Biochemical Pharmacology, Vol. 29, pp. 2089–2094.

0006-2952/80/0801-2089 $02.00/0

COMMENTARY

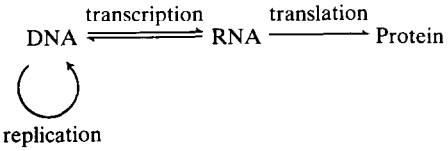
AN ALGORITHMIC APPROACH TO SEQUENCE → REACTIVITY OF PROTEINS

SPECIFICITY OF PROTEIN INHIBITORS OF SERINE PROTEINASES*

MICHAEL LASKOWSKI, JR.
Department of Chemistry, Purdue University, West Lafayette, IN 47907, U.S.A.

The Olympian View

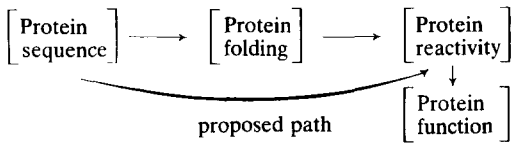
The central dogma of molecular biology



is a starkly simple and beautiful statement. However, those concerned with fundamental biological research wish to elaborate on it. Two different approaches to such an elaboration are possible—mechanistic and algorithmic. The mechanists deal with such important matters as how the various listed steps (as well as the later organelle, cell and organismic assembly) take place. The algorithm constructors build algorithms such as the base pairing rules for DNA or for RNA complementary to the DNA and the translation algorithm—the genetic code.

The ultimate success of the algorithmic approach might be achieved when it becomes possible to predict from reading the DNA sequence of their genomes that rats have long tails while guinea pigs do not. This objective seems to me still relatively distant, but highly worthwhile.

Since I am a protein chemist, I propose to concentrate only on that section of the algorithm which deals with proteins. This can be stated as



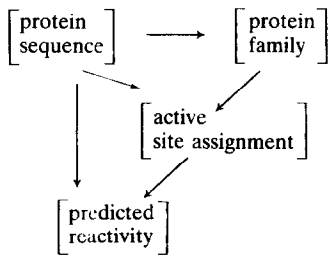
Clearly, were we able to develop the protein sequence → protein function algorithm, we would become the darlings of our skeptical competitors, penny-pinching granting agencies and even of the general public. Unfortunately, such a pure algorithm is likely to be most difficult to obtain without introduction of additional information. We already know a great many cases where proteins of closely related specificity and amino acid sequence do entirely different 'jobs'. For example, some trypsin-like serine proteinases digest food, others clot blood, dissolve the unneeded blood clots, produce and eliminate protein hormones and allow spermatozoa to penetrate ova. It is likely to prove very difficult (although maybe not impossible) to decide from sequence alone for which of these tasks a given enzyme molecule is especially fitted. It should prove to be a far easier task to predict the detailed specificity of an enzyme on various peptide and ester substrates. It is for this reason that, in spite of the continual din of 'Structure–Function' Symposia, I like the two distinct concepts, protein reactivity and protein function. I hope that it is obvious that by the vague term reactivity I mean primarily the various interactions with biologically important molecules and not reactivity toward some chemical reagents taken off the shelf.

In dealing with the protein sequence → protein reactivity algorithm, most workers prefer the hierarchical order. They reason that it is absolutely required to determine the sequence → folding algorithm first, and then to predict the reactivity from folding. One could not argue that knowing the exact folding would not be helpful in predicting reactivity. However, while it would be immensely helpful to have a sequence → folding algorithm, it is not essential. The recent progress in developing such algorithms is not highly encouraging, in spite of the fact that their development attracted the attention of many of the most talented protein chemists. From the point of view of being utilized as a starting point of sequence to reactivity algorithm, the present sequence to folding algorithms have three shortcomings. First, they are not accurate enough. Second, they predict the regular structures—α helices, β sheets and bends. These, while they have a lot to do with the overall structure of proteins, have less to do with the active sites, which are often at places where regular structures are distorted or totally irregular. Third, even when correct, the algorithms often predict regular structures displaced by 1–3 residues from positions where these are actually found. If the prediction of an active site is to be based on these—the active site residue may well be predicted wrong by a few residues—yet, for what

* Work from my laboratory is supported on a long-term basis by the National Institutes of Health Grant GM 10831.

follows, it is essential that the active site be predicted *exactly* right.

