The Conversion of Prothrombin to Thrombin

II. DIFFERENTIATION BETWEEN THROMBIN- AND FACTOR Xγ-CATALYZED PROTEOLYSES*

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SUMMARY

Prothrombin is activated by activated Factor Xγ (Factor Xγ and phospholipid) in the presence of diisopropylfluorophosphate which preferentially inhibits thrombin. Under these conditions a single activation fragment, designated Fragment 1-2, is released which represents the complete nontrombin portion of the prothrombin molecule. The same fragment is seen as a transient species in the absence of diisopropylfluorophosphate when the activation reaction is monitored by disc electrophoresis in 8 M urea. The NH₂-terminal residue of both prothrombin and Fragment 1-2 is alanine, suggesting that Fragment 1-2 is derived from the NH₂-terminal end of the prothrombin molecule. Fragment 1-2 is not cleaved by activated Factor Xγ (Factor Xγ and phospholipid), but is cleaved by thrombin to form two fragments which are identical by electrophoresis, ion exchange chromatography, and amino acid composition to Fragment 1, the fragment released from prothrombin on incubation with thrombin, and Fragment 2, the fragment released by activated Factor Xγ when Intermediate 1 is converted to Intermediate 2 (Paper I; Owen, W. G., Esmon, C. T., and Jackson, C. M. (1974) J. Biol. Chem. 249, 594-605). It is proposed that the formation of Intermediate 1 during prothrombin activation is solely the result of thrombin-catalyzed proteolysis of prothrombin.

Rapid activation of prothrombin requires four components in addition to prothrombin itself: two proteins, Factor Xγ, and Factor Vγ, phospholipid, and Ca²⁺. Of these components, only Xγ catalyzes the proteolytic conversion of prothrombin to thrombin in the absence of the other components (1-4). Studies in both our laboratory (5) and other laboratories (6-12) have resulted in the following description of this process (5).

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† The abbreviations used are: Xγ, the activated form of Factor Xγ; Vγ, Factor Vγ; dansyl, 5-dimethylaminonaphthalene-1-sulfonyl, iPrPF, diisopropylfluorophosphate.

METHODS

Prothrombin, Xγ, Vγ, phospholipid, and thrombin were prepared as described previously (5). Prothrombin was activated with Xγ, Vγ, phospholipid, and Ca²⁺. This set of activation reagents...
is abbreviated \([X, V, \text{ phospholipid, } Ca^+]\). All activations were performed in 0.1 M NaCl, 0.01 M \(CaCl_2\), 0.02 M Tris-HCl, pH 7.5. Specific conditions for activation are given in the figure legends. Reaction products from prothrombin activation mixtures were separated on columns of QAE-(quaternaryaminoethyl) Sephadex Q-50 (5) and Bio-Gel P-100. Disc gel electrophoresis was performed by the technique of Ornstein (16) and Davis (17). Sodium dodecyl sulfate gel electrophoresis was carried out by the procedure of Laemmli (18) except that the separating gels contained 0.27% \(N, N^\prime\)-methylenebisacrylamide. Urea disc gel electrophoresis was performed by the addition of 0.48 g of urea to each milliliter of separating or stacking gel solution prior to polymerization. No sample gel was used. Samples were prepared by heating aliquots of the reaction mixture at 100° for 1 to 2 min in 6 to 8 M urea. The sample was layered directly on top of the stacking gel. The gels were rapidly fixed and stained at 70°.

Soybean trypsin inhibitor (type II-S, Sigma Chemical Co., St. Louis, Mo.) was linked to Sepharose 4B with cyano groups by the method of Cantreleas (19). Amino acid analyses were performed on protein samples after hydrolysis in 6 N HCl in sealed evacuated ampules for 24 and 72 hours at 110°.

Single column analyses employed Durrum DC-1 resin (Durrum Chemical Co., Palo Alto, Calif.) and a Beckman 120C analyzer. Disopropylfluorophosphate (Sigma Chemical Co.) was used as a 1 M solution in anhydrous 2-propanol. NH₂-terminal amino acids were determined by the procedure of Gray (20). The dansylamino acids were identified by chromatography on polyamid sheets (21).

