2263.
Kassner, R. J. (1973), *J. Am. Chem. Soc. 95,* 2674.
Perutz, M. F. (1972), *Nature (London) 237,* 495.
Perutz, M. F., Heidner, E. J., Ladner, J. E., Beetlestone, J. G., Ho, C., and Slade, E. F. (1974a), *Biochemistry 13,* 2187.
Perutz, M. F., Ladner, J. E., Simon, S. R., and Ho, C. (1974b), *Biochemistry 13,* 2163.
Riddick, J. A., and Bunger, W. B. (1970), *Tech. Chem. (N.Y.) 1,* 567.
Rougee, M., and Brault, D. (1973), *Biochem. Biophys. Res. Commun. 53,* 1364.
Stynes, D. V., and James, B. R. (1974), *J. Am. Chem. Soc. 96,* 2733.
Wang, J. H., Nakahara, A., and Fleisher, E. B. (1958), *J. Am. Chem. Soc. 80,* 1109.
Zerner, M., Gouterman, M., and Kobayashi, H. (1966), *Theor. Chim. Acta 6,* 363.

Studies on Self-Association of Proteins. Self-Association of α-Chymotrypsin at Its Isoelectric Point in Buffer Solutions of Ionic Strength 0.1†

M. W. Pandit and M. S. Narasinga Rao*,‡

ABSTRACT: The self-association of α-chymotrypsin at its isoelectric point has been studied in two buffer solutions of μ (ionic strength) = 0.1: phosphate buffer (pH 6.9) and Tris buffer (pH 8.3). The weight-average molecular weight (by the Archibald method) and sedimentation coefficient were determined as a function of protein concentration. The molecular weights measured were the same in both the buffers. In sedimentation velocity experiments unimodal peaks were obtained at all the protein concentrations. The molecular weight data could be fitted to a nonideal indefinite self-association equilibrium or a hexamerization equilibrium with all the intermediate species coexisting. The sedimentation data could be fitted to an octamerization equilibrium.

The self-association of α-chymotrypsin (EC 3.4.4.5) has been studied extensively (see Pandit and Rao, 1974a, for earlier references). In buffer solutions of μ = 0.2[1] and above association proceeds essentially to dimerization or trimerization (Steiner, 1954; Egan et al., 1957; Rao and Kegeles, 1958; Winzor and Scheraga, 1964). On the other hand, at its isoelectric point in buffer solutions of μ = 0.05, extensive association occurs (Massey et al., 1955; Nichol and Bethune, 1963; Pandit and Rao, 1974a). Sedimentation velocity data under these conditions fit a monomer–hexamer equilibrium (Gilbert, 1955, 1959; Pandit and Rao, 1974a). However, the molecular weight data by the Archibald method can be best described by an indefinite self-association equilibrium (Pandit and Rao, 1974a).

The self-association of α-chymotrypsin at acid pH values and μ = 0.1 has been studied by the light scattering method (Steiner, 1954). Essentially dimerization was observed. A decrease in pH or increase in ionic strength favored association. However, no measurements have been reported at μ = 0.10 at the isoelectric point of the protein. In this investigation the self-association of α-chymotrypsin has been studied at μ = 0.1 at its isoelectric point, by measuring the weight average molecular weight and the sedimentation coefficient as a function of protein concentration.

This enzyme has an isoelectric point of pH 8.3 in uni-univalent buffers (Anderson and Alberty, 1948; Rao and Kegeles, 1958). However, its isoelectric point in phosphate buffer of μ = 0.20 is pH 6.2 and this increases to pH 6.9 in phosphate buffer of μ = 0.10 (Rao and Kegeles, 1958).

In this investigation two buffer solutions were used, phosphate buffer of pH 6.9 and μ = 0.10 and Tris buffer of pH 8.3 and μ = 0.10. It has been reported that in Tris buffer of pH 8.3 and μ = 0.05, considerable autolysis of the protein occurs (Pandit and Rao, 1974a). However, no such autolysis was observed in Tris buffer solution of μ = 0.10.

