The Identification of Distinctive Forms of Human α_2 -Macroglobulin by using the Numerical Relationship between Trypsin Binding in α - and β -Modes

By R. MALCOLM TOPPING* and ARTHUR H. CRAVEN†

*School of Molecular Sciences and †School of Mathematical and Physical Sciences,
University of Sussex, Brighton BN1 9QJ, Sussex, U.K.

(Received 15 March 1978)

Interactions between the serine proteinase trypsin and the protein proteinase inhibitors in human blood were expressed in terms of a coupled set of non-linear differential equations, which has been solved for each of 110 samples of serum obtained from colleagues and from a variety of hospital sources. Optimization of nine unknown theoretical parameters and 21 experimental rate measurements of the hydrolytic activity of trypsin in free and bound states after admixture with various amounts of a given serum was achieved by an iterative procedure using initial estimates of the parameters derived from the 'four-straight-line' model described in the preceding paper [Topping & Seilman (1979) *Biochem. J.* 177, 493–499.] Such a procedure yielded the following information for each sample of serum examined: (a) the concentrations of α_1 -antitrypsin and α_2 -macroglobulin; (b) the unequivocal assignment of α_2 -macroglobulin into one of seven categories on the basis of trypsin binding in two kinetically differentiated modes (α and β); (c) the hydrolytic activities of trypsin (versus Bz-Arg-OEt) when bound to α_1 -antitrypsin, and to α_2 -macroglobulin in the α - and β -modes. Molecular interpretations of the binding of trypsin to α_2 -macroglobulin are discussed and the potential clinical value of recognizing the nature of such binding is reported.

The importance of the controlled operation of proteolytic enzymes in the maintenance of physiological harmony is widely recognized (Fritz & Tschesche, 1971; Fritz *et al.*, 1974; Reich *et al.*, 1975; Ribbons & Brew, 1976). One aspect of such control is exerted by the family of protein proteinase inhibitors, two members of which, α_1 A and α_2 M, assume particular importance as a result of their wide inhibitory spectrum. Serine proteinases, such as trypsin, elastase, collagenase, chymotrypsin, plasmin and thrombin, all of which participate in biochemical processes vital to normal functioning, also all have their activities modified by interaction with either α_1 A or α_2 M or both. An investigation into the partition of the serine proteinase trypsin between the principal inhibitory sites of human serum has suggested that the multiple interactions may be best interpreted by using a postulate that human α_2 M is capable of binding trypsin in two kinetically differentiated ways (Topping & Seilman, 1979). The kinetically dominant mode involves binding of up to two molecules of enzyme per molecule of α_2 M (α -sites) and is followed by the binding of a variable number of molecules of enzyme in a subsidiary mode (β -

sites). When bound in the α -mode, the ability of trypsin to hydrolyse the substrate Bz-Arg-OEt is invariably greater than when bound in the β -mode. Only when virtually all the binding capability of α_2 M has been satisfied does α_1 A contribute to the inhibitory pattern. This proposed sequence of events has given rise to a 'four-straight-line' model for the partition of trypsin between the inhibitory sites of human serum. Such a model, based on eqns. (4)–(6) of the preceding paper (Topping & Seilman, 1979) with the assumption that $k_{+1} \gg k_{+2} \gg k_{+3}$, provides a useful basis for predicting the fate of the enzyme when introduced into human serum. The model makes no allowance for minor trypsin inhibitors such as the inter- α -trypsin inhibitor, which constitutes about 5% of the α_1 A in the Pi^{MM} phenotype (Mittman, 1972). Such neglect introduces negligible error into the computed characteristics of a partition profile, because of the low concentrations of such inhibitors compared with α_1 A and their inability to compete effectively with α_2 M.

The present paper describes a first refinement of this model, which treats eqns. (4)–(6) of the preceding paper (Topping & Seilman, 1979) as a coupled set and allows the values of the equation parameters (k_{+1} , k_{+2} , k_{+3} , the concentrations of α - and β -sites on α_2 M and the concentration of α_1 A) and the values for

Abbreviations used: α_1 A, α_1 -antitrypsin; α_2 M, α_2 -macroglobulin; Bz-Arg-OEt, α -N-benzoyl-L-arginine ethyl ester.

the hydrolytic activity (relative to free trypsin) of trypsin (substrate Bz-Arg-OEt) when bound in the α -mode (r_α) or β -mode (r_β) to α_2 M or when bound to α_1 A (r_γ) to fluctuate so as to provide optimal fit of theory to experiment.