**RESULTS**

Demonstration of New Activation Fragment by Activating Prothrombin with \([X, V, \text{ Phospholipid, } Ca^+]\) in Presence of Disopropylfluorophosphate—Parallel prothrombin activations were performed with \([X, V, \text{ phospholipid, } Ca^+]\) in the presence and absence of 10 mM iPr₂PF. Prothrombin was incubated with \([X, V, \text{ phospholipid, } Ca^+]\) for 12 min, an interval known from previous experiments to be sufficient to convert all the starting prothrombin to thrombin in the absence of iPr₂PF (5). After incubation, the reaction mixtures were chromatographed on separate analytical QAE-Sephadex columns. Each column had 2 cm of soybean trypsin inhibitor-Sepharose over the QAE-Sephadex gel bed to remove \(X\) from the reaction mixtures. Otherwise, in the process of chromatography, small amounts of thrombin were formed from remaining prothrombin or reaction intermediates (Fig. 1). As iPr₂PF also inhibits \(X\) (22), although much less effectively than it inhibits thrombin (23), the \(X\) activity is decreasing continually during the activation reaction. As a consequence, activation in the presence of iPr₂PF results in small amounts of prothrombin and Intermediate 1 remaining after 12 min. In contrast to activation without iPr₂PF, these components are detected both in the chromatogram and in a sodium dodecyl sulfate electrophoresis gel of the reaction mixture in the process of chromatography, small amounts of thrombin were formed from remaining prothrombin or reaction intermediates. Inactivation (Fig. 1). As iPr₂PF also inhibits \(X\), although much less effectively than it inhibits thrombin (23), the \(X\) activity is decreasing continually during the activation reaction. As a consequence, activation in the presence of iPr₂PF results in small amounts of prothrombin and Intermediate 1 remaining after 12 min. In contrast to activation without iPr₂PF, these components are detected both in the chromatogram and in a sodium dodecyl sulfate electrophoresis gel of the reaction mixture that contained iPr₂PF.

The sodium dodecyl sulfate electrophoresis gels shown above the peaks of the two chromatograms differ only in the appearance of an additional band in the Fragment 2 peak. This new product has an apparent molecular weight by sodium dodecyl sulfate
thrombin is rapidly inhibited by iPrzPF.

A careful comparison of the elution profiles from the QAE-Sephadex columns indicates two other important differences. First, the ratio of the areas of the Fragment 2 to Fragment 1 peaks is 0.7 in the absence of iPrzPF and 1.0 in the presence of iPrzPF, consistent with an increased amount of a product which behaves chromatographically like Fragment 2. As some unreacted prothrombin is present with the Fragment 1, this ratio is actually greater than 1. Second, resolution of the two Fragment 2 peaks is reduced. The well resolved Fragment 2 doublet is a reproducible feature of complete activation mixtures (5).

The difference in the thrombin peaks between the two chromatograms (Fig. 1) does not reflect real differences in the thrombin, since chromatography of mixtures at lower ionic strength results in elution of only a single thrombin peak (5). Also, the initial ionic strength of the reaction mixture is higher than the results with purified Fragment 1-2 below. This result is consistent with the formation of Fragment 1-2 in a reaction mixture in which thrombin is rapidly inhibited by iPrzPF.

Characterization of Fragment 1-2—Fragment 1-2 could be prepared on a large scale and in high yield only when the prothrombin concentration was reduced in the activation mixture and a separate soybean trypsin inhibitor-Sepharose column was used to remove the Xa completely from the Fragment 1-2. As some unreacted prothrombin is present with the Fragment 1, this ratio is actually greater than 1. Second, resolution of the two Fragment 2 peaks is reduced. The well resolved Fragment 2 doublet is a reproducible feature of complete activation mixtures (5).

Fragment 1-2 (1.35 mg) from gel filtration (Fig. 3) was incubated with thrombin (30 pg) in 0.02 M Tris-HCl, pH 7.5, 0.1 M NaCl (Fraction 1-2, 1 mg in 1 ml, was incubated with thrombin (7) (50 µg) in 0.02 M Tris-HCl, pH 7.5, 0.1 M NaCl for 1.35 hours at 23°C. The reaction mixture was chromatographed on a column (0.9 x 24 cm) of QAE-Sephadex Q-50 (5). Four peaks were eluted: the added thrombin (Fraction 14), Fragment 1 (Fraction 50), and the characteristic two peaks of Fragment 2 (Fractions 64 and 69). The ratio of the areas of the Fragment 2 to Fragment 1 peaks was 0.7, identical with the ratio for these two peaks in Fig. 1A. Furthermore, the elution positions for the Fragment 1 and the Fragment 2 derived from Fragment 1-2 are identical with those of the fragments derived from prothrombin on activation with [Xa, V, phospholipid, Ca²⁺] or Xa alone (Fig. 1A).