Materials and Methods

α-Chymotrypsin. Worthington α-chymotrypsin, 3× crystalline, CDI 7-JC, was used without further purification.

Chemicals. The chemicals used were guaranteed reagent grade or chemically pure grade.

Archibald Molecular Weight. The molecular weight measurements were made with a Spinco Model E ultracentrifuge equipped with schlieren optics and a RTIC unit. Solutions prepared in phosphate buffer were dialyzed in the cold for 12 hr. However, for measurements in Tris buffer the protein was directly dissolved in the buffer solution and used. For the false bottom, 0.1 ml of fluorocarbon oil (FC 43) was used. The temperature was maintained at 25 ± 1° with the RTIC unit. The experimental details were the same as described earlier (Pandit and Rao, 1974a).

Sedimentation Velocity. The measurements were made at 25 ± 1°. From the pictures taken at different intervals of centrifugation, the sedimentation coefficient was calculated by determining the movement of the second moment of the

† From the Regional Research Laboratory, Hyderabad 500009, India. *Received January 20, 1975.* This is part III in the series Studies on Self-Association of Proteins.

‡ Present address: Protein Technology Discipline, Central Food Technological Research Institute, Mysore 570013, India.

[1] Abbreviations used are: Tris, 2-amino-2-hydroxymethylpropane-1,3-diol; μ, ionic strength.

boundary (Goldberg, 1953) and reducing it to $s_{20,w}$.

Partial Specific Volume. A value of 0.736 was used (Schwert and Kaufman, 1951).

Protein Concentration. This was determined spectrophotometrically using a value of 20.6 for $E_{280\,nm}^{1\%}$ (Pandit and Rao, 1974a).

Buffer Solution. Phosphate buffer of pH 6.9 and μ = 0.10 was used. Tris buffer of pH 8.3 and 0.02 M was prepared and the ionic strength made to 0.10 by the addition of KCl.

Results and Discussion

The molecular weight was calculated from both the top and bottom meniscus. Pictures were taken at 15, 30, and 45 min after the attainment of the operating speed. Since the speeds used were low there was not much variation in the molecular weight values obtained at different time intervals; the values from the top and bottom meniscus also did not differ significantly. Therefore, in each experiment an average value was calculated.

The apparent weight-average molecular weight, $M_{w(a)}$, obtained at several protein concentrations, C, is given in Figure 1; C has been expressed on a grams per liter scale. $M_{w(a)}$ increased with C suggesting that self-association occurred under the experimental conditions. The highest molecular weight was about 10×10^4. Extrapolation of $M_{w(a)}$ vs. C data to $C \rightarrow 0$, to obtain the monomer molecular weight, was a little uncertain. However, a linear extrapolation gave a value of 23,000–25,000, in good agreement with the reported values (Rao and Kegeles, 1958).

The values of $M_{w(a)}$ obtained in phosphate and Tris buffers could be fitted to the same curve. There were no significant differences. Under the experimental conditions used variation in pH and the nature of the buffer salt did not influence association.

$M_{w(a)}$ vs. C data were analyzed to determine the nature of association and to evaluate the equilibrium constant(s). For these analyses the value of monomer molecular weight is needed. In analogy with the previous calculations a value of 23,000 was used (Rao and Kegeles, 1958; Pandit and Rao, 1974a). The following types of analyses were made.

Ideal Monomer–n-Mer Equilibrium. The simplest type of association would be an ideal monomer–n-mer equilibrium without any intermediate species present in the equilibrium mixture. For this analysis the equation of McKenzie et al. (1967) was used:

$$\log K_d^n = \log n + (n-1)\log(C/M_1) - (n-1)\times \log(nM - M_1) + n\log(nM_1 - M_w) - \log(M_w - M_1) \quad (1)$$

where K_d^n is the dissociation constant on a molar scale, n is the value of the n-mer, C is the concentration in grams per liter, M_1 is the molecular weight of the monomer, and M_w is the measured molecular weight at C. When the right-hand side of eq 1 is plotted against C, it should yield a straight line parallel to the x axis, if n has been correctly chosen. Then the intercept would be log K_d^n. Such plots for values of n, from 4 to 7, are given in Figure 2. None of the plots could be considered linear; the ideal monomer–n-mer equilibrium thus did not fit the data.

