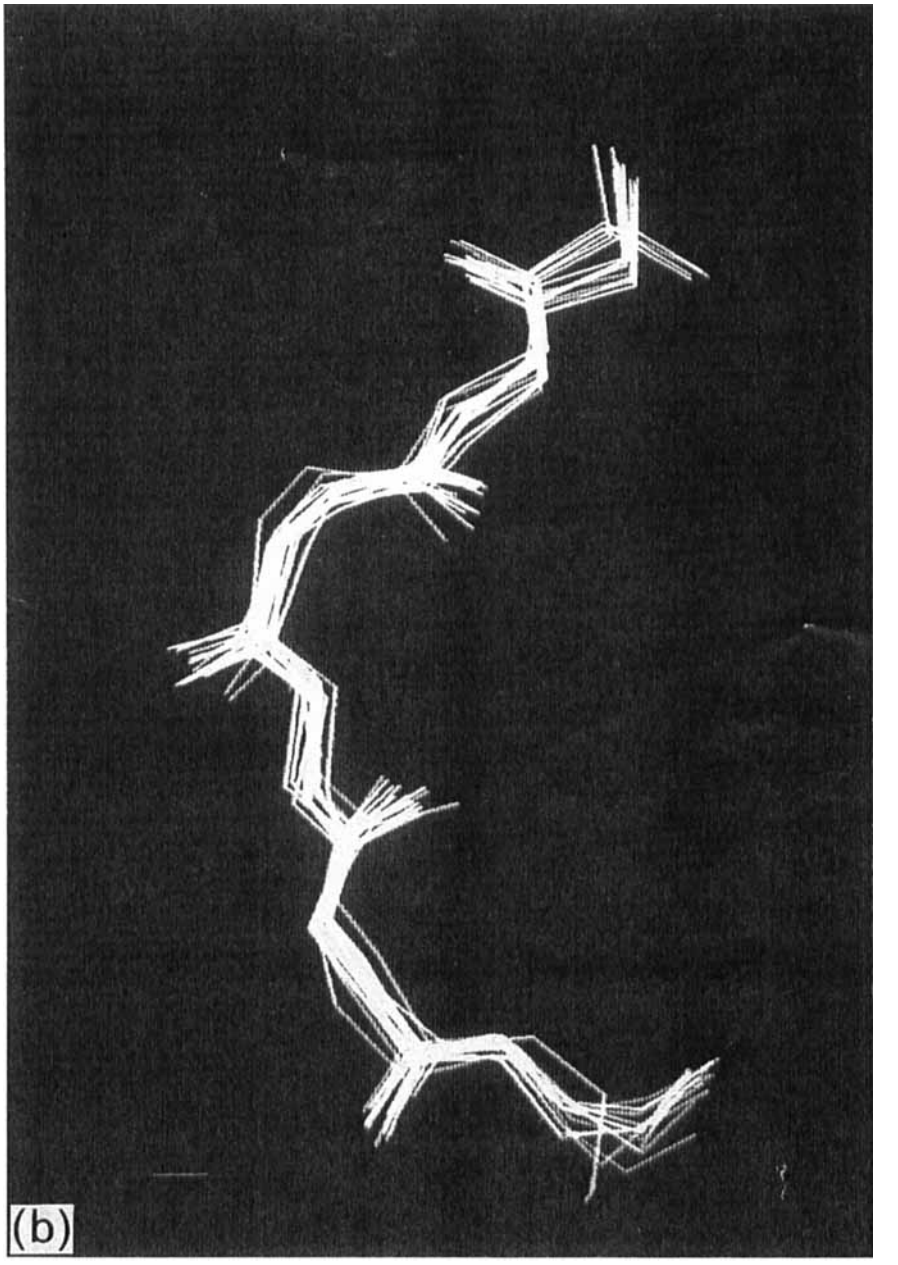

FIGURE 3 Continued.