A sample of the isolated Fragment 1-2 (40 µg/200 µl) was incubated with thrombin (4 µg) for 40 min at 23°C. Fig. 4A shows sodium dodecyl sulfate electrophoresis gels of the starting Fragment 1-2 and the products formed by thrombin. Fig. 4B shows the same materials as seen by pH 9.5 disc electrophoresis. In both electrophoresis systems the reaction products underwent co-electrophoresis with isolated Fragment 1 and Fragment 2 from ordinary activation mixtures. A trace of remaining Fragment 1-2 which was not detected by electrophoresis at pH 9.5 can be seen in the sodium dodecyl sulfate electrophoresis gel.
Sodium dodecyl sulfate electrophoresis gels from this experiment are shown above the column peaks (Fig. 5).

**Amino Acid Composition of Fragments 1, 2 and of Fragments 1, 2 Formed by Thrombin-catalyzed Cleavage of Fragment 1-2—**

Amino acid compositions of Fragment 1-2 from the Bio-Gel P-100 column (Fig. 3) and Fragment 1 and Fragment 2 from the QAE-Sephadex column (Fig. 5) are given in Table I. The compositions of isolated Fragments 1 and 2 from prothrombin activated with [Xa, V, phospholipid, Ca++] (5) are compared with Fragments 1 and 2 derived from cleavage of Fragment 1-2 (Table II). No significant differences were found between Fragments 1 derived from Fragment 1-2 (Table I) and Fragment 1 described previously (Table II). Likewise, Fragment 2 derived from Fragment 1-2 is identical with Fragment 2 from prothrombin activation mixtures (Table II).

**Formation of Fragment 1-2 during Prothrombin Activation by Factor Xa Alone in Presence of Diisopropylfluorophosphate—** Fragment 1-2 was successfully isolated from prothrombin activation mixtures in which Xa alone was the activator only if both the Xa and thrombin were removed from the mixture prior to chromatography on QAE-Sephadex. Prothrombin in 10 mM iPr2PF was incubated with Xa. Approximately one-half the starting prothrombin was still present when the activation was terminated as assessed by sodium dodecyl sulfate gel electrophoresis. The partial activation mixture was passed through a column (0.9 x 17 cm) which consisted of a 7-cm bed of sulfopropyl Sephadex SP-50 layered over a 10-cm bed of soybean trypsin inhibitor-Sepharose. The mixed column was equilibrated in 0.02 M Tris-HCl, pH 7.5, 0.1 M NaCl. Under these conditions, Xa was adsorbed to the soybean trypsin inhibitor-Sepharose column, and the thrombin was adsorbed to the sulfopropyl Sephadex (24). The effluent from this column was brought to 1 mM in iPr2PF and then chromatographed on QAE-Sephadex, as described in Fig. 1. Two features of this chromatogram (Fig. 6) are particularly significant. First, neither Fragment 1 nor Fragment 2 is seen in the sodium dodecyl sulfate electrophoresis gel of the final reaction mixture, nor in the column peaks in which they are usually found. Second, a comparison of the mobility of Fragment 1-2 in sodium dodecyl sulfate electrophoresis gels (gels above Fractions 66 to 78) with the mobility of the thrombin (gels above Fractions 1 to 50) shows that Fragment 1-2 is formed by thrombin-catalyzed cleavage of Fragment 1-2 from proteolytic cleavage of Fragment 1.2 with thrombin.

**TABLE II**

Comparison of amino acid compositions of fragments derived from prothrombin and fragment 1-2

| Source | Fragment 1 | Fragment 2 |
|--------|-----------|-----------|
| Prothrombin Fragment 1-2 | Prothrombin Fragment 1-2 |
| Residues/mole | Residues/mole | Residues/mole | Residues/mole |
| Asp | 16.2 | 14.7 | 17.3 | 17.0 |
| Thr | 10.3 | 9.8 | 4.6 | 4.8 |
| Ser | 14.2 | 11.2* | 9.2 | 10.0 |
| Glu | 23.4 | 22.0 | 15.5 | 15.2 |
| Pro | 10.1 | 10.7 | 9.7 | 10.3 |
| Gly | 12.0 | 12.5 | 12.1 | 13.3 |
| Ala | 10.2 | 10.9 | 9.6 | 10.0 |
| ½ Cys | 9.1 | N.D. | 4.0 | N.D. |
| Val | 9.1 | 9.2 | 4.9 | 5.5 |
| Ile | 1.0 | 0.7 | 0.0 | 0.0 |
| Leu | 3.7 | 3.4 | 1.1 | 1.1 |
| Tyr | 10.1 | 10.4 | 8.6 | 9.2 |
| Phe | 3.9 | 3.9 | 3.5 | 4.1 |
| Lys | 3.9 | 4.4 | 3.0 | 3.2 |
| His | 5.0 | 5.2 | 2.0 | 2.2 |
| Arg | 1.9 | 2.6 | 0.0 | 0.0 |
| 14.0 | 14.5 | 7.3 | 7.6 |

* Analyses performed on replicate 24-hour hydrolysates.