Nonideal Monomer–n-Mer Equilibrium. Several equations are available to test this model (Adams, 1967). The following equation was used (Adams, 1965):

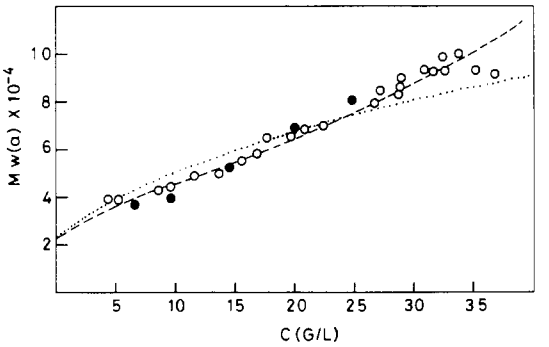

FIGURE 1: Variation of weight-average molecular weight with protein concentration: (○) phosphate buffer (pH 6.9) and μ = 0.10; (●) Tris buffer (pH 8.3) and μ = 0.10; (- - -) curve calculated with $4K$ = 0.259 and BM_1 = −0.0025; (· · ·) curve calculated with $4K$ = 0.381 and BM_1 = 0.

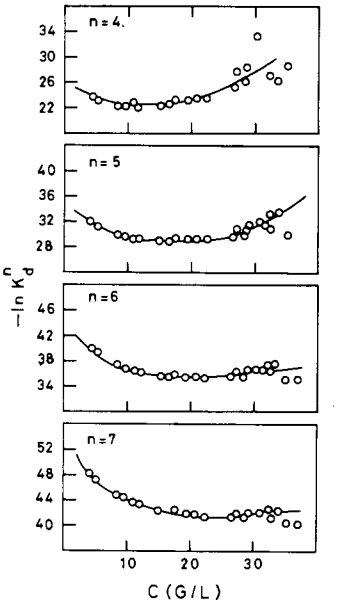

FIGURE 2: Plots of $-\ln K_d^n$ vs. C.

$$n = \left[\frac{1}{(M_1/CM_{w(a)}) - BM_1} - C_1\right] \Big/ (C - C_1) \quad (2)$$

where BM_1 is the nonideal term and C_1 is the concentration of the monomer; the other terms have the same meaning as in eq 1. Adams and Williams (1964) have shown that:

$$C = \alpha \exp(-BM_1 C) \quad (3)$$

where α is the monomer concentration not corrected for nonideality. α was obtained from the $M_{w(a)}$ vs. C data by the method of Steiner (1952). Using different values of BM_1, positive, zero, and negative, the right-hand side of eq 2 was calculated. None of the plots gave a straight line with zero slope. Thus, the nonideal monomer–n-mer hypothesis also did not fit the data.

Ideal or Nonideal Indefinite Self-Association Equilibrium. Of the various equations available to test this model (Adams, 1967; Van Holde and Rossetti, 1967; Chun et al., 1972), the equation of Van Holde and Rossetti (1967) was used since it can use the experimental $M_{w(a)}$ vs. C data directly and does not need derived quantities such as $M_1/M_{n(a)}$, etc. $M_{n(a)}$ is the apparent number-average molecular weight. The equation of Van Holde and Rossetti (1967) is:

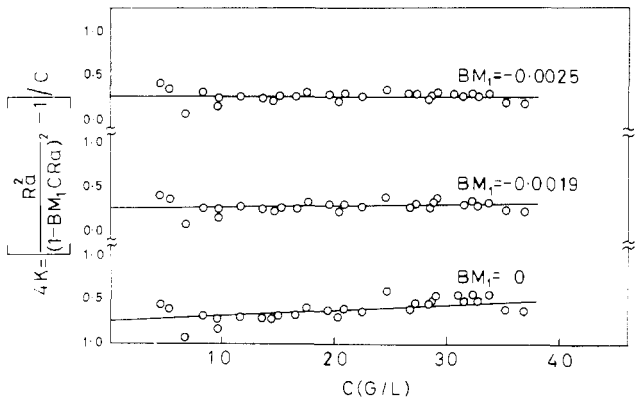

FIGURE 3: Plots of $\{[R_a^2/(1 - BM_1CR_a)^2] - 1\}/C$ vs. C.

$$4K = \left[\frac{R_a}{(1 - BM_1CR_a)^2} - 1\right]\Big/C \qquad (4)$$

where K is an intrinsic association constant and $R_a = M_{w(a)}/M_1$. For the correctly chosen value of BM_1, the right-hand side of eq 4 would give a line with zero slope and intercept equal to $4K$.

Such plots for various values of BM_1 are given in Figure 3. The plot of $BM_1 = -0.0025$ gave a line with zero slope. Other plots had a slope. In the case of $BM_1 = -0.0025$ the intercept ($4K$) was 0.259.

Using the values of $4K$ and BM_1, $M_{w(a)}$ was calculated as a function of C using eq 4. The calculated curve along with the experimental data are given in Figure 1. The fit between the two was good. A similar curve calculated with $BM_1 = 0$ and $4K = 0.381$ did not fit the data satisfactorily. The standard deviation for the case $BM_1 = -0.0025$ was 0.0519 and for $BM_1 = 0$ it was 0.0984, indicating that the former gave a better fit with the experimental data. To facilitate comparison with the discrete self-association model to be discussed later the standard deviation of $M_{w(a)}/M_1$ vs. C data rather than that of $M_{w(a)}$ vs. C data was calculated.

Chun et al. (1972) have suggested a graphical method to evaluate both K and BM_1 and they applied the method successfully to the self-association of bovine glutamic dehydrogenase. Similarly, Tang and Adams (1973) have used this method in their study of β-lactoglobulin self-association. The method needs the quantity $M_1/M_{n(a)}$, which is obtained by graphical integration of $M_1/M_{w(a)}$ vs. C data. Although $M_1/M_{w(a)}$ at $C \rightarrow 0$ is 1, there was considerable uncertainty about the way the curve could be drawn in the region $C < 5$ g/l.; this uncertainty was reflected in the values of $M_1/M_{n(a)}$ obtained. When all the $M_1/M_{n(a)}$ and $M_1/M_{w(a)}$ values were used for plotting two straight lines were obtained. However, if the data below $C = 10$ g/l. were ignored, a single straight line with a slope of -0.003 was obtained. This value of BM_1 agreed reasonably well with the values obtained by the method of Van Holde and Rossetti (1967).

Thus, nonideal self-association equilibrium with a negative value of BM_1 fitted the data satisfactorily. The significance of the negative nonideal term was not obvious. Hancock and Williams (1969) observed that a negative BM_1 value was needed to describe $M_{w(a)}$ vs. C data of chymotrypsinogen A self-association.

Discrete Self-Association Equilibrium. When self-association occurs up to n-mers with the intermediate species co-existing in the equilibrium mixture the following equation can be used:

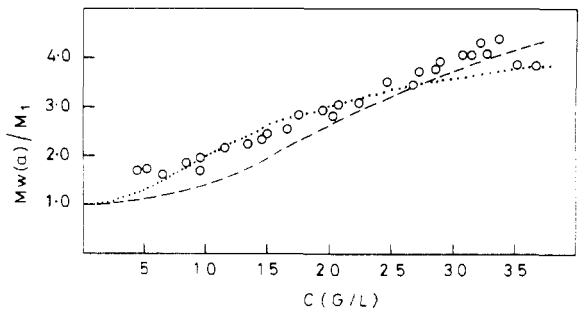

FIGURE 4: Variation of $M_{w(a)}/M_1$ with C: (O) experimental data; (• • •) curve calculated for $n = 6$ with K_n values given in Table I; (- - -) curve calculated for $n = 8$ with K_n values given in Table I.