Thus I propose that in the search for a sequence → reactivity algorithm, the approach should be altered. I propose instead



This approach eliminates the direct need for determination or prediction of folding. It substitutes for it the law of homology—homologous proteins have similar three-dimensional structures and similar mechanisms of action. The law of homology is not exact, but it is far better than any current folding algorithm. The introduction of the law of homology is associated with a formidable cost—the loss of generality. However, it is quite likely that no general protein sequence (or folding) → reactivity algorithm can be developed. Proteins differ a great deal from nucleic acids in the great complexity of their folding and in their functional versatility. It is probably a situation which defies general algorithms. Most authorities estimate that there are of the order of 10^3 homologous protein families; thus, approximately 10^3 algorithms will be needed. As the number of families is so relatively, but not impossibly, large, we should, at first, start developing those algorithms which will be the most interesting and the easiest to obtain. In the remainder of this commentary, I shall argue that protein inhibitors of serine proteinases are one of the best systems to initiate this approach.

Such an ideal system should be composed of very small proteins since not only are they easier to understand, but also it is easier to obtain and sequence the numerous homologs needed. Furthermore, the reactivity we talk about should be chemically well defined, not a black box (i.e. not a muscle twitch), and the mechanism of interaction should be known as well as possible. Finally, we should have an easy source of interesting homologs for study.

Proteinase inhibitors fulfill the first two objectives admirably. However, it is their special property that they fulfill the third so well. Proteinase inhibitors frequently remain active after semi-synthetic replacement of residues in their reactive sites. Probably as a consequence of this chemical property, they do not strongly conserve their reactive sites during evolution, thus providing us with a truly large number of interesting inhibitors with altered specificity. In most proteins we would view amino acid replacements as leaving the activity unchanged or turning it off. In inhibitors many replacements alter specificity in an interesting way. None of this is meant to imply that proteinase inhibitors are the only system on which such an algorithm can be constructed. Indeed, the work of others on serine proteinases, globins, cytochromes, etc. has implicitly or explicitly produced similar algorithms.

*Protein proteinase inhibitors**

Protein proteinase inhibitors are ubiquitous; they were isolated from various tissues of animals, plants and micro-organisms. Of those thus far described, a large majority are strictly specific for the inhibition of serine proteinases. This group of inhibitors can, in turn, be divided into two subgroups. The first subgroup is made up of extremely stable (both to denaturation and to wanton proteolysis) proteins, containing one or more disulfide bridges, which are either very small (40–200 amino acid residues) or consist of several small inhibitory domains or homology regions. All of these inhibitors interact with their cognate enzymes according to a standard mechanism detailed below. This group, which includes a large majority of known inhibitors, is listed in Table 1. The second subgroup is made up of relatively large, rather unstable inhibitors, frequently devoid of intramolecular disulfides, and primarily present in mammalian blood plasma, e.g. α_1 proteinase inhibitor, α_1 antichymotrypsin, α_2 antiplasmin and antithrombin III. The mechanism of interaction of these inhibitors with their cognate enzymes is less well understood—it may or may not be closely similar (but not identical) to what is described below. We will not concern ourselves with this group any further.

* The account of proteinase inhibitors given here is highly simplified and woefully incomplete. For more complete reviews see Refs. 1–3.

Table 1. Families of those protein inhibitors of serine proteinases which obey the standard mechanism*

(I)	Bovine pancreatic trypsin inhibitor (Kunitz) family
(II)	Pancreatic secretory trypsin inhibitor (Kazal) family
(III)	*Streptomyces* subtilisin inhibitor family
(IV)	Soybean trypsin inhibitor (Kunitz) family
(V)	Soybean proteinase inhibitor (Bowman–Birk) family
(VI)	Potato I inhibitor family
(VII)†	Potato II inhibitor family
(VIII)†	*Ascaris* trypsin inhibitor family
(IX)	Other families

* From Ref. 3.

† Information on families VII and VIII is rather marginal. It may be that establishment of these families is not yet justified.

