Henderson, C. E., Perham, R. N., & Finch, J. T. (1979) *Cell (Cambridge, Mass.) 17*, 85–93.
Jaenicke, R. (1978) *Naturwissenschaften 65*, 569–577.
Jaenicke, R. (1979) *FEBS-Symp. 52*, 187–198.
Jaenicke, R. (1982) *Biophys. Struct. Mech.* (in press).
Jaenicke, R., & Rudolph, R. (1980) in *Protein Folding* (Jaenicke, R., Ed.) pp 525–548, Elsevier/North-Holland, Amsterdam.
Koike, M., & Koike, K. (1976) *Adv. Biophys. 9*, 187–227.
Koike, M., Reed, L. J., & Carroll, W. R. (1963) *J. Biol. Chem. 238*, 30–39.
Perham, R. N. (1975) *Philos. Trans. R. Soc. London, Ser. B 272*, 123–136.
Perham, R. N., & Roberts, G. C. K. (1981) *Biochem. J. 199*, 733–740.
Perham, R. N., Harrison, R. A., & Brown, J. P. (1978) *Biochem. Soc. Trans. 6*, 47–50.
Perham, R. N., Duckworth, H. W., & Roberts, G. C. K. (1981) *Nature (London) 292*, 474–477.
Reed, L. J. (1974) *Acc. Chem. Res. 7*, 40–46.
Wawrzynczak, E. J., Perham, R. N., & Roberts, G. C. K. (1981) *FEBS Lett. 131*, 151–154.
Zettlmeissl, G., Rudolph, R., & Jaenicke, R. (1979) *Biochemistry 18*, 5567–5571.
Zettlmeissl, G., Rudolph, R., & Jaenicke, R. (1982) *Eur. J. Biochem.* (in press).

Enzymatic Condensation of Nonassociated Peptide Fragments Using a Molecular Trap†

Gene A. Homandberg,‡ Akira Komoriya,§ and Irwin M. Chaiken*

ABSTRACT: We have tested the feasibility of achieving protease-catalyzed condensation between nonassociating peptide fragments through mediation of a molecular trap. In this study, two subfragments of bovine pancreatic ribonuclease S-peptide, containing residues 1–10 and 11–15, were rejoined by clostripain catalysis to form the 1–15 peptide. The extent of this stereospecific condensation was enhanced by adding ribonuclease S-protein (residues 21–124), which acts as a trap in binding 1–15 but not 1–10 or 11–15 and which thus shifts the equilibrium to favor 1–15 formation. The resultant (1–15)·(21–124) noncovalent complex, defined as [des-16–20]-ribonuclease S, was detected by the enzymatic activity characteristic of the naturally derived ribonuclease S complex. Reaction of 1 mM S-protein and 20 mM fragments leads to 80% of the ribonuclease activity expected from the amount of 21–124 present. This indicates that 4% of the fragments 1–10 and 11–15 were condensed, compared to a maximal condensation of 5% based on the amount of trap. The less than theoretical yield is due largely to slow proteolytic degradation of 21–124 to a form which is no longer able to bind the condensation product 1–15. Yields were increased to 15% by addition of further trap. The successful synthesis of 1–15 emphasizes the usefulness of molecular traps to promote stereospecific fragment condensation between nonassociating peptide fragments for the synthesis and semisynthesis of polypeptides.

It is now recognized that by perturbing the equilibrium constant for peptide bond hydrolysis, peptide bonds can be synthesized between peptide fragments by using "proteolytic" enzymes. For example, inclusion of 90% glycerol in solutions of the noncovalently associated protein fragment complexes RNases S[1] (Homandberg & Laskowski, 1979; Homandberg et al., 1980), staphylococcal nuclease T (Homandberg & Chaiken, 1980; Komoriya et al., 1980) and the cytochrome *c* complex [(1–38)·(39–104)] (Homandberg et al., 1980; Juillerat & Homandberg, 1981) has been shown to promote resynthesis of the interfragment peptide bonds by the same proteolytic enzyme used initially under aqueous conditions to hydrolyze those bonds. The glycerol cosolvent decreases the free energy change associated with peptide bond hydrolysis principally by suppressing the ionization of the carboxylate liberated by the hydrolysis (Homandberg et al., 1978). For fragment condensation, this method has many advantages over chemical condensation, since it is stereospecific, has a high yield (25–50%/equilibration), does not require blocking of side chain groups, and proceeds under gentle conditions. However, an important limitation of the above procedure is its reliance on the existence of a noncovalently associated fragment complex. This fragment association must orient the carboxyl and amino groups to be attached so that they are close to one another as well as accessible to the proteolytic enzyme. Further, the complex must be stable in the presence of the organic cosolvent used to shift the equilibrium toward peptide bond synthesis.