Experimental

Materials and methods

The materials and kinetic procedures are described in the preceding paper (Topping & Seilman, 1979).

Mathematical procedures

The reaction eqns. (4)–(6) of the preceding paper (Topping & Seilman, 1979) form a set of coupled first-order non-linear differential equations with six unknown parameters, the initial concentrations of the three types of inhibitory site and their corresponding specific rate constants for binding to trypsin. Once these equations are solved, a further three parameters (r_α , r_β and r_γ) are needed to calculate the hydrolytic activity of trypsin in its various states, which can then be compared, for any chosen value of serum volume, with the corresponding experimental observation. The object of the mathematical procedures is to establish the values of these parameters for any given blood sample.

The four-straight-line model described in the preceding paper (Topping & Seilman, 1979) effectively decouples the equations by assuming infinite separation between the rate constants, and thus allows independent solution of the equations. Such a procedure provides initial estimates of the concentrations of inhibitory sites from the volumes of serum corresponding to each discontinuity in the slope of the experimental curve. Initial estimates for the residual activity factors are also obtained by extrapolating each segment of the 'curve' back to the ordinate.

By using these initial estimates it is possible to begin an iterative procedure to determine the values of the nine unknown parameters for any given blood sample. The differential equations are solved for a range of serum volumes by using standard Runge-Kutta methods with time-step lengths adjusted to take account of the different reaction rates. A reaction is considered complete when the concentration of one reactant has fallen to less than one-millionth of the original concentration. On completion of the reactions the values of hydrolytic activity are calculated and compared with the experimental values giving an error vector, each element of which corresponds to a particular serum volume. By following Powell's (1964) minimization technique the values of the parameters are adjusted, and the iteration is repeated until the error vector reaches a minimum in the least-squares sense. Although it is not possible to prove that this is the only minimum, a second mini-

mum has not been found, even though the numerical procedure has been subjected to very large perturbations of the initial estimates.

Results and Discussion

A basic assumption of the four-straight-line model for the interactions between trypsin and human serum is that the linearity of the segments of the profile indicates a wide separation of rate constants between competing processes. A consequence of this assumption is that attainment of the plateau region of the profile corresponds to total capture of enzyme by the α -sites of α_2 M (Topping & Seilman, 1979). Previous work has suggested that, under conditions similar to those used in the present work, only approx. 40% of trypsin is captured by α_2 M to give a complex in which 80% of the tryptic activity is preserved, while the remaining trypsin is bound to other inhibitors (Ganrot, 1966, 1967). The results of imposing such constraints on our model are shown in Table 1 and Fig. 1. If the parameters of eqns. (4)–(6) of the preceding paper (Topping & Seilman, 1979) and the values for r_α , r_β and r_γ are allowed to vary freely so as to provide optimum fit of model to experiment, the results (column A, Table 1) indicate a good correspondence (Fig. 1; line A is theoretical, open circles are experimental). The single constraint that the complex between α_2 M and trypsin retains 80% tryptic activity results in optimum values of the remaining parameters, as in column B (Table 1), which generate line B (Fig. 1). An additional constraint that binding in the β -mode is non-existent (i.e. that α_2 M binds trypsin in the currently accepted fashion) leads to a further perturbation as shown in column C (Table 1) and line C (Fig. 1). Clearly neither constraint permits an acceptable fit to the observed data and continuous curvature inevitably results from severe competition between inhibitors for available enzyme. A slight curvature frequently observed (experimentally) between linear segments of a profile is, however, closely reproduced by the computer simulation based on the optimization procedures, and suggests that under the conditions of our experiments effective competition between inhibitory sites for enzyme is at most marginally significant.

Table 2 contains a summary of the data produced by computer optimization of the competing processes represented by eqns. (4)–(6) of the preceding paper (Topping & Seilman, 1979) and, for each sample of serum, 21 experimentally determined rate measurements of the activity of trypsin in free and bound states. No boundary limits or restrictions of any kind were imposed on the optimization procedures. The following conclusions may be drawn from inspection of these results.