Before going on it should be pointed out that many inhibitor molecules consist of multiple domains, each with a single inhibitory reactive site. The multiple reactive sites are brought together by a variety of means, e.g. noncovalent association (S-SI inhibitor, potato I inhibitor), multiple disulfide bridged chains [testudin (I. Kato and W. J. Kohr, unpublished observations)], tandem gene elongation to produce disulfide bridged homology regions (Bowman–Birk inhibitors), and tandem gene elongation to produce tandem domains (ovomucoid, ovoinhibitor). In most of these situations, the reactive sites on the various domains are not identical. It thus makes little molecular sense to talk about the specificity of an ovomucoid from a certain bird species. What we want to talk about is the specificity of each individual domain.

Each inhibitory domain contains in it a single, special peptide bond located on the surface of the molecule—*the reactive site.* This bond serves as the substrate for the cognate enzyme—surprisingly the k_{cat}/K_m value for this interaction is generally very high (10^3–10^6 M^{-1} sec^{-1}), frequently higher than for good synthetic peptide substrates (esters and anilids are generally better) for the enzyme. Thus, one requirement for an inhibitor is that it should be a good substrate, as judged by the high k_{cat}/K_m criterion. Fortunately for our work, the specificity of serine proteinases is one of the most studied problems in biochemistry so that a huge amount of data is available. The sequences surrounding most reactive sites are those we would expect of good substrates for their cognate enzymes.

The appropriate sequence may not be sufficient to ensure high k_{cat}/K_m. The sequence must also be sterically accessible to the active site of the enzymes. Both enzymes and inhibitors are relatively rigid and full of protrusions. These protrusions may get in the way of one another and thus prevent the interaction between the active site of the enzyme and the reactive site of the inhibitor. This type of steric hindrance was demonstrated by computer confrontation of molecular models of pancreatic trypsin inhibitor (Kunitz) [4] and of soybean trypsin inhibitor (Kunitz) [5] with the molecular model of subtilisin. In neither case was it possible to get the desired interaction. Gratifyingly, neither inhibitor (or semi-synthetic analog with altered P_1) (see Fig. 1 for definition) inhibits subtilisin when tested in solution in the laboratory. It is quite likely that several additional failures to inhibit can be explained by steric hindrance.

However, inhibitors are not simply substrates with high k_{cat}/K_m. If this were so, they might rapidly become exhausted after all of the molecules are hydrolyzed. In order to prevent this, the hydrolysis must be operationally reversible. Inhibitors arrange for this reversibility first of all by placing the reactive site in a disulfide loop. Thus, hydrolysis of the reactive site peptide bond does not lead to the production of two fragments, but rather to the opening of a disulfide bridged ring. This is, however, not enough. In addition, the residues surrounding the reactive site heavily interact with the remainder of the inhibitor molecule so that upon hydrolysis there is relatively little gain in motional freedom and, therefore, a small increase in entropy. Thus, the

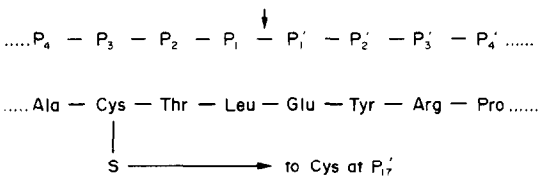


Fig. 1. Diagrammatic representation of a reactive site of a protein proteinase inhibitor (top line) in Berger–Schechter notation and (bottom line) the amino residues surrounding the reactive site of the third domain of turkey (*Meleagris gallopavo*) ovomucoid. This domain strongly inhibits chymotrypsin, elastase and subtilisin, but not trypsin. The arrow indicates the reactive site [6].

equilibrium constant for reactive site peptide bond hydrolysis is near unity at neutral pH and the inhibitor with the peptide bond hydrolyzed is thermodynamically as good an inhibitor as the inhibitor with the peptide bond intact.