* Not corrected for destruction.

* N.D., not determined.

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**TABLE I**

Amino acid compositions of fragment 1-2 and fragments 1 and 2 from proteolytic cleavage of fragment 1-2 with thrombin

| Residue | Fragment 1-2 | Fragment 1 | Fragment 2 |
|---------|-------------|-----------|-----------|
| Residues/mole | Residues/mole | Residues/mole |
| Asp | 0.108 | 32.4 | 0.0711 | 14.7 | 0.0408 | 17.0 | 31.7 |
| Thr | 0.0500 | 15.0 | 0.0474 | 9.8 | 0.0136 | 4.8 | 14.6 |
| Ser | 0.0760 | 22.8 | 0.0548 | 11.2 | 0.0241 | 10.0 | 21.2 |
| Glu | 0.126 | 37.8 | 0.106 | 22.0 | 0.0366 | 15.2 | 37.2 |
| Pro | 0.0652 | 19.6 | 0.0621 | 10.7 | 0.0250 | 10.3 | 21.0 |
| Gly | 0.0805 | 24.1 | 0.0664 | 12.5 | 0.0321 | 13.5 | 25.8 |
| Ala | 0.0665 | 20.0 | 0.0628 | 10.9 | 0.0292 | 10.0 | 20.9 |
| ½ Cys | 0.0444 | 13.3 | ND | ND | ND | ND | 14.7 |
| Val | 0.0822 | 14.5 | 0.0444 | 9.2 | 0.0133 | 5.8 | 14.7 |
| Met | 0.0258 | 0.8 | 0.0322 | 0.7 | 0.0 | 0.0 | 0.7 |
| Ile | 0.0163 | 4.9 | 0.0165 | 3.4 | 0.0070 | 1.1 | 4.5 |
| Leu | 0.0642 | 19.2 | 0.0502 | 10.4 | 0.0223 | 9.2 | 19.6 |
| Tyr | 0.0242 | 7.3 | 0.0159 | 3.9 | 0.0101 | 4.1 | 8.0 |
| Phe | 0.0232 | 7.0 | 0.0212 | 4.4 | 0.0075 | 3.2 | 7.6 |
| Lys | 0.0238 | 7.1 | 0.0215 | 4.4 | 0.0064 | 2.2 | 7.4 |
| His | 0.00719 | 2.2 | 0.0120 | 2.6 | 0 | 0 | 2.6 |
| Arg | 0.0748 | 22.5 | 0.0704 | 14.5 | 0.0194 | 7.6 | 22.1 |
| GluN | 0.0248 | 7.5 | 0.0215 | 4.4 | 0.0120 | 2.6 | 7.4 |

* ND, not determined.
above Fractions 15 to 30) suggests that in more heavily loaded gels, Fragment 1-2 may not be distinguished from thrombin or Intermediate 2 (5).

Failure of Factor X_a to Form Fragments 1 and 2 from Isolated Fragment 1.2—Fragment 1.2 (30 μg in 300 μl) was incubated with [X_a, 1 μg; V, 2 μg; phospholipid, 1 μg; CaCl_2, 10 mM] for 12 min at 23°C. The activation mixture was analyzed by sodium dodecyl sulfate electrophoresis. Neither Fragment 1 nor Fragment 2 was detected in the fixed, stained gel. An equimolar sample of prothrombin would have been activated completely in less than 2 min under these conditions (5).

In a second attempt to cleave Fragment 1-2 with X_a, 30 μg of Fragment 1-2 in 300 μl was incubated with 1.5 μg of X_a for 40 min at 23°C. No cleavage of Fragment 1-2 was detected. Since Factor X_a did not cleave Fragment 1-2 either alone or in conjunction with the other activation components, and since activation can be carried out without the formation of appreciable Fragment 1 (Figs. 2, 6) it appears that Fragment 1 is released from prothrombin only as a result of proteolysis by thrombin.