Table 1. Optimization of models for interactions between trypsin and human serum with experimental results

Column A contains the results obtained by allowing the computer complete freedom in selecting values for the listed parameters, so as to provide optimal fit to experiment. When this process is repeated with a constraint imposed on r_α (column B) or with constraints imposed on binding in the β -mode and on r_α (column C), the fit is impaired (Fig. 1, lines A, B and C). P_A , P_B and P_C are here represented as the percentages of trypsin bound to α_2M (α - and β -sites) and to α_1A respectively at serum volumes 0.25 ml and 0.40 ml (Fig. 1). The quantities $[\alpha_2M_{\alpha(1,2)}]$ and $[\alpha_2M_{\beta(1,2,\dots,n)}]$ represent the concentrations (μ equiv.) of α - and β -sites on α_2M respectively, where one equivalent is capable of binding 1 mol of trypsin.

	A	B	C
	No constraints	One constraint*	Two constraints*
$[\alpha_1A]$ (μM)	43.9	51.98	51.56
$[\alpha_2M_{\alpha(1,2)}]$ (μ equiv.)	6.52	2.40	12.5
$[\alpha_2M_{\beta(1,2,\dots,n)}]$ (μ equiv.)	19.94	14.9	0*
r_α	0.47	0.80*	0.80*
r_β	0.24	0.38	—
r_γ	0.00	0.00	0.00
k_{+1}/k_{+3}	5×10^3	51.7	5.7
k_{+2}/k_{+3}	10^2	14.6	—
P_A at serum volume (ml)			
0.25	90.5	26.2	55.4
0.40	92.6	29.2	56.2
P_B at serum volume (ml)			
0.25	9.3	58.4	—
0.40	7.2	56.7	—
P_C at serum volume (ml)			
0.25	0.2	15.4	44.6
0.40	0.2	14.1	43.8

Fig. 1. Computer simulation of a partition profile by using the models of Table 1

Optimization of the parameters of eqns. (4)–(6) of the preceding paper together with r_α , r_β and r_γ (Table 1, column A) leads to good correspondence between theory (line A) and experiment (open circles). Models that invoke more nearly equipartition of trypsin between α_1A and α_2M , or which do not recognize binding in the ' β -mode', lead to continuous curvature and provide a less satisfactory simulation of experiment (lines B and C).

(1) Every sample of human serum examined to date falls into one of seven categories on the basis of the ratio of trypsin bound to α_2M in the α -mode to that bound in the β -mode, when all binding sites on α_2M are occupied. The ratio is invariably close to integral (Table 2, column 4, and Fig. 2) and the seven categories correspond to nominal ratios of 1:1, 1:2, 1:3, 1:4, 1:5, 1:6 and 1:7. A number of reports of the existence of different molecular varieties of α_2M have appeared in the literature, although the inherent instability of α_2M , for example during preparation, freezing and storage (Haverback *et al.*, 1962; Lebreton de Vonne *et al.*, 1972), is recognized as a serious obstacle to the correct interpretation of the various varieties and fragments that have been observed in different laboratories. Electrophoresis on polyacrylamide gel indicated at least five such varieties (Saunders *et al.*, 1971), and crossed antigen–antibody electrophoresis showed that the partition of α_2M molecules with different migration rates varied from serum to serum, especially during disease (Ganrot & Laurell, 1966).

(2) Concentrations of α_2M are calculated by assuming a maximal binding in the plateau region of a profile of two molecules of enzyme per molecule of α_2M . Only by invoking this idea can the relative trypsin-binding capacities of α_1A and the α -sites on α_2M (approx. 6.5:1), which result from optimization, be reconciled with the accepted average molar ratio in human serum of α_1A and α_2M (approx. 13:1). This result does not depend on the degree of activity of the enzyme preparations used, provided that α_1A and $\alpha_2M_{\alpha(1,2)}$ show the same discrimination in

Table 2. *Computer analysis of partition profiles*

Significant characteristics of human serum derived for each sample from the optimization of the hydrolytic activity of trypsin in free and bound states at 21 different trypsin/serum ratios with the theory, based on eqns. (4)–(6) of the preceding paper (Topping & Seilman, 1979), of the partition of trypsin between the serum inhibitors. As the 'best fit' of theory to experiment was found to be not significantly affected by small variations in the specific rate constants, a fixed proportion of $k_{+1}/k_{+2}/k_{+3}$ of $5 \times 10^3:10^2:1$ was used throughout the Table. The serum samples, classified for convenience by reference to the ratio of β - to α -sites on α_2 M, were drawn roughly equally from colleagues and from a variety of hospital sources.

