the height of this barrier, the I state was incubated for an extended period of time under conditions which minimize aggregation (Table 1). Although the ability to recover activity in the presence of the pro region slowly decayed with time, large amounts of activity were recovered even after a month of incubation (Fig. 2*a*). By contrast, no activity was recovered in the absence of the pro region in 800 h of incubation. From the detection limit in the protease assay, the first order rate constant for conversion of the I state to the native state is estimated to be $<10^{-9}$ s^{-1}. Thus, even with the three native disulphide bonds in place, the native conformation does not seem to be kinetically accessible from the I state. On the basis of this data, the free energy barrier for conversion of I to the native state in the absence of the pro region is in excess of 27 kcal mol^{-1}.

The kinetics of folding in the presence of the pro region (Fig. 2*b*) are consistent with a simple single turnover mechanism: I + Pro ⇔ I-Pro ⇒ Nat-Pro. Strong product inhibition[4] presumably accounts for both the single-turnover kinetics and the observed stoichiometry (Fig. 1) of the refolding reaction. The apparent rate constant for conversion of the I-Pro complex is roughly 0.016 s^{-1}. These results indicate that the pro region increases the rate of folding (k) by over seven orders of magnitude (k(+pro)/k(−pro) ≥ 1.7×10^7).

Structural characterization shows the I state to have properties intermediate between the native and denatured states. By gel filtration (Fig. 3*a*), the I state chromatographs as a single species with an apparent Stokes radius (28.4 Å) between that of native (20.4 Å) and denatured enzyme (37.5 Å). Circular dichroism (CD) spectroscopy (Fig. 3*b*) shows that the I state contains nearly as much secondary structure (70% β sheet) as the native state (N, 75% β sheet) and much more than the fully denatured state (D, 0% β sheet). Aromatic region CD and fluorescence spectroscopy both indicate that the I state has little or no organized tertiary structure (data not shown).

The presence of well defined secondary structure and absence of tertiary interactions indicated by the CD and fluorescence spectra, together with the expanded hydrodynamic radius, are hallmarks of the 'molten globule' or A states of various proteins under non-native conditions[7–10]. But the I state may be the first example of a long-lived 'molten globule'-like conformation of a polypeptide chain under conditions in which the native state is also stable.

In principle, a *trans*-acting factor could promote folding either by decreasing the rate of off-pathway folding reactions or by increasing the rate of a limiting on-pathway reaction. The molecular chaperonins, a ubiquitous class of proteins which are thought to bind to partially folded proteins and prevent irreversible aggregation and misfolding, seem to aid folding through the former mechanism[5,6]. By contrast, the pro region functions by directly stabilizing the rate-limiting folding transition state, thereby increasing the rate of folding by over seven orders of magnitude. The inability of denatured α-lytic protease to fold to the native state in the absence of the pro region is not due to competing off-pathway reactions under the conditions used in the experiments described above: folding competence is retained in the absence of the pro region (Fig. 2*a*).

It is generally assumed that protein folding is under thermodynamic control[11,12]. By lowering the height of a limiting energy barrier, the pro region provides a means to access new regions of conformational space. A 'new' state (the enzymatically active state) is found which has considerably different properties from the low energy state (I) reached in the absence of the pro region. Without the pro region, both states are stable for weeks under identical conditions with no detectable interconversion; hence one of these states must be kinetically trapped.

Perhaps our most important finding is that the energy barriers separating minima in polypeptide chain conformational space can exceed 27 kcal mol^{-1}. If such barriers were present on the folding free energy surfaces of other proteins, large regions of conformational space would be kinetically inaccessible. It would then be conceivable that in such cases the native conformation might be at a local and not a global free energy minimum. □

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RETRACTION

Amyloid plaques, neurofibrillary tangles and neuronal loss in brains of transgenic mice overexpressing a C-terminal fragment of human amyloid precursor protein

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THE paper published under this title[1] has been retracted: see Scientific Correspondence[2].

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