$$\frac{M_{w(a)}}{M_1} = \frac{C_1 + 2K_2C_1^2 + 3K_3C_1^3 + \ldots + nK_nC_1^n}{C_1 + K_2C_1^2 + K_3C_1^3 + \ldots + K_nC_1^n} \qquad (5)$$

where C_1 is the monomer concentration and K_2, K_3, and K_n are association constants for the formation of dimer, trimer, and n-mer from the monomer. For the nonideal case, $C_1 = \alpha \exp(-BM_1C)$.

Inspection of Figure 1 indicated that the highest weight-average molecular weight obtained experimentally was approximately 10×10^4, which was 4–5 times the minimum molecular weight of 2.3×10^4. Thus, the equilibrium mixture should contain at least up to pentamers. Therefore, a value of $n = 6$ was chosen. Preliminary values of $K_2 \ldots K_6$ were obtained by the graphical procedure of Steiner (1952). These were refined by the process of iteration. For any one set of calculations five K values were held constant and the sixth was varied between certain limits. By this procedure, for the ideal case, a set of $K_2 \ldots K_6$ values was obtained (Table I) which gave the calculated $M_{w(a)}/M_1$ vs. C curve shown in Figure 4. The fit was not as good as in the case of nonideal indefinite self-association equilibrium; the standard deviation was 0.0947. However, over a fairly large range of concentration the fit was reasonably good. Thus, a hexamerization equilibrium appeared equally probable. The solution with $n = 6$ could not be considered as unique. Possibly other sets of n and K_n values would fit the data equally well. For this reason, analysis for the more complicated case of nonideal discrete self-association was not attempted.

Sedimentation Velocity Experiments. The self-association was studied by the sedimentation velocity method also. The patterns obtained at several protein concentrations are given in Figure 5. At all concentrations unimodal peaks were obtained. The patterns were, however, asymmetrical. Resolution into bimodal peaks was not observed; this contrasts with the behavior in buffer solutions of $\mu = 0.05$ (Massey et al., 1955; Nichol and Bethune, 1963; Pandit and Rao, 1974a).

The $\bar{s}$ value was determined as a function of protein concentration and is plotted as $\bar{s}/(s_1)_0$ vs. C in Figure 6; here $(s_1)_0$ is the sedimentation coefficient of the monomer at $C \rightarrow 0$. A value of 2.5 was used for $(s_1)_0$. The ratio $\bar{s}/(s_1)_0$ increased up to $C \sim 25$ g/l. and then decreased due to hydrodynamic effects. The profile was that of an associating protein system. The sedimentation coefficient data were analyzed to determine the self-association model. Since the peaks were unimodal at all concentrations a simple monomer-n-mer model was excluded (Gilbert, 1955). A monomer-n-mer model with coexisting intermediate species was chosen. The following equation was used to calculate $\bar{s}/(s_1)_0$ as a function of C (Gilbert and Gilbert, 1973):

Table I: Association Constants for Hexamerization or Octamerization Equilibrium.

n	K_2	K_3	K_4	K_5	K_6	K_7	K_8
6	4×10^{-2}	8×10^{-4}	4×10^{-4}	3×10^{-5}	1.5×10^{-5}	—	—
8	3×10^{-2}	6.75×10^{-4}	1.35×10^{-5}	2.53×10^{-7}	4.56×10^{-9}	7.97×10^{-11}	1.5×10^{-8}