A more general need in peptide fragment condensation is to join fragments that are nonassociating. In such a case, the method of enzymatic peptide fragment condensation suffers from a considerably unfavorable equilibrium in the synthesis direction. To increase yields for condensing nonassociating fragments, one can employ mass action as well as solvent-induced perturbation of equilibrium constants. Unfortunately, for many peptides, the high concentrations required are difficult to achieve. A more likely way to achieve such enzymatic fragment condensation would be to couple the unfavorable reaction of a synthesis

† From the Laboratory of Chemical Biology, National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20205. *Received January 7, 1982.*
‡ Present address: Winter Research Building, Mount Sinai Medical Center, Milwaukee, WI 53233.
§ Present address: Laboratory of Chemoprevention, National Cancer Institute, National Institutes of Health, Bethesda, MD 20205.

[1] Abbreviations: RNase, RNase A, RNase S, and SRNase S, bovine pancreatic ribonuclease, ribonuclease A, ribonuclease S, and semisynthetic ribonuclease S, respectively; RNase-(1–15), the sequence of residues 1–15 of native RNase; RNase-S-(1–20) and -(21–124), the fragments of RNase containing residues 1–20 and 21–124, respectively.

0006-2960/82/0421-3385$01.25/0

$$A + B \rightleftharpoons C \quad (1)$$

with a second, more favorable reaction

$$C + D \rightleftharpoons CD \quad (2)$$

In such a condition, D takes the role of a molecular trap for the product.

The objective of the present study was to test the effectiveness of a molecular trap to drive enzymatic fragment condensation of nonassociating native sequence peptides. As the test case, we used the system of [des-16–20]RNase S (Potts et al., 1963; Hofmann et al., 1966), a derivative of bovine pancreatic RNase containing the polypeptides RNase-(1–15) and RNase-S-(21–124). This complex is fully as active enzymatically as native RNase A or RNase S. The dissociation constant for the RNase S complex is 10^{-7} M. Clostripain hydrolysis of RNase-(1–15) at the single arginyl bond Arg-10–Gln-11 yields the two subfragments 1–10 and 11–15. These subfragments would not be expected to associate appreciably either with one another or with RNase-S-(21–124) (Allende & Richards, 1962). However, it was considered that any 1–15 re-formed by the subfragment condensation

$$\text{synthetic-(1–10)} + \text{synthetic-(11–15)} \rightleftharpoons \text{synthetic-(1–15)} \quad (3)$$

then would associate with the 21–124 trap

$$\text{synthetic-(1–15)} + \text{ribonuclease-S-(21–124)} \rightleftharpoons \text{RNase S complex} \quad (4)$$

to drive the condensation toward desired coupled product 1–15. We have found in this study that the above process can be affected, with consequent synthesis of appreciable amounts of 1–15. Some of the complications in such a process and means of dealing with them are discussed.

Experimental Procedures

Materials

Bovine pancreatic RNase A, RNase S, RNase-S-(21–124), RNase-S-(1–20), cytidine 2′,3′-phosphate, and morpholineethanesulfonic acid were from Sigma Chemical Co. Clostripain and the arginyl-specific protease from mouse submaxillary gland were from Boehringer Mannheim. Manual amino acid sequencing supplies were from Pierce Chemical Co.

RNase-S-(21–124) also was prepared from RNase A as described by Richards & Vithayathil (1959). All RNase-S-(21–124) was freed from RNase A and S contamination by passage through a Sepharose–5′-[(4-aminophenyl)-phosphoryl]uridine 2′(3′)-phosphate column in 0.4 M ammonium acetate, pH 5.2 (Chaiken & Taylor, 1976). The 21–124 fragment used in condensation reactions was maximally contaminated with 0.03% ribonuclease A and/or S as determined by enzymatic activity. All N^{α}-*tert*-butyloxycarbonyl L-amino acids were purchased from Vega-Fox Biochemicals. The coupling reagent dicyclohexylcarbodiimide was obtained from Aldrich Chemical Co.

Methods

Chemical Synthesis of Fragments 1–10 and 11–15. The RNase subfragments 1–10 and 11–15 were synthesized by using the Merrifield solid-phase procedure with resin cleavages and deprotection of the peptide by anhydrous hydrogen fluoride for 1 h at 0 °C. For removal of N^{ϵ}-trifluoroacetyl groups at positions Lys-1 and Lys-7 of the fragment 1–10, the HF-cleaved peptide was treated with 1 M piperidine in 8 M urea as previously described (Dunn et al., 1974). Standard 1% cross-linked chloromethylated polystyrene (SX-1, Bio-Rad) was used for the synthesis of 11–15. The fragment 1–10 was made by using the recently described solid-phase support [4-(oxymethyl)phenyl]acetamidomethyl-polystyrene, or hydroxymethyl-PAM resin (Kent et al., 1974; Komoriya et al., 1980).