TABLE I
Crystal structures of serine protease inhibitors

Inhibitor	Family*	Enzyme Complexed	Inhibitory Loop Sequence						PDB code[17]	Reference
			P_3	P_2	P_1	$P_{1'}$	$P_{2'}$	$P_{3'}$		
Bovine Pancreatic Trypsin Inhibitor (BPTI)	Kunitz	–	P	C	K	A	R	I	5PTI	18
		Porcine Kallikrein A							2KAI	19
		Bovine Trypsinogen							2TGP	20
		Bovine β-Trypsin							2PTC	20
3rd Domain of Japanese Quail Ovomucoid Inhibitor	Kazal	–	C	P	K	D	Y	R	1OVO	21
3rd Domain of Silver Pheasant Ovomucoid Inhibitor	Kazal	–	C	T	M	E	Y	R	2OVO	22
3rd Domain of Turkey Ovomucoid Inhibitor (TOM)	Kazal	Bovine α-Chymotrypsin	C	T	L	E	Y	R	1CHO	23
		Streptomyces griseus Proteinase B							3SGB	24
Pancreatic Secretory Trypsin Inhibitor (PSTI)	Kazal	Bovine Trypsinogen	C	P	K	I	Y	N	1TGS	25
Chymotrypsin Inhibitor 2 From Barley Seeds (CI-2)	Potato Inhibitor 1	–	V	T	M	E	Y	R	2CI2	26
		Subtilisin Novo							2SNI	27
Eglin-C	Potato Inhibitor 1	Subtilisin Carlsberg	V	T	L	D	L	R	1CSE	28
		Thermitase							1TEC	29
Polypeptide Chymotrypsin Inhibitor-1 (PCI-1) From Russet Burbank Potato Tubers	Potato Inhibitor 1	*Streptomyces griseus* Proteinase B	C	P	L	N	C	N	4SGB	15
Streptomyces Subtilisin Inhibitor (SSI)	Subtilisin	–	C	P	M	V	Y	D	2SSI	30

*Classification according to Laskowski and Kato.[16]

TABLE II
Consensus main chain conformation of the inhibitory loops of serine protease inhibitors

	P_3	P_2	P_1	$P_{1'}$	$P_{2'}$	$P_{3'}$
θ	−118°	−71°	−100°	−86°	−106°	−124°
ψ	−171°	151°	37°	152°	104°	98°

Mean values of the main chain torsion angles (ϕ, ψ) for the inhibitory loop residues P_3 to $P_{3'}$, calculated over the structures 5PTI, 1OVO, 2OVO, 2CI2, 2SSI, 2PTC, 1CHO, 1TEC, 1TGS, 2SNI, and 4SGB (codes listed in Table I).

consistent with the view that all of the inhibitors studied interact with their enzymes by a common mechanism,[16] and the fact that they display relative selectivity rather than absolute specificity towards the different protease enzymes. It also indicates that this main chain conformation provides the essential supporting structure required for inhibitory activity in these inhibitors. Since the inhibitory loop main chain is conserved, it is only the side-chains of these loop residues which vary between the different inhibitors, and so it must be the nature and conformation of these which determine the enzyme specificity.

As means of obtaining smaller and more pharmaceutically acceptable inhibitors of serine protease enzymes, it would thus appear possible to design molecules based upon hexapeptides, where the main chain conformation was constrained with the mean ϕ, ψ angles of the natural inhibitors' P_3 to $P_{3'}$ residues (see Table II).

In order to gain a likely qualitative appreciation of the specificity of the 'hexapeptide' inhibitors, reference can be made to the P_3 and $P_{3'}$ residues of the natural inhibitors, to determine the numbers of ion-pair and hydrogen bond interactions formed by these residues. The approximate number of these interactions in the various complexes were determined using simple distance criteria: $\leqslant 3.2$ Å between donor and acceptor atoms for hydrogen bonds, and $\leqslant 4$ Å between oppositely charged groups for ion-pairs. The analyses show that residue P_1 forms, on average, 49% of the total number of hydrogen bonds between the inhibitor and the enzyme, and that the inhibitory loops as a whole form 67–100% of all the intermolecular hydrogen bonds in the complexes. There are very few ion pair interactions involved at the interfaces, and all of those that are found involve charge residue side-chains at P_1 or $P_{1'}$.

It can be concluded therefore that is should be feasible to design an effective peptidyl serine protease inhibitor whose enzyme binding site comprises only 6 amino acid residues, held in particular main chain conformation, with side chains selected to fit the enzyme's active site.

Acknowlegements

KSW acknowledges receipt of an SERC Quota award, and DJB a Univeristy of London Central Research Fund grant.