Serum sample	α_1 A (μ M)	α_2 M (μ M)	β -sites/ α -sites	r_α	r_β	r_γ
30	27.60	3.39	1.08	0.30	0.19	0.07
52	32.97	3.12	1.00	0.375	0.27	0.14
89	62.50	5.13	1.02	0.33	0.24	0.14
44	9.90	4.17	1.01	0.66	0.49	0.39
			Mean 1.03			
			S.D. 0.04			
22	47.39	5.73	2.14	0.37	0.13	0.05
34	66.14	2.94	1.95	0.41	0.245	0.065
35	46.87	2.71	1.88	0.31	0.19	0.05
50	54.74	2.86	1.91	0.40	0.29	0.09
B 5	48.43	2.34	1.89	0.38	0.20	0.03
B 7	36.51	2.97	2.01	0.44	0.28	0.06
B 8	41.66	2.99	1.98	0.46	0.35	0.06
B 9	44.63	3.05	1.91	0.49	0.21	0.02
B19	26.04	2.73	1.90	0.54	0.36	0.00
B22	19.06	2.79	1.99	0.41	0.16	0.00
B29	16.56	2.55	1.88	0.48	0.20	0.00
B30	34.37	2.60	1.97	0.44	0.16	0.00
B13	27.60	2.34	2.00	0.55	0.13	0.05
B10	38.54	2.99	2.00	0.55	0.28	0.05
B17	33.33	2.76	2.01	0.56	0.26	0.05
B25	43.75	3.12	2.00	0.56	0.22	0.05
B11	67.70	2.50	2.13	0.22	0.12	0.04
B15	38.90	2.66	2.14	0.57	0.18	0.02
40	37.50	4.04	2.10	0.35	0.18	0.09
48	64.11	3.12	2.08	0.31	0.16	0.00
56	38.49	4.22	1.97	0.29	0.18	0.03
62	47.39	3.91	2.07	0.305	0.19	0.06
65	55.57	4.69	1.96	0.36	0.20	0.05
76	58.33	4.11	2.04	0.215	0.15	0.03
88	44.68	2.94	2.02	0.32	0.17	0.055
115	72.23	4.61	2.01	0.325	0.185	0.08
B 2	10.99	1.95	2.00	0.67	0.52	0.34
B26	5.89	2.08	2.00	0.55	0.46	0.20
L 3	68.75	2.79	2.01	0.54	0.19	0.08
64	51.82	3.67	1.96	0.55	0.32	0.06
			Mean 2.00			
			S.D. 0.07			
B 1	43.23	2.34	3.11	0.51	0.20	0.02
B 6	43.23	2.50	2.84	0.42	0.13	0.03
B27	31.25	2.34	3.00	0.41	0.15	0.02
B21	29.16	2.55	3.08	0.485	0.17	0.00
B18	28.64	2.08	3.00	0.45	0.23	0.075
42	72.91	3.75	3.16	0.31	0.19	0.05
46	38.17	3.91	3.00	0.335	0.10	0.01
L 1	36.47	2.60	3.00	0.46	0.12	0.05
L 2	43.23	3.39	3.00	0.46	0.15	0.05
24	31.77	3.20	2.90	0.54	0.25	0.06
25	39.58	2.86	2.73	0.41	0.20	0.05
28	37.50	3.12	3.00	0.37	0.22	0.06
29	35.94	4.43	3.00	0.38	0.125	0.00
31	41.66	2.60	3.00	0.40	0.26	0.03