The cleaved and deprotected synthetic peptides were applied to a Sephadex G-25 column (2 × 25 cm) equilibrated with 0.2 M acetic acid. The fractions of the major peptide peak were combined and lyophilized. This crude peptide was purified further by cation-exchange chromatography using sulfopropyl-Sephadex C-25 (2 × 25 cm) equilibrated with 50 mM ammonium acetate, pH 5.2. A linear salt gradient to 200 mM ammonium acetate, pH 5.2, was used. The expected amino acid compositions of purified synthetic subfragments 1–10 and 11–15 were obtained from exhaustive acid hydrolysates (6 N HCl, in vacuo, 110 °C, 24 h) by using a Beckman Model 121 MB automatic analyzer.

Condensation of Synthetic Fragments 1–10 and 11–15. The optimal conditions used for the condensation were 1 mM in RNase-S-(21–124) and 5–20 mM in each synthetic fragment. The components were dissolved in 100 mM morpholineethanesulfonate, pH 6.0, containing 10 mM $CaCl_2$ and 10 mM ascorbate (to activate clostripain). Clostripain was added last for a final concentration of 0.05–0.1 mg/mL (about 10% w/w). The pH was readjusted to 6, a value which, although not optimal for the protease activity, is thermodynamically optimal for synthesis of a peptide bond in organic cosolvent (Homandberg et al., 1978).

Assay for Synthesis. The extent of synthesis was followed by evolution of RNase enzymatic activity. Aliquots of the synthesis solutions were added to 0.9 mM cytidine 2′,3′-phosphate substrate solutions. The absorbance change at 286 nm was compared to those obtained from standard solutions of RNase A. Since it was shown that the native peptides of residues 1–10 and 11–20 do not form active complexes with RNase-S-(21–124) at millimolar concentrations even in the presence of substrate (Allende & Richards, 1962; Finn & Hofmann, 1965), any observed activity above the 0.03% contamination level was taken as due to condensation of the fragments which result in the formation of the active complex [des-16–20]RNase S.

Isolation and Characterization of the Product. The condensation product, synthetic-(1–15), was separated initially from excess unreacted fragments in the form of the noncovalent complex [des-16–20]SRNase S. The clostripain was first inactivated by adjustment of synthesis solutions to 5 mM in ethylenediaminetetraacetic acid. Synthesis mixtures then were passed through Sephadex G-25 (0.9 × 30 cm) equilibrated with 50 mM ammonium bicarbonate. The clostripain and [des-16–20]SRNase S complex eluted in the void volume followed by the great bulk of excess synthetic-(1–10) and -(11–15). A rechromatography removed further contaminating synthetic-(1–10). For some purifications, the complex was isolated by adsorption onto Sepharose–5′-[(4-aminophenyl)phosphoryl]uridine 2′(3′)-phosphate. In either case, the eluted complex was lyophilized, redissolved in water to a concentration of 10 mg/mL, and adjusted to a 5% (v/v) trichloroacetic acid concentration at 0 °C. After equilibration to room temperature, the precipitate was centrifuged and the supernatant was fractionated on a Bio-Rad P-4 (400 mesh, 0.7 × 30 cm) column equilibrated with 1% (v/v) acetic acid.

Results

Clostripain-Catalyzed Fragment Condensation. The reaction to couple subfragments synthetic-(1–10) and -(11–15) was attempted first with solutions of 0.1 mM RNase-S-(21–

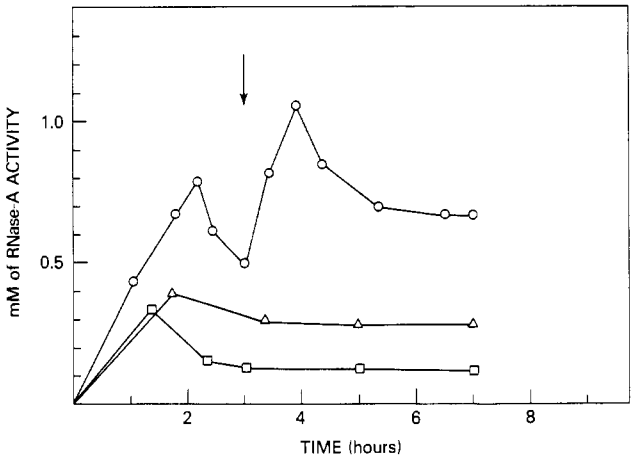

FIGURE 1: Clostripain-catalyzed condensation of synthetic-(1–10) and -(11–15) using RNase-S-(21–124) as the molecular trap. Reaction mixture contained 1 mM RNase-S-(21–124), 1 mg/mL clostripain, and the following synthetic peptides: (○) 20 mM each of 1–10 and 11–15; (△) 20 mM each of [ε-TFA-Lys-1,7](1–10) and (11–15); and (□) 5 mM each of 1–10 and 11–15. After 3 h of reaction (arrow), 1 mM further RNase-S-(21–124) was added in the experiment indicated by open circles. Enzymatic activity was assessed against cytidine 2′,3′-phosphate. A millimolar unit of activity is the activity (ΔOD_{287} per minute) representative of 1 μmol of RNase S/mL of sample.