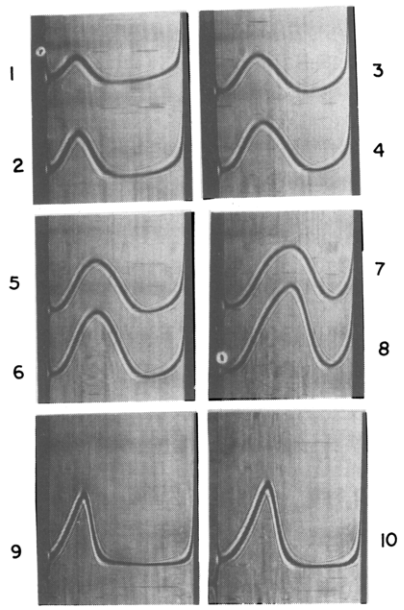

FIGURE 5: Sedimentation velocity patterns of α-chymotrypsin in phosphate buffer of pH 6.9 and ionic strength 0.1 at various protein concentrations. Bar angle and time after reaching maximum speed are given in parentheses; a 12-mm centerpiece was used in all cases except 9 and 10 in which a 3-mm centerpiece was used. Sedimentation proceeded from left to right: (1) 3.2 g/l. (50°, 54 min); (2) 6.0 g/l. (50°, 54 min); (3) 10.0 g/l. (55°, 72 min); (4) 14.0 g/l. (55°, 72 min); (5) 17.8 g/l. (60°, 63 min); (6) 21.5 g/l. (60°, 63 min); (7) 26.0 g/l. (60°, 76 min); (8) 33.5 g/l. (60°, 76 min); (9) 42.8 g/l. (60°, 35 min); (10) 50.4 g/l. (55°, 54 min).

$$\bar{s}/(s_1)_0 = (1 - gC) \sum_n [K_n C_1{}^n (n)^{2/3}]/C \qquad (6)$$

where $\bar{s}$ is the weight-average sedimentation coefficient at concentration C ($=\Sigma_n n K_n C_1{}^n$); $(s_1)_0$ is the sedimentation coefficient of the monomer at $C \rightarrow 0$; K_n is the association constant for the formation of n-mer from the monomer; C_1 is the monomer concentration; and g is a constant relating $\bar{s}$ with C. A value of $g = 0.0081/g$ was used. The assumption that $(s_n)_0/(s_1)_0 = n^{2/3}$ was also made.

First using the relationship $\bar{s}/(s_1)_0 = (1 - gC)[(s_n)_0/(s_1)_0]$, a series of plots of $\bar{s}/(s_1)_0$ vs. C was made for values of $n = 1, 2, \ldots 8$. By plotting the experimental $\bar{s}/(s_1)_0$ values on the same graph it was observed that the equilibrium mixture should contain at least up to hexamers. For $n = 6$, using K_n values given in Table I, $\bar{s}/(s_1)_0$ was calculated as a function of C. The calculated curve with the experimental data is given in Figure 6. The fit was not satisfactory. Next, the calculation was repeated for $n = 7$ using several sets of $K_2 \ldots K_7$ values. None of them yielded a curve which would fit the data satisfactorily. The calculations were repeated for $n = 8$. By successive approximation a set of $K_2 \ldots K_8$ values was obtained (Table I) which yielded a calculated curve that fitted the data well (Figure 6). The standard deviation was 0.0353 for $n = 6$ and 0.0107 for $n = 8$, indicating that the latter was a better model.

Since octamerization equilibrium fitted the sedimentation data well, an $M_{w(a)}/M_1$ vs. C curve was calculated

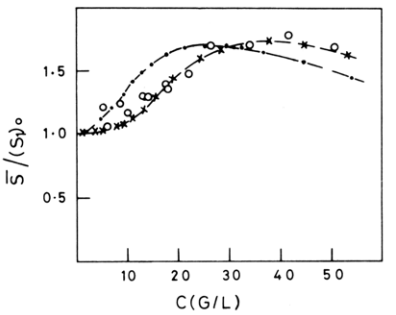

FIGURE 6: Variation of $\bar{s}/(s_1)_0$ with C: (O) experimental data; (- · -) curve calculated for $n = 6$ with K_n values given in Table I; (- × -) curve calculated for $n = 8$ with K_n values given in Table I.

using these K_n values. The calculated curve is given in Figure 4. The fit was rather poor; the standard deviation was 0.1446. Based on these results, it cannot be unequivocally stated that the conclusions from molecular weight and sedimentation data are incompatible with each other. We believe that by a process of iteration it would be possible to obtain a set of n and K_n values which would describe both the sets of data equally well.