Having arranged for all that, nature is not yet finished. If the k_{cat}/K_m value were partitioned into its k_{cat} and K_m components in a normal fashion, e.g. $k_{cat}/K_m = 10^3$ M^{-1} sec^{-1}; $k_{cat} = 1$ sec^{-1}, $K_m = 10^{-3}$ M, we would have little inhibition and very rapid reactive site hydrolysis at neutral pH. In fact, inhibitors partition the high k_{cat}/K_m value very unusually into a very low k_{cat} and exceptionally low K_m. For example, in the bovine β trypsin, soybean trypsin inhibitor (Kunitz) at neutral pH, the k_{cat}/K_m value is approximately 10^6 M^{-1} sec^{-1}, k_{cat} is approximately 10^{-6} sec^{-1} (a million times slower than normal) and K_m is 10^{-12} M (a billion times lower than normal). It is this low K_m that is responsible for the extremely strong inhibition. We do not, as yet, understand clearly what sequence characteristics of the inhibitor and of the enzyme are responsible for this unusual partitioning. In absence of this knowledge, we cannot solve the problem of what is a good inhibitor deductively; we must—as do almost all algorithm constructors—consult the experimental data. A very large number (approximately 200) of inhibitors (more properly inhibitory domains) have been sequenced and the specificities of the sequenced inhibitors (partially) determined. The work suffers from two major difficulties. First, it is far easier to talk about inhibition qualitatively ("inhibits or does not inhibit") while, in fact, such a distinction is not very reliable. What we should have is equilibrium constants for enzyme–inhibitor complex formation, rates of formation and of dissociation of complexes, and the rates of reactive site hydrolysis and resynthesis. Such data are very difficult to obtain and are available for very few systems, but we are trying to get this information for more systems. Second, there are truly very many serine proteinases and, therefore, the task of determination of specificity seems almost endless. In this article I will talk only about trypsin (bovine β trypsin), chymotrypsin (bovine chymotrypsin Aα), elastase (porcine elastase I) and subtilisin (subtilisin BPN'). However, such a restriction omits the exciting findings that frequently bovine trypsin is inhibited strongly, human trypsin less strongly [7] and trypsin 1 from the starfish *Dermasterias imbricata* very weakly [8]. Accordingly, trypsin 1, human trypsin

Table 2. Inhibitory specificity to be expected from various amino acid residues at position P_1

Residue		Enzymes inhibited	Footnotes
A	Ala	Elastase	*
C	Cys	Unknown	†
D	Asp	Unknown	*
E	Glu	Probably an analog of Staphyloccocal proteinase V8	‡
F	Phe	Chymotrypsin, trypsin weakly	§
G	Gly	Unknown	
H	His	Unknown	
I	Ile	Unknown	
K	Lys	Trypsin strongly, chymotrypsin weakly	‖
L	Leu	Chymotrypsin, elastase, subtilisin	¶
M	Met	Chymotrypsin, elastase, subtilisin	¶
N	Asn	Unknown	
P	Pro	Unknown	
Q	Gln	Unknown	**
R	Arg	Trypsin strongly, chymotrypsin weakly	‖
S	Ser	Elastase (chymotrypsin C only)	††
T	Thr	Unknown	
V	Val	Elastase	‡‡
W	Trp	Chymotrypsin	§§
Y	Tyr	Chymotrypsin	‖‖
—	Deletion	Inactive	¶¶

* In first homology regions of soybean inhibitor CII [9] and garden bean inhibitor II [10], both Bowman–Birk family; third domain of chicken ovomucoid—inactive [6].

† Present in first domains of Japanese quail [6] and of francolin (I. Kato and W. J. Kohr, unpublished observations) ovomucoids.

‡ While we have not found Staphyloccocal proteinase V8 to be inhibited, it specifically hydrolyzes the reactive site bonds, suggesting that a related enzyme will be inhibited [11].

§ Present in the second homology region of lima bean inhibitor IV [12], chicken ovoinhibitor (domain V) (I. Kato, unpublished observations), and in semi-synthetic derivatives of soybean trypsin inhibitor (Kunitz) [13, 14] and of pancreatic trypsin inhibitor (Kunitz) [15].

‖ Extremely common.

¶ Extremely common; when present in Kazal, S-SI and Ascaris families, all three enzymes are inhibited; in Bowman–Birk inhibitors only chymotrypsin is inhibited.