NH_2-terminal Amino Acid Analysis of Fragment 1.2—Dansylalanine was the only residue present in sufficient quantity on the thin-layer chromatogram to represent an NH_2-terminal amino acid. This observation, coupled with the data of Magnusson (15) which indicate that thrombin (NH_2-terminal threonine and isoleucine) comes from the COOH-terminal end of prothrombin and the previously determined alanine NH_2-terminus of prothrombin (25-27) (confirmed in this laboratory), indicates that Fragment 1.2 arises from the NH_2-terminal end of the prothrombin polypeptide chain.

Fragment 1.2 Formation during Prothrombin Activation—In view of the previous observation that Intermediate 2 and Fragment 1-2 undergo co-electrophoresis in sodium dodecyl sulfate gels, unambiguous demonstration of Fragment 1-2 during activation could not be obtained by this technique. Disc electrophoresis in urea, however, resolves Fragment 1-2 and Intermediate 2. A time course of prothrombin activation by [X_a, V, phospholipid, Ca^{2+}] in the absence of iPrPF is shown in Fig. 7. The identity of each of the bands in the urea gels was determined by co-electrophoresis with the isolated intermediates and fragments. The bands from the top to the bottom of the gels are: 1, thrombin, which aggregates under these electrophoresis conditions (28); 2, Intermediates 1 and 2, which are not resolved in this system and are characteristically multiple patterns similar to the pattern described for thrombin (28); 3, prothrombin; 4, Fragment 1-2; 5, Fragment 1 (diffuse and broad); and 6, Fragment 2 (very sharp). It is seen from the time course that Fragment 1-2 is formed during prothrombin activation in the absence of iPrPF. However, as a consequence of proteolysis by thrombin, only Fragments 1 and 2 are found in the final reaction mixture.

FIG. 7. Time course of activation of prothrombin (P) by [X_a, V, phospholipid, Ca^{2+}] in the absence of iPrPF as monitored by disc electrophoresis in urea. Prothrombin, 800 μg in 1 ml was activated with [X_a, 0.4 μg; V, 3 μg; phospholipid, 4 μg]. Aliquots of 100 μl were added to 100 μl of 12% urea in a boiling water bath at the time intervals shown below the gels. The tubes containing the activation mixture were removed from the boiling water bath after 1 to 4 min. Each gel contained 20 μg of protein. F-1.2, Fragment 1-2.

FIG. 8. Time course of activation of prothrombin by X_a in the absence of iPrPF as monitored by disc electrophoresis in urea. Prothrombin, 800 μg in 1 ml was activated with X_a (15 μg) at 23°C. Aliquots of 50 μl were added to tubes with 50 μl of 12% urea in a boiling water bath at the times shown below the gels. The tubes containing the activation mixture were removed from the boiling water bath after 1 to 4 min. Each gel contained 20 μg of protein.

DISCUSSION

Prothrombin activation in the presence of iPrPF results in the formation of a stable activation fragment (Fragment 1-2) which consists of the covalently linked Fragments 1 and 2 described previously (5). This fragment is readily cleaved by thrombin to form Fragments 1 and 2. However, it was not cleaved by X_a either alone or in the presence of V, phospholipid, and Ca^{2+}. In view of these results, formation of the "activation intermediate" designated Intermediate 1, which can be observed during prothrombin activation, must occur primarily and probably exclusively as a result of proteolysis by thrombin and not X_a. Demonstration of an NH_2-terminal alanine residue in Fragment 1-2, the same NH_2 terminus as prothrombin, supports a previous proposal (15) that thrombin (NH_2-terminal threonine and isoleucine) is derived from the carboxyl end of the prothrombin polypeptide chain. Since Fragment 1 is removed from prothrombin to give Intermediate 1, a single polypeptide chain (5, 12), Fragment 1 must be derived from the NH_2-terminal end of the prothrombin polypeptide chain.
On the basis of these observations and the results presented previously (5), Scheme 1 is proposed to describe the process of prothrombin activation. The symbol, $\diamond$, in the schematic map, represents the position in prothrombin at which peptide bonds are cleaved during prothrombin activation. The lengths of the line segments, which represent the activation products, are proportional to the number of amino acid residues in each activation product (5). An activation pathway is defined by the order of bond cleavages.