Table 2—continued

Serum sample	α_1A (μM)	α_2M (μM)	β -sites/ α -sites	r_α	r_β	r_y
32	37.50	3.12	3.00	0.46	0.24	0.06
33	37.50	3.10	3.03	0.35	0.17	0.04
S 1	31.82	3.05	3.10	0.50	0.155	0.07
S 2	34.06	2.86	2.96	0.45	0.17	0.05
36	60.41	2.81	3.05	0.46	0.28	0.08
39	29.16	2.55	3.08	0.53	0.20	0.09
41	28.70	4.19	3.03	0.35	0.18	0.02
51	43.23	2.37	3.05	0.42	0.245	0.03
53	43.90	3.26	3.06	0.47	0.24	0.00
54	32.91	3.41	2.90	0.31	0.16	0.00
68	55.73	3.46	3.00	0.48	0.21	0.00
67	25.99	2.97	3.04	0.315	0.135	0.00
73	41.66	5.00	3.17	0.35	0.12	0.03
70	58.33	5.08	3.10	0.40	0.16	0.025
72	46.40	3.15	2.97	0.35	0.21	0.07
82	20.31	3.67	3.05	0.425	0.29	0.05
83	25.00	3.15	2.97	0.415	0.275	0.04
84	25.00	3.12	3.00	0.34	0.18	0.07
86	21.14	3.10	3.09	0.42	0.215	0.03
87	21.30	3.15	2.97	0.395	0.22	0.08
91	20.47	3.15	2.97	0.37	0.12	0.05
103	44.42	2.94	3.25	0.365	0.20	0.11
114	60.62	2.81	3.04	0.34	0.22	0.07
BR 2	29.16	2.50	3.17	0.57	0.27	0.08
B16	62.50	2.71	2.83	0.50	0.12	0.05
126	31.14	3.02	3.10	0.37	0.18	0.02
129	60.26	2.14	3.16	0.49	0.20	0.035
			Mean 3.02			
			S.D. 0.10			
18	36.47	2.60	4.00	0.42	0.22	0.09
26	42.18	2.08	3.91	0.39	0.21	0.05
27	25.94	2.42	3.97	0.34	0.16	0.09
37	41.66	2.08	4.00	0.41	0.21	0.05
38	25.00	2.79	3.99	0.34	0.17	0.04
43	29.06	2.73	4.09	0.30	0.15	0.03
49	17.24	2.50	4.18	0.40	0.16	0.02
55	37.50	2.47	4.04	0.38	0.22	0.03
71	34.11	2.25	4.05	0.37	0.20	0.05
74	24.63	2.08	4.00	0.34	0.125	0.02
97	27.76	2.86	3.84	0.34	0.15	0.00
110	34.74	2.81	3.94	0.33	0.185	0.02
112	31.40	2.47	3.95	0.315	0.13	0.00
119	43.64	2.66	4.22	0.35	0.15	0.06
124	22.24	2.68	4.18	0.315	0.165	0.00
127	34.74	2.73	4.18	0.43	0.25	0.01
131	47.65	3.52	4.07	0.30	0.145	0.015
B4/132	38.17	2.24	4.07	0.43	0.18	0.055
134	60.41	2.24	4.12	0.32	0.12	0.05
136	40.05	3.18	3.93	0.38	0.18	0.05
111	39.79	2.21	4.13	0.44	0.195	0.06
			Mean 4.04			
			S.D. 0.10			
19	37.50	2.86	5.00	0.49	0.24	0.00
47/9/77	46.87	2.08	4.94	0.60	0.31	0.03
47/8/76	60.93	1.93	4.91	0.55	0.22	0.07
96	22.08	2.37	4.88	0.39	0.14	0.015
107	55.57	2.34	4.92	0.49	0.26	0.06
133	88.64	2.14	4.85	0.44	0.19	0.10

Table 2—continued

Serum sample	α_1A (μM)	α_2M (μM)	β -sites/ α -sites	r_α	r_β	r_γ
135	52.60	2.63	4.85	0.46	0.19	0.03
104	29.58	2.66	5.20	0.39	0.20	0.05
			Mean 4.94			
			S.D. 0.11			
69	7.92	1.95	6.07	0.43	0.20	0.12
108	37.55	2.11	5.91	0.39	0.18	0.05
116	22.24	1.95	6.10	0.33	0.09	0.00
118	34.74	1.98	6.01	0.34	0.14	0.075
128	70.57	2.11	5.96	0.33	0.115	0.01
130	38.44	1.72	5.98	0.535	0.225	0.10
			Mean 6.01			
			S.D. 0.07			
106	54.79	1.98	6.89	0.53	0.32	0.05
	Mean 39.50	Mean 2.92				
	S.D. 15.26	S.D. 0.76