** Present in chachalaca ovomucoid third domain [6], but this domain does not inhibit any of numerous enzymes tested—probably not due to P_1 Gln, but due to other residues.

†† Present in bobwhite quail ovomucoid third domain [6].

‡‡ Present in goose ovomucoid third domain [6].

§§ In semi-synthetic derivatives of soybean trypsin inhibitor (Kunitz) [13, 14] and of pancreatic trypsin inhibitor (Kunitz) [15].

‖‖ Present in Japanese quail ovoinhibitor fifth domain [16], and in adzuki bean (Bowman–Birk) inhibitor, second homology region [17].

¶¶ Conclusion is based on semi-synthetic studies and on a lack of inhibitory activity of β_1-bungarotoxin [18].

and bovine trypsin hydrolyze the reactive sites of trypsin inhibitors rapidly, slowly and very slowly—a clear example that inhibition is related to k_{cat}/K_m partitioning. I also omit the physiologically important distinctions between the inhibition of trypsin, plasmin, thrombin, Factor Xa, acrosin and other trypsin-like enzymes. Similarly, many other enzymes, e.g. *Streptomyces griseus* proteinases A and B, proteinase K, α lytic proteinase and aspergillopeptidase B, are omitted for the sake of simplicity (or lack of a broad enough data base). With a sufficient number of workers these can become incorporated.

Sequencing of inhibitors, which obey the standard mechanism, revealed an important fact. All of these are not homologous. Instead they belong to a number of separate families that are listed in Table 1. However, given a sequence of a protein, it is quite easy to decide whether or not it belongs to one of the well characterized families, especially if it is a mem-

ber of families I–V (where relatively many examples are available). Once the assignment to a specific family is made, it is readily possible to assign the reactive site position by homology.* We (and others) routinely make such assignments and so far we have never been wrong. The turkey ovomucoid third domain reactive site was assigned in this way. Recently (W. Ardelt, unpublished observations), we have shown experimentally that it is this site which inhibits chymotrypsins A, B and C, subtilisins (BPN′ and Carlsberg), *S. griseus* proteinases A and B, proteinase K, and elastase I. Once the reactive site is assigned, we can look up residue P_1 in Table 2 and make a reasonably accurate prediction of inhibitory specificity. Such a prediction will, however, be far from perfect, because while the specificity is dominated by residue P_1, it is not dictated by it. Additional residues modulate specificity. Let us examine some of these.

The residue P_2 (when allowed to vary—in Family I, P_2 must be Cys) exerts a significant effect on inhibition. In trypsin inhibitors, Thr (which is frequently found there) seems to lead to stronger inhibition than alternatives (K. A. Wilson and R. W Sealock, unpublished observations). A negative charge at this residue (avoided by nature) greatly weakens inhibition [9]. In many families P_3 must be Cys so we have little information here. A negative charge at P_4 greatly weakens inhibition of the enzymes which have an extended binding site (subtilisin and elastase). The third domains of Gambel's quail and of chestnut bellied scaled quail ovomucoids [6] differ only in the residue shown

	P_4	P_3	P_2	P_1 ↓	P_1'
Scaled	. . .Asp–	Cys–	Thr–	Leu–	Glu. . .
Gambel's	. . .Ala–	Cys–	Thr–	Leu–	Glu. . .

Yet, Gambel's quail third domain is a strong inhibitor of chymotrypsin, elastase and subtilisin, while scaled quail third domain inhibits chymotrypsin strongly, elastase weakly and subtilisin almost not at all (M. Empie, unpublished observations). This is in accord with the general opinion that the binding site in chymotrypsin is narrow and in elastase and subtilisin extended.

On the other side of the reactive site, P_1' residues generally vary widely with little effect on inhibitory activity. There are two important exceptions. In the Bowman–Birk inhibitor family P_1' is always Ser [9]. Semi-synthetic studies show that its replacement weakens the inhibitor [9]. Pro occurs very rarely as a P_1' residue but a striking case is that of the second domain of golden and silver pheasant ovomucoids (W. J. Kohr, unpublished observations).