The existence of pathway A is supported by the following evidence: (a) the appearance and disappearance of Intermediate 1 during prothrombin activation, Fig. 3 of the preceding paper (5); (b) the peptide bond linking Fragment 1 with Fragment 2 is not cleaved by Factor X; (c) isolation of Intermediate 1 and Fragment 1 after incubation of prothrombin with thrombin; (d) isolation of Intermediate 2 and the demonstration that it can be converted to thrombin (5); and (e) the observed appearance and disappearance of Intermediate 2 during activation of isolated Intermediate 1 (Fig. 11 of Ref. 5). In the latter situation the ambiguity of co-electrophoresis of Intermediate 2 and Fragment 1-2 cannot exist.

Either pathway B or C would result in the formation of Fragment 1-2. To date no intermediates have been isolated from prothrombin activated with $[^{40}X, V, phospholipid, Ca^{2+}]$ which can be identified unambiguously as coming from pathway B or C. Until such an intermediate is isolated or sufficient kinetic data obtained, the question of whether pathway B or C (or both) is the kinetic pathway of prothrombin activation will remain open.

REFERENCES

1. **MILSTONE, J. H.** (1961) *Fed. Proc.* **20**, 742-748
2. **BARTON, P. G., JACKSON, C. M., AND HANAHAN, D. J.** (1967) *Nature* **214**, 923-924
3. **HERMANN, H. C., ENSOUFF, M. P., HERMANN, P. W., SWANK, A. C. W., AND MACKAY, Y. G.** (1967) *Nature* **215**, 248-251
4. **SIEGERS, W. H., SAKURAGAWA, N., McCOY, L. E., SEDENSKY, J. A., AND DOMBRUSE, F. A.** (1972) *Thromb. Res.* **1**, 293-310
5. **OWEN, W. G., ENSOUFF, C. T., AND JACKSON, C. M.** (1974) *J. Biol. Chem.* **249**, 594-605
6. **LANCHANTIN, C. F., FRIEDMAN, J. A., AND HART, D. W.** (1975) *J. Biol. Chem.* **240**, 3276-3282
7. **ARONSON, D. L., AND MENACHE, D.** (1966) *Biochemistry* **5**, 2635-2640
8. **LANCHANTIN, C. F., FRIEDMAN, J. A., AND HART, D. W.** (1968) *J. Biol. Chem.* **243**, 476-486
9. **LANCHANTIN, C. F., FRIEDMAN, J. A., AND HART, D. W.** (1969) *J. Biol. Chem.* **244**, 865-875
10. **SIEGERS, W. H., MURANO, G., AND McCOY, L.** (1970) *Thromb. Diath. Haemorrh.* **23**, 26-36
11. **STERN, S. S., AND BLOUT, E. R.** (1972) *Biochemistry* **11**, 4502-4515
12. **HELDEBRANT, C. M., BUTKOWSKI, R. J., BAJAJ, S. P., AND MANN, K. G.** (1973) *Fed. Proc.* **32**, 318
13. **MANN, K. G., HELDEBRANT, C. M., AND FASS, D. N.** (1971) *J. Biol. Chem.* **246**, 6106-6114
14. **FASS, D. N., AND MANN, K. G.** (1973) *J. Biol. Chem.* **248**, 3280-3287
15. **MAGNUSSON, S.** (1970) *Thromb. Diath. Haemorrh.* **38** (suppl), 97-104
16. **ORNSTEIN, L.** (1964) *Ann. N. Y. Acad. Sci.* **121**, 321-349
17. **DAVIS, B. J.** (1964) *Ann. N. Y. Acad. Sci.* **121**, 404-427
18. **LAEHMII, U. K.** (1970) *Nature* **227**, 680-685
19. **CUATRECASAS, L. (1970) J. Biol. Chem. 245, 3069-3083**
20. **GRAY, W. R.** (1972) *Methods Enzymol.* **35**, 121-138
21. **WOODS, K. R., AND WANG, K. T.** (1967) *Biochim. Biophys. Acta* **133**, 389-397
22. **JACKSON, C. M., AND HANAHAN, D. J.** (1968) *Biochemistry* **7**, 4506-4517
23. **LEVISON, J. E., AND ENSOUFF, M. P.** (1969) *Brit. J. Haematol.* **17**, 173-178
24. **LUNDSTAD, R. L.** (1971) *Biochemistry* **10**, 2901-2906
25. **MILLER, K.** (1958) *J. Biol. Chem.* **231**, 957-965
26. **MAGNUSSON, S.** (1968) *Acta Chem. Scand.* **12**, 355-356
27. **THOMAS, W. R., AND SEDGEWICK, W. H.** (1970) *Biochim. Biophys. Acta* **55**, 555-557
28. **ROSENBERG, R. D., AND WASH, D. F.** (1970) *J. Biol. Chem.* **245**, 3049-3054
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