Fig. 2. Classification of human α_2M by β -sites/ α -sites ratio. A plot is shown of the concentration of β -sites against the concentration of α -sites on α_2M for each of 110 samples of human serum. The lines D, E, F, G, H, J and K have slopes of 1, 2, 3, 4, 5, 6 and 7 respectively.

binding various forms of the enzyme, e.g. when both α_1A and $\alpha_2M_{\alpha(1,2)}$ bind active forms of the enzyme and both fail to bind inactive forms. The significance of this result, that under appropriate conditions the

molar binding ratio of trypsin to α_2M may be 2:1, has been discussed in the context of the widely contested debate on such molar ratios (Topping & Seilman, 1979).

(3) Although the molar binding ratio of trypsin to α_2M discussed above does not depend on the activity of the enzyme preparations used, the absolute values of α_2M and of α_1A quoted in Table 2 do depend on the trypsin activity. Although this activity will be inevitably somewhat less than 100%, the assumption of full activity yields values for concentrations of α_1A and α_2M that when averaged ($[\alpha_1A]=39.50\mu M$; $[\alpha_2M]=2.92\mu M$) show good correspondence to the results of immunochemical assay ($[\alpha_1A]=36\mu M$; $[\alpha_2M]=2.8\mu M$) (Laurell, 1974).

(4) In the present paper, discussion of the activities of trypsin when bound to α_2M in the α - and β -modes (relative to free trypsin) will be limited to the observation that the former is invariably greater than the latter, whatever the ratio of β -sites to α -sites. The activity of trypsin when bound to α_1A is usually (but not always) virtually zero. The values (r_γ) quoted in Table 2 may include also any contribution to hydrolytic activity from background other than serum components, and as a result only values of r_γ greater than 0.05 should be regarded as significant. When, as is occasionally observed, the serum itself possesses hydrolytic activity towards the substrate Bz-Arg-OEt, a compensating correction is applied to the profile before computer analysis.

Molecular interpretations of binding of enzyme to α_2M in α - and β -modes

The very considerable amount of work published on the molar binding ratios of proteinases and α_2M (Topping & Seilman, 1979, and references cited

therein), in which ratios of 1:1, 2:1 and 3:1 have been proposed, frequently in contradiction, appears in marked contrast with the present work, in which kinetic evidence suggests that up to 16 molecules of trypsin may, under suitable conditions, be associated with a single molecule of α_2 M. As yet, we have no explanation for the apparent failure to recognize, by isolation, complexes in which proteinase is bound to α_2 M in what we have termed the β -mode. However, the potential metastability of such states has been a primary reason for our concern to examine the whole serum by using non-destructive kinetic methods rather than subject the complex mixtures of sensitive materials to isolation procedures. Attempts to relate such extraordinary stoichiometry to some kind of structural ensemble must accommodate the following (albeit limited) information derived from analysis of the partition profiles.

(a) The ratio of α -sites to β -sites on α_2 M is always integral and varies from 1:1 to 1:7.

(b) All α_2 M possesses two α -sites per molecule.

(c) Both α -sites on α_2 M are occupied by trypsin in preference to any β -site.

(d) Although trypsin invariably shows a greater binding affinity to an α -site than to a β -site, its ability to hydrolyse the substrate Bz-Arg-OEt is more effectively inhibited by association with the β -site than the α -site ($r_\alpha > r_\beta$).

(e) The two α -sites on a molecule of a given type of α_2 M inhibit the enzyme to the same degree and/or have the same rates of acquisition of enzyme. The same is true of the group of β -sites, provided that all the sites function independently. At least one of these conditions must obtain in order that the four-straight-line model can provide an acceptable correspondence with the experimental data.

