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1. Brown, D. D. *Harvey Lect.* **76,** 27-44 (1982).
2. Rhodes, D. & Klug, A. *Cell* **46,** 123-132 (1986).
3. Miller, J., McLachlan, A. D. & Klug, A. *EMBO J.* **4,** 1609-1614 (1985).
4. Rosenberg, U. B. *et al. Nature* **319,** 336-339 (1986).
5. Vincent, A., Colot, H. V. & Rosbach, M. *J. molec. Biol.* **186,** 149-166 (1985).
6. Hartshorne, T. A., Blumberg, H. & Young, E. T. *Nature* **320,** 283-287 (1986).
7. Rhodes, D. *EMBO J.* **4,** 3473-3482 (1985).
8. Sakonju, S. & Brown, D. D. *Cell* **31,** 395-405 (1982).
9. Fairall, L., Rhodes, D. & Klug, A. *Cell* (submitted).
10. Sakonju, S., Bogenhagen, D. F. & Brown, D. D. *Cell* **19,** 13-25 (1980).
11. Bodenhagen, D. F., Sakonju, S. & Brown, D. D. *Cell* **19,** 27-35 (1980).
12. Gait, M. J., Matthes, H. W. D., Singh, M., Sproat, B. S. & Titmus, R. C. *Nucleic Acids Res.* **10,** 6243-6254 (1982).
13. Conner, B. N., Yoon, C., Dickerson, J. L. & Dickerson, R. E. *J. molec. Biol.* **174,** 663-695 (1984).
14. Wang, A. H.-J., Fujii, S., van Boom, J. H. & Rich, A. *Proc. natn. Acad. Sci. U.S.A.* **79,** 3968-3972 (1982).
15. McCall, M. J., Brown, T. & Kennard, O. *J. molec. Biol.* **183,** 385-396 (1985).
16. Sussman, J. L., Holbrook, S. R., Church, G. M. & Kim, S. H. *Acta crystallogr.* A**33,** 800-804 (1977).
17. Hendrickson, W. A. & Konnert, J. H. in *Biomolecular Structure, Conformation, Function and Evolution* Vol. 1 (ed. Srinivasan, R.) 43-57 (Pergamon, Oxford, 1981).
18. Wilson, A. J. C. *Nature* **150,** 152 (1942).
19. Arnott, S. & Hukins, D. W. L. *Biochem. biophys. Res. Commun.* **47,** 1504-1509 (1972).
20. Calladine, C. R. & Drew, H. R. *J. molec. Biol.* **178,** 773-782 (1984).
21. Arnott, S., Hukins, D. W. L. & Dover, S. D. *Biochem. biophys. Res. Commun.* **48,** 1392-1399 (1972).
22. Arnott, S., Hukins, D. W. L., Dover, S. D., Fuller, W. & Hodgson, A. R. *J. molec. Biol.* **81,** 107-122 (1973).
23. Nilsson, L., Clore, G. M., Gronenborn, A. M., Brünger, A. T. & Karplus, M. *J. molec. Biol.* **188,** 455-475 (1986).
24. Drew, H. R. & Travers, A. A. *Cell* **37,** 491-502 (1984).
25. Haran, T. thesis, Weizmann Inst. (1986).
26. Shakked, Z. *et al. J. molec. Biol.* **166,** 183-201 (1983).
27. Kadonaga, J. T., Jones, K. A. & Tjian, R. *Trends biochem. Sci.* **11,** 20-23 (1986).
28. Rabinovich, D. & Shakked, Z. *Acta crystallogr.* A**40,** 195-200 (1984).
29. Watson, J. D. & Crick, F. H. C. *Nature* **171,** 737-738 (1953).
30. Franklin, R. E. & Gosling, R. G. *Acta crystallogr.* **6,** 673-677 (1953).
31. Langridge, R. *et al. J. molec. Biol.* **2,** 38-64 (1960).
32. Fuller, W., Wilkins, M. H. F., Wilson, H. R. & Hamilton, L. D. *J. molec. Biol.* **12,** 60-80 (1965).
33. Arnott, S. *Nature* **320,** 313 (1986).
34. Huber, P. W. & Wool, I. G. *Proc. natn. Acad. Sci. U.S.A.* **83,** 1593-1597 (1986).

