phages to animal viruses, as well as moving from Illinois to Columbia, Q β and its relatives still have their surprises and puzzles. Last week in *Nature*, de Wachter and Fiers (221, 233; 1969) reported that Q β RNA molecules fall into two classes, which differ by one base in the 5'-terminal base sequences. In this issue of *Nature* (see page 321), Kamen reports the long-awaited proof that at least the last base at the opposite end of the Q β RNA molecule, the 3'-end, is added by some mechanism independent of base pairing and after the rest of the molecule has been synthesized.

All viral RNA so far analysed has the same 3'-terminal sequence, CCA, but in the case of Q β —the only RNA phage the replication of which has been studied *in vitro*—all the evidence suggests that RNA made *in vitro* has the 3'-terminal sequence CC. In other words, the final A residue is not replicated. For the past eighteen months it has been generally assumed that the final A residue is added by some enzyme in the host bacteria, which is absent from the *in vitro* systems. One obvious candidate for the job is the enzyme which is responsible for the turnover of the 3'-terminal CCA sequence common to all tRNA molecules.

Kamen's experiment says nothing about the enzyme involved in adding the final A residue, but it establishes that Q β RNA molecules lacking the 3'-terminal A residue are infective and therefore able to replicate. This in turn implies that the A residue is added to RNA molecules by a host cell enzyme.

Essentially what Kamen did was extract RNA from Q β phage particles. These molecules have the complete CCA 3' sequence. By a stepwise chemical reaction he removed first the terminal A and then the first C residue and tested the ability of the intact RNA molecule and the two types of modified molecules to replicate in spheroplasts (bacteria from which the cell wall has been removed to allow the phage RNA entry). Only the intact RNA and the molecules lacking the terminal A residue were infective; moreover, all the progeny molecules had the terminal A residue restored. No RNA lacking more than the last A residue was infective.

If the terminal A residue can be removed without abolishing infectivity, at least this base must be added independent of any Watson-Crick base pairing replication mechanism. The problem now is to establish what enzyme is responsible for this reaction and why the terminal A residue is required.

PROTEIN CHEMISTRY

Denaturation and Refolding

from our Molecular Biology Correspondent

THE renaturation of globular proteins from a completely unfolded to a fully refolded and biologically active state has become a commonplace. The question which has recently been exercising protein chemists in this regard is the nature of the kinetic pathways by which this refolding to the unique native conformation proceeds. In the absence of disulphide bonds, or if these have been preserved intact, refolding is rather rapid—on a time scale of seconds—but when regeneration of disulphide bonds is involved, the refolding, in terms, for instance, of recovery of enzymatic activity, is

on a scale of hours or even days. This is evidently a consequence of the possibility of incorrect disulphide pairings, which leads to subsidiary potential minima in which the system becomes transiently trapped.

The systematic study of refolding reactions in such systems has been undertaken by Isemura and his associates. In their most recent paper (Yutani *et al.*, *J. Biochem., Tokyo*, **64**, 449; 1968), they consider the refolding of reduced egg lysozyme. The return of the native structure can be measured by spectroscopic methods, such as the disappearance of the ultraviolet difference spectrum arising from the change of environment of aromatic side chains, and the optical rotation or circular dichroism. As work from the same laboratory has previously shown, the refolding is catalysed by metal ions (in this case copper) in a low concentration. The refolding in standard conditions is complete after about 20 h, the difference spectrum having then vanished, and the characteristic Cotton effects in the circular dichroism, due to the backbone conformation and anomalous aromatic side chains, fully restored. The most striking feature of the results is the immediate return of the greater part (some 70 per cent) of the secondary structure when the denaturant, 8 M urea, is diluted out. At this stage there is no measurable enzymatic activity, or regeneration of disulphide bonds. Thereafter, the titre of sulphhydryl groups gradually diminishes, and activity appears. The return of activity was approximately paralleled by the reappearance of a feature in the circular dichroism (260 m μ band) previously suspected to reflect simply the number and configuration of disulphide bonds. It now seems to have more complex origins, for its reappearance essentially accompanies the formation of the fourth and last disulphide bridge. It is concluded that the enzyme has some activity when only two disulphide bonds have reformed—and indeed earlier studies showed that one such bond could be broken in the native protein, with retention of much of the activity.

Contrasting with this work is a kinetic study of the rapid reversible denaturation process, involving no rupture or formation of disulphide bond, described by Pohl (*Europ. J. Biochem.*, **7**, 146; 1968), working with trypsin. Here a simple temperature-jump method is used, in which the temperature of thermostat fluid around the sample is suddenly changed. Within an appropriate pH range, the temperature jump brings about a shift in the equilibrium between the native and denatured states of the protein, and the relaxation kinetics can be obtained by following either the change in the ultraviolet difference spectrum or the optical rotation. As with earlier results on chymotrypsin, the absorbance change is characterized by a single rate process and is independent of protein concentration. From experiments at different temperatures, a forward and a reverse rate constant are obtained, and the corresponding energies of activation give the enthalpy of denaturation. The results bear out the conclusions on other single-chain proteins, which have generally indicated that denaturation is a two-state equilibrium with no intermediates between the native and the unfolded states. The enthalpy is temperature dependent, and this effect resides in the renaturation process. The effect of pH on the kinetics is consistent with the liberation of two carboxyl groups in the unfolded state.