	P_4	P_3	P_2	P_1 ↓	P_1'
Golden	. . .Leu–	Cys–	Asn–	Lys–	Pro. . .
Silver	. . .Leu–	Cys–	Asn–	Lys–	Ala. . .

Silver pheasant ovomucoid inhibits trypsin, golden pheasant ovomucoid does not.

The prohibition against Pro appears to extend to the P_2' position (at least in Kazal inhibitors) since tinamou ovomucoid (W. J. Kohr, unpublished observations) with the first domain reactive site sequence

	P_3	P_2	P_1 ↓	P_1'	P_2'
Tinamou	. . .Cys–	Pro–	Lys–	Thr–	Pro. . .

does not inhibit trypsin. The strict prohibitions against Pro in P_1' and P_2' can be understood fairly readily. The residues surrounding the reactive site of inhibitors must have a required set of Ramachandran angles ϕ and ψ [5]. Proline cannot adopt the required ϕ angle at P_1' and P_2'. On the other hand, it has the required angle at P_4, P_3, P_2 and P_3' and P_4', and it is frequently found there in many active inhibitors.

This set of modulating rules concentrated only on residues surrounding the reactive site in the sequence. Yet X-ray crystallographic studies of the few enzyme–inhibitor complexes [5, 20, 21] studied show that, aside from these few, more residues of the inhibitor (about 5–7) not adjacent to the reactive site in the sequence touch the enzyme. We have numerous examples to show that these contacts also make important contributions to inhibition. I did not list here the few definite cases where we can assign (or guess at) roles of such individual residues because these are highly tentative and also because they apply only to individual inhibitor families, not to all inhibitors following the standard mechanism.

I should caution the reader that the present set of rules is not complete. As a dramatic, but far from only, example, I wish to point out that they predict that the chicken ovomucoid first domain [6] should inhibit trypsin. It does not. (The inhibitory site for trypsin in chicken ovomucoid is on the second domain, which we also predict should inhibit trypsin.) There are several other interesting questions of inhibitor specifity to which we cannot as yet give an answer.

However, let me restate again what can be done. Given a completely new protein sequence, we can with reasonable confidence say whether or not it belongs to any of the first five inhibitor families listed in Table 1 (with somewhat less certitude we could also decide whether it belongs to one of the other listed families). If the answer is no, we cannot reach any other conclusions about its inhibition of serine proteinases as it may belong to some as yet uncharacterized inhibitor family. If the answer is yes, we can unambigously assign in it (by homology) the position of the reactive site. With this information in hand we can apply our algorithm and conclude (but we will sometimes be wrong) whether it will be active and, if so, what enzymes it will inhibit. While a scoring method for judging the validity of this algorithm was not yet developed, it seems that the predictions are reasonably good.

I believe that similar algorithms may be developed

* What is actually easy to assign is the 'putative reactive site'. This has always turned out to be the reactive site for those inhibitors which were found to be active. However, some proteins, clearly homologous to inhibitors, do not inhibit any enzymes against which they have thus far been tested and indeed may not be active against any enzyme (they are 'dead' domains). In such cases, it may not be strictly proper to talk about the reactive site but rather about the 'putative reactive site', for example, in golden pheasant ovomucoid second domain.

by others (some already exist) for other proteins, thus increasing the generality of this woefully non-general approach.

The genetic code is a beautifully general algorithm. However, it translates from one totally abstract language, whose functional consequences we do not understand, to another equally abstract language. The protein sequence → reactivity algorithms translate from an abstract to a concrete language we understand. But the genetic code is a complete dictionary. We, on the other hand, provide a dictionary for only a dozen or so words and even then not a very good dictionary. Proteins are far more complicated than nucleic acids.

Acknowledgements—The following visitors, collaborators and (then or now) graduate students worked on proteinase inhibitors in my laboratory over the past twenty years. I am deeply grateful to them all, as it is they and not I, who did the real work: W. Ardelt, M. Welch Baillargeon, U. DeVonis Biddlingmeyer, W. C. Bogard, S. Crema, M. Empie, D. A. Estell, P. Fankhauser, W. R. Finkenstadt, M. Hall, M. A. Hamid, S. Herbert, H. F. Hixson, Jr., G. A. Homandberg, I. Kato, R. Duran Klopp, W. J. Kohr, D. Kowalski, T. R. Leary, J. Lebowitz, J. A. Luthy, C. J. March, J. A. Mattis, R.E. McKee, J. E. McKie, Jr., A. Morawiecki, C. W. Niekamp, K. Ozawa, M. Praissman, R. J. Peanasky, B. L. Schatzley, R. W. Sealock, J. Schrode, D. Wang, G. H. Widener, Jr. and K. A. Wilson.