The molecular weight data would fit well either a nonideal indefinite self-association or a discrete self-association equilibrium. A similar observation has been made in the self-association of papain at pH 7.8 and $\mu = 0.05$ (Pandit and Rao, 1974b). Hancock and Williams (1969) have reported that $M_{w(a)}$ vs. C data of chymotrypsinogen A at pH 8.3 and $\mu = 0.05$ would fit well either a monomer–dimer–trimer or an indefinite self-association equilibrium. Rao and Kegeles (1958) found that $M_{w(a)}$ vs. C data of α-chymotrypsin at pH 6.2 and $\mu = 0.20$ would fit a monomer–dimer–trimer equilibrium. We have analyzed their data for the indefinite self-association equilibrium and find this fits the data well with $BM_1 = +0.002$ (on a grams per liter concentration scale).

Thus, it would appear that a clear-cut choice between the models is not possible in many cases. For unequivocal conclusions, the methods of analysis require data of precision in the region of low concentration in both sedimentation equilibrium and sedimentation velocity experiments (Chun et al., 1972; Reisler and Eisenberg, 1971; Gilbert and Gilbert, 1973). To get precise data in this region is rather difficult. Computer simulation of concentration distribution in sedimentation equilibrium (Howlett et al., 1973) or sedimentation velocity (Gilbert and Gilbert, 1973) appears to offer advantages in this regard.

A comparison of self-association of α-chymotrypsin at $\mu = 0.05$ and $\mu = 0.10$ shows that in both cases the curve calculated for the indefinite self-association model fitted the experimental data better than those based on other models. Furthermore, for association at $\mu = 0.05$, $BM_1 = 0$ and at $\mu = 0.10$, $BM_1 = -0.0025$. Thus, at higher ionic strength the association was nonideal. One would expect that increasing the ionic strength would reduce nonideal effects. An explanation for this apparent anomaly can be attempted only when it is possible to choose a unique model of association

exclusive of other models. The calculations reported in our earlier paper (Pandit and Rao, 1974a) and this paper do not lead to such a clear-cut choice.

Acknowledgment

The authors are grateful to Dr. G. A. Gilbert and Dr. L. M. Gilbert for helpful discussions and for help with calculations on the sedimentation velocity data. The Model E ultracentrifuge used in this study was a gift from the Wellcome Trust, London.