MATTERS ARISING

The mechanism of activation of porcine pepsinogen

IN a recent paper we studied the early events in the activation of porcine pepsinogen using fluorescence-detected stopped flow kinetics[1]. We characterized two first-order decays when working with both native pepsinogen and a covalent conjugate between the fluorescent moiety 6-(*p*-toluidinyl)-naphthalene-2-sulphonyl- and pepsinogen[1]. This result was demonstrated to be consistent with a model in which concurrent first-order transformations are undergone by two species of zymogen which are related by an ionization-dependent equilibration between them. (A sequential model was ruled out by an analysis of the data.) We hypothesized that the two species initiate two concurrent pathways of activation. Subsequently, James and Sielecki, in an extrapolation from their new crystallographic structure of porcine pepsinogen, also proposed two alternative pathways for activation[2]. They did not explicitly suggest, however, that the two paths are followed simultaneously under a given set of conditions. It will be noted that their proposal bears a close resemblance to the model we had already worked out[1] based on the kinetic analysis described above. Most recently we have demonstrated that the *p*H-dependent segregation of pepsinogen molecules between alternative pathways persists at least through the process which exposes the active site[3]. This was shown by following the kinetics of binding of a fluorescent analogue of pepstatin to pepsinogen upon acidification. It was again observed that there are two first-order processes consistent with the concurrent reaction model described above.

The crystal structure of pepsinogen reported by James and Sielecki[2] permits a much clearer understanding of the origins of the changes in intrinsic fluorescence that we obtained[1]. They noted that the environment of at least four tyrosyl residues must be perturbed upon release of Lys 36P (residues 1P–44P comprise the activation peptide) from its electrostatic interaction with the carboxylates of the catalytically-active residues Asp 32 and Asp 215. The fluorescence changes that we observed occur on the millisecond time scale, and are followed by no further change for several seconds[1]. This strongly suggests that very soon after acidification the activation peptide is released from its initial position in which it blocks the catalytic site[2].

The distinguishing conformational features of the two molecular species of acidified pepsinogen, which form a Bronsted acid-base conjugate pair, remain to be identified. Further studies by us, as well as results obtained by crystallographic analysis, may provide a means to this end.

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1. Auer, H. E. & Glick, D. M. *Biochemistry* **23,** 2735-2739 (1984).
2. James, M. N. G. & Sielecki, A. R. *Nature* **319,** 33-38 (1986).
3. Glick, D. M., Auer, H. E., Rich, D. H., Kawai, M. & Kamath, A. *Biochemistry* **25,** 1858-1864 (1986).

JAMES AND SIELECKI REPLY—Acid activation of porcine pepsinogen has long been known to involve conformational changes[1,2] that can be reversed by rapidly returning the *p*H of the solution to neutrality. Conformational changes on a time scale of 5 ms–2 s have also been detected by stopped flow fluorescence kinetics.[3] Analysis of these data suggests that there are two concurrent activation pathways for pepsinogen. Exposure of the active site of the enzyme occurs on roughly the same time scale (1–2 s). The kinetic data for the binding of a fluorescent pepstatin analogue are also interpretable in a two concurrent pathway model.[4]

The crystal structure of porcine pepsinogen[5] has shown that the pepsin active site is fully formed in the zymogen but that it is blocked competitively by the specific folding of the activation peptide (Leu1P–Leu44P). This blocking takes place in a manner different from that expected for productive substrate binding. The two alternative steps in the proposed activation pathway[5] are concerned only with repositioning the activation peptide so that the scissile bond (Leu16P–Ile17P) can approach the catalytic aspartates. This cleavage step has a relatively longer half-time of ~28 s (ref. 6). Unfortunately, a static crystal structure cannot provide definitive data on dynamic events that are occurring at millisecond or second time scales. However, the three-dimensional structure allows us to propose the two alternative pathways for productive binding of the scissile peptide bond of pepsinogen in the unimolecular cleavage reaction. Whether our alternative binding modes are associated with relatively rapid fluorescence changes and the concurrent pathways so deduced remains to be seen.

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1. Perlmann, G. E. *J. molec. Biol.* **6,** 452-463 (1963).
2. McPhie, P. *J. biol. Chem.* **247,** 4277-4281 (1972).
3. Auer, H. E. & Glick, D. M. *Biochemistry* **23,** 2735-2739 (1984).
4. Glick, D. M., Auer, H. E., Rich, D. H., Kawai, M. & Kamath, A. *Biochemistry* **25,** 1858-1864 (1986).
5. James, M. N. G. & Sielecki, A. R. *Nature* **319,** 33-38 (1986).
6. Al-Janabi, J., Hartsuck, J. A. & Tang, J. *J. biol. Chem.* **247,** 4628-4632 (1972).