REFERENCES

1. M. Laskowski, Jr. and R. W. Sealock, in *The Enzymes* (Ed. P. D. Boyer), 3rd Edn, Vol. 3, pp. 375–483. Academic Press, New York (1971).
2. H. Tschesche, *Angew. Chem. (Int. edn)* **13,** 10 (1974).
3. M. Laskowski, Jr. and I. Kato, *A. Rev. Biochem.* **49,** 597 (1980).
4. J. D. Robertus, J. Kraut, R. A. Alden and J. J. Birktoft, *Biochemistry* **11,** 4293 (1972).
5. Y. Mitsui, Y. Satow, Y. Wanatabe, S. Hiromo and Y. Iitaka, *Nature, Lond.* **277,** 447 (1979).
6. I. Kato, W. J. Kohr and M. Laskowski, Jr., in *Regulatory Proteolytic Enzymes and Their Inhibitors* Proc. Eleventh FEBS Meeting (Ed. S. Magnusson), pp. 197–206. Pergamon Press, New York (1978).
7. M. Laskowski, Jr., I. Kato, T. R. Leary, J. Schrode and R. W. Sealock, in *Bayer Symposium V—Proteinase Inhibitors* (Eds. H. Fritz, H. Tscheshe, L. J. Greene and E. Truscheit), pp. 597–611. Springer, Berlin (1974).
8. D. A. Estell, K. A. Wilson and M. Laskowski, Jr., *Biochemistry* **19,** 131 (1980).
9. T. Ikenaka and S. Odani in Regulatory Proteolytic Enzymes and Their Inhibitors, Proc. Eleventh FEBS Meeting (Ed. S. Magnusson), pp. 207–216. Pergamon Press, New York (1978).
10. K. A. Wilson and M. Laskowski, Sr., *J. biol. Chem.* **250,** 4261 (1975).
11. I. Kato, W. J. Kohr and M. Laskowski, Jr., *Fedn Proc.* **36,** 764 (1977).
12. F. C. Stevens, S. Wuertz and J. Krahn, in *Bayer Symposium V—Proteinase Inhibitors* (Eds. H. Fritz, H. Tscheshe, L. J. Greene and E. Truscheit), pp. 344–354. Springer, Berlin (1974).
13. T. R. Leary and M. Laskowski, Jr., *Fedn Proc.* **32,** 465 (1973).
14. D. Kowalski, T. R. Leary, R. E. McKee, R. W. Sealock, D. Wang and M. Laskowski, Jr., in *Bayer Symposium V—Proteinase Inhibitors* (Eds. H. Fritz, H. Tscheshe, L. J. Greene and E. Truscheit), pp. 311–24. Springer, Berlin (1974).
15. H. Jering and H. Tschesche, *Eur. J. Biochem.* **61,** 453 (1976).
16. M. Laskowski, Jr., I. Kato and W. J. Kohr, in *Versatility of Proteins* (Ed. C. H. Li), pp. 307–318. Academic Press, New York (1978).
17. C. Ishikawa, S. Nakamura, K. Wanatabe and K. Takahashi, *Fedn Eur. Biochem. Soc. Lett.* **99,** 97 (1979).
18. K. Kondo, K. Narita and C. Y. Lee, *J. Biochem, Tokyo* **83,** 101 (1978).
19. R. E. McKee, Ph.D. Thesis, Purdue University, West Lafayette, IN (1975).
20. A. Ruhlmann, D. Kukla, D. Schwager, K. Bartels and R. Huber, *J. molec. Biol.* **77,** 417 (1973).
21. R. M. Sweet, H. T. Wright, J. Janin, C. H. Chothia and D. M. Blow, *Biochemistry* **13,** 4212 (1974).