References

Adams, E. T., Jr. (1965), *Biochemistry 4,* 1646.
Adams, E. T., Jr. (1967), Fractions, No. 3, Beckman Instruments, Palo Alto, Calif., p 1.
Adams, E. T., Jr., and Williams, J. W. (1964), *J. Am. Chem. Soc. 86,* 3454.
Anderson, A. E., and Alberty, R. A. (1948), *J. Phys. Colloid Chem. 52,* 1345.
Chun, P. W., Kim, S. J., Williams, J. D., Cope, W. T., Tang, L. H., and Adams, E. T., Jr. (1972), *Biopolymers 11,* 197.
Egan, R., Michel, H. G., Schlueter, R., and Jandorf, B. J. (1957), *Arch. Biochem. Biophys. 66,* 366.
Gilbert, G. A. (1955), *Discuss. Faraday Soc. 20,* 68.
Gilbert, G. A. (1959), *Proc. R. Soc. London, Ser. A 276,* 354.
Gilbert, L. M., and Gilbert, G. A. (1973), *Methods Enzymol. 27D,* 273.
Goldberg, R. J. (1953), *J. Phys. Chem. 57,* 194.
Hancock, D. K., and Williams, J. W. (1969), *Biochemistry 8,* 2598.
Howlett, G. J., Nichol, L. W., and Andrews, P. R. (1973), *J. Phys. Chem. 77,* 2907.
Massey, V., Harrington, W. F., and Hartley, B. S. (1955), *Discuss. Faraday Soc. 20,* 24.
McKenzie, H. A., Sawyer, W. H., and Smith, M. B. (1967), *Biochim. Biophys. Acta 147,* 73.
Nichol, L. W., and Bethune, J. L. (1963), *Nature (London) 198,* 880.
Pandit, M. W., and Rao, M. S. Narasinga (1974a), *Biochemistry 13,* 1048.
Pandit, M. W., and Rao, M. S. Narasinga (1974b), *Biochim. Biophys. Acta 371,* 211.
Rao, M. S. Narasinga, and Kegeles, G. (1958), *J. Am. Chem. Soc. 80,* 5724.
Reisler, E., and Eisenberg, H. (1971), *Biochemistry 10,* 2659.
Schwert, G. W., and Kaufman, S. (1951), *J. Biol. Chem. 190,* 807.
Steiner, R. F. (1952), *Arch. Biochem. Biophys. 39,* 333.
Steiner, R. F. (1954), *Arch. Biochem. Biophys. 53,* 457.
Tang, L. H., and Adams, E. T., Jr. (1973), *Arch. Biochem. Biophys. 157,* 520.
Van Holde, K. E., and Rossetti, G. P. (1967), *Biochemistry 6,* 2189.
Winzor, D. J., and Scheraga, H. A. (1964), *J. Phys. Chem. 68,* 338.

Degradation of Fibrinogen by Plasmin. Isolation of an Early Cleavage Product†

Elizabeth J. Harfenist* and Robert E. Canfield

ABSTRACT: As part of a project aimed at developing sensitive, specific, and quantitative tests for in vivo proteolysis of fibrinogen or fibrin a peptide which is cleaved from human fibrinogen at an early stage of digestion by plasmin has been isolated and characterized. This peptide, which has been designated fragment H, appears to be fairly resistant to further plasmin digestion and to have a molecular weight of approximately 20,000 as determined by gel filtration and by polyacrylamide gel electrophoresis in sodium dodecyl sulfate. It has a unique amino acid composition consisting of a high content of hydrophilic residues, especially serine, glycine, and proline, and a remarkably low content of hydrophobic residues. Edman degradation shows that it consists mainly of a single peptide chain whose NH_2-terminal sequence is Met-Glu-Leu-Glu-Arg-Pro-Gly-Gly-Asn-Glu-, and in addition there appears to be a minor contaminating chain. A product with the same characteristics has been isolated from a plasmin digest of the reduced carboxymethylated Aα chain of fibrinogen indicating that fragment H is produced by cleavage of the Aα chain. Polypeptides, apparently identical with fragment H, have also been isolated from plasmin digests of different samples of fibrin.

Three enzymes, thrombin, activated factor XIII, and plasmin, play important roles in the formation, stabilization, and subsequent lysis of a thrombus. In each case selected regions of the fibrinogen or fibrin molecule serve as substrates for the enzyme (Doolittle, 1973). Specific and sensitive methods to quantitate the levels of the reaction products of these enzymes in the circulation would be helpful in studying their roles in physiologic and pathologic states. One example of this approach has been the development of a radioimmunoassay for fibrinopeptide A, the 16 amino acid fragment which is released from the Aα chain of fibrinogen by thrombin to initiate the clotting process (Nossel et al., 1971). By means of this assay the plasma level of

† From the Department of Medicine, College of Physicians and Surgeons, Columbia University, New York, New York 10032. *Received February 3, 1975.* Supported by program project Grant HL-15486 from the National Institutes of Health.