References

1. Stroud, R.M. (1974) *Scientific American*, **231**, No. 1, 74.
2. Neurath, H. (1989) *Trends Biochem. Sci.*, **14**, 268.
3. Travis, J. and Salvesen, G.S. (1983) *Ann. Rev. Biochem.*, **52**, 655.
4. Weiss, S.J. (1989) *N. Engl. J. Med.*, **320**, 365.
5. Morse, J.O. (1978). *N. Engl. J. Med.*, **299**, 1045 and 1099.
6. Johnson, D. and Travis, J. (1979) *J. Biol. Chem.*, **254**, 4022.

7. Editorial (1990) *Lancet*, **335,** 1372.
8. Shock, A. and Laurent, G.J. (1990) *Mol. Aspects Med.*, **11,** 425.
9. Henson, P.M. and Johnston, R.B. (1987) *J. Clin. Invest.*, **79,** 669.
10. Nadel, J.A. (1989) *Drugs*, **37,** Suppl. 1, 51.
11. Trainor, D.A. (1987) *Trends Pharmacol. Sci.*, **8,** 303.
12. Powers, J.C. and Zimmerman, M. (1989) In Sandler, M. and Smith, H.J. (eds.), *Design of Enzyme Inhibitors as Drugs*, pp. 596–619: Oxford, New York, Tokoyo: Oxford Univeristy Press.
13. Editorial (1988) *The Medical Letter*, **30,** 29.
14. Wibley, K.S. and Barlow, D.J. (1990) *J. Pharm. Pharmacol.*, **42,** (suppl), 71P.
15. Greenblatt, H.M., Ryan, C.A., and James, M.N.G. (1989) *J. Mol. Biol.*, **205,** 201.
16. Laskowski Jr., M. and Kato, I. (1980) *Ann. Rev. Biochem.*, **49,** 593.
17. Bernstein, F.C., Koetzle, T.F., Williams, G.J.B., Meyer, E.F., Brice, M.D., Rodgers, J.R., Kernard, O., Shimanouchi, T. and Tasumi, M. (1977) *J. Mol. Biol.*, **112,** 535.
18. Wlodawer, A., Walter, J., Huber, R. and Sjolin, L. (1984) *J. Mol. Biol.*, **180,** 301.
19. Chen, Z. and Bode, W. (1983) *J. Mol. Biol.*, **164,** 283.
20. Marquart, M., Walter, J., Deisenhofer, J., Bode, W. and Huber, R. (1983) *Acta. Crystallogr. Sect. B.*, **39,** 480.
21. Papamokos, E., Weber, E., Bode, W., Huber, R., Empie, M.W., Kato, I. and Laskowski Jr., M. (1982) *J. Mol. Biol.*, **158,** 515.
22. Bode, W., Epp, O., Hyber, R., Laskowski Jr., M. and Ardelt, W. (1985) *Eur. J. Biochem.*, **147,** 387.
23. Fujinaga, M., Sielecki, A.R., Read, R.J., Ardelt, W., Laskowski Jr., M. and James, M.N.G. (1987) *J. Mol. Biol.*, **195,** 397.
24. Read, R.J., Fujinaga, M., Sielecki, A.R., James, M.N.G. (1983) *Biochemistry*, **22,** 4420.
25. Bolognesi, M., Gatti, G., Menegatti, E., Guarneri, M., Marquart, M., Papamokos, E.. and Huber, R. (1982) *J. Mol. Biol.*, **162,** 839.
26. McPhalen, C.A. and James, N.M.G. (1987) *Biochemistry*, **26,** 261.
27. McPhalen, C.A. and James, M.N.G. (1988) *Biochemistry*, **27,** 6582.
28. Bode, W., Papamokos, E., and Musli, D. (1987) *Eur. J. Biochem.*, **166,** 673.
29. Gros, P., Fujinaga, M., Dijkstra, B.W., Kalk, K.H. and Hol, W.G.J. To be published.
30. Mitsui, Y., Satow, Y., Watanabe, Y. and Iitaka, Y. (1979). *J. Mol. Biol.*, **131,** 697.
31. Barlow, D.J., Perkins, T.D.J. and Wibley, K.S., Unpublished Data.
32. Remington, S.J. and Mathews, B.W. (1980) *J. Mol. Biol.*, **140,** 77.
33. Lee, B. and Richards, F.M. (1971) *J. Mol. Biol.*, **55,** 379.
34. Schecter, I. and Berger, A. (1967) *Biochem. Biophys. Res. Commun.*, **27,** 157.