A purely schematic description of the binding of enzyme to α_2 M, which attempts to reconcile factors (a)–(e) above, may be based on an arrangement of molecules of the enzyme into two independent rows on the 'surface' of the inhibitor. One end of each row is anchored to the α_2 M structure and subsequent members of a row interact with both the preceding member and with the inhibitor matrix. The concept of α -site or binding in the α -mode then translates to binding in the terminal position and β -site is equivalent to non-terminal binding. Such an arrangement inevitably requires the enzyme molecule being bound to occupy an α -site in preference to a β -site, as only terminal positions can be occupied. This process takes place with equal readiness from either α -site on α_2 M and, in some species of α_2 M, is capable of linking up to eight molecules of trypsin into a row. As the terminal position of a row may be considered to be more 'exposed' than any position within the row, then the higher activity of α -sites when bound to a terminal position (to an α -site) would be not unreasonable. The four-straight-line model is

compatible with such an arrangement, provided that for a given species of α_2 M all enzyme bound in terminal positions is equally capable of hydrolysing the substrate, and enzyme bound in any other position also shows equivalent (but lower) activity. As in this scheme the β -sites are no longer functionally independent, the second condition permitted under (e) above, that the β -sites have the same rates of acquisition of enzyme, no longer applies.

The 'trap' hypothesis for binding of enzyme to α_2 M, which was proposed in the belief that α_2 M binds trypsin in a 1:1 molar ratio (Barrett & Starkey 1973), cannot be easily adapted to explain multiple binding to trypsin. Even if the 'trap', once sprung, could be reset by regeneration of the susceptible peptide-link 'trigger', the idea of incorporating a large number of trypsin molecules within the matrix of the α_2 M molecule seems less plausible than an association of the enzyme with the surface of α_2 M in such a way as to provide varying access of substrates and inhibitors, subject to size, by diffusion to the active site of the bound enzyme.

In summary, the results detailed in Table 2 and Fig. 2 provide unequivocal evidence of a recognizable pattern in the complex multiple interactions between trypsin and human serum, which allows the classification of human α_2 M into seven distinctive categories. The molecular interpretation of this kinetic behaviour is open to speculation. One possibility has been outlined above, but clearly further work is needed to resolve this aspect of the problem. The lack of a complete mechanistic understanding of the inhibitory capabilities of human serum, however, has not prevented empirical correlation of selected characteristics of the partition profiles (in particular the α/β ratios) with a variety of pathological disorders (B. G. Rigden, C. W. G. Turton, S. Whiting, A. H. Craven & R. M. Topping, unpublished work). The results strongly indicate that the analysis of enzyme-inhibitor interactions, as described in the present paper, may prove of significant clinical value.

We thank Dr. B. G. Rigden and Dr. C. W. G. Turton for co-ordinating clinical aspects of the work and Ms. Susan Whiting for excellent technical assistance.

References

- Barrett, A. J. & Starkey, P. M. (1973) *Biochem. J.* **133**, 709–724
- Fritz, H. & Tschesche, H. (eds.) (1971) *Proc. Int. Res. Conf. Proteinase Inhibitors*, Walter de Gruyter, New York
- Fritz, H., Tschesche, H., Greene, L. J. & Truscheit, E. (eds.) (1974) *Bayer Symp. 5th: Proteinase Inhibitors* Springer-Verlag, Berlin
- Ganrot, P. O. (1966) *Clin. Chim. Acta* **13**, 518–521
- Ganrot, P. O. (1967) *Ark. Kemi* **26**, 577–582
- Ganrot, P. O. & Laurell, C.-B. (1966) *Clin. Chim. Acta* **14**, 137–138

- Haverback, B. J., Dyce, B., Bundy, H. F., Wirtschafter, S. K. & Edmondson, H. A. (1962) *J. Clin. Invest.* **41**, 972-980
- Laurell, C.-B. (1974) *Protides Biol. Fluids Proc. Colloq.* **22**, 3-12
- Lebreton de Vonne, T., Mouray, H., Berthillier, G. & Got, R. (1972) *Biochim. Biophys. Acta* **257**, 365-371
- Mittman, C. (ed.) (1972) *Pulmonary Emphysema and Proteolysis*, pp. 141-144, Academic Press, New York and London
- Powell, M. J. D. (1964) *Comput. J.* **7**, 155-162
- Reich, E., Rifkin, D. B. & Shaw, E. (eds.) (1975) *Cold Spring Harbor Conf. Cell Proliferation: Proteases and Biological Control*, vol. 2
- Ribbons, D. W. & Brew, K. (eds.) (1976) *Miami Winter Symp.* **11**
- Saunders, R., Dyce, B. J., Vannier, W. E. & Haverback, B. J. (1971) *J. Clin. Invest.* **50**, 2376-2383
- Topping, R. M. & Seilman, S. (1979) *Biochem. J.* **177**, 493-499