Table I: Amino Acid Composition of the Product of Enzymatic Fragment Condensation

	amino acid residue (mol/mol of peptide)		
amino acid	fragment 1–10	fragment 11–15	product
Lys	2.17 (2)	0.03 (0)	2.00 (2)
His	0.0 (0)	0.92 (1)	0.95 (1)
Arg	0.75 (1)	0.02 (0)	0.88 (1)
Asx	0.02 (0)	1.12 (1)	1.15 (1)
Thr	1.17 (1)	0.01 (0)	1.00 (1)
Ser	0.0 (0)	1.06 (1)	0.93 (1)
Glx	2.09 (2)	0.99 (1)	2.70 (3)
Ala	3.02 (3)	0.05 (0)	3.20 (3)
Met	0.0 (0)	1.01 (1)	1.06 (1)
Phe	1.00 (1)	0.01 (0)	0.91 (1)

124) and 0.1–1 mM of each synthetic subfragment. No synthesis was observed at these concentrations. Addition of cytidine 2′-phosphate, a potential complex-stabilizing component, to a final concentration of 2 mM had no effect. However, increasing the concentrations of RNase-S-(21–124) to 1 mM and fragments to 1–20 mM did allow condensation. The reaction progress curves of Figure 1 indicate that several events occur during the condensation. The initial increase in activity reflects synthesis of 1–15 and its complexation with RNase-S-(21–124). However, the following descending portions of the curves most likely reflect a competing proteolytic inactivation of 21–124 (see below). The plateau level of synthesis shown in Figure 1 is constant for several days. If a second equivalent of RNase-S-(21–124) is added, the rise and partial fall of activity repeat. As noted additionally in Figure 1, we observed reduced but finite condensation of 11–15 with 1–10 peptide which was trifluoroacetylated at Lys residues 1 and 7. The reduced condensation of blocked vs. unblocked 1–10 may reflect reduced binding of blocked product by the trap, although differences in substrate nature or solubility of the blocked peptide (turbidity was noted) cannot be ruled out.

Isolation of Resynthesis Product. The resynthesis product 1–15 was dissociated from RNase-S-(21–124) by addition of

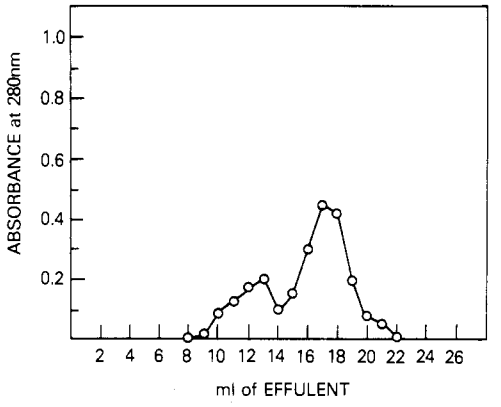

FIGURE 2: Chromatographic isolation of resynthesis product 1–15. The isolated complex, [des-16–20]SRNase S, derived from condensation of 1–10 and 11–15 using 21–124 as the trap was dissociated with trichloroacetic acid into the resynthesis product 1–15 [soluble in 5% (v/v) Cl_3AcOH solution] and 21–124 (precipitated). The supernatant was applied to a Bio-Rad P-4 (400 mesh, 0.7 × 30 cm) column equilibrated with 1% acetic acid.

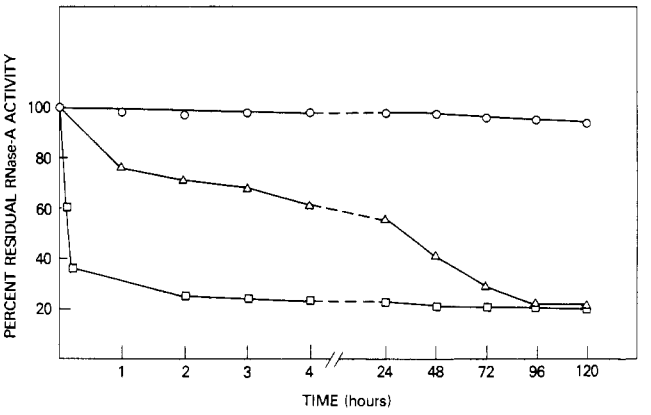

FIGURE 3: Evaluation of RNase-S-(21–124) as a proteolytically suitable molecular trap. Clostripain (1 mg/mL) was reacted with 11 mg/mL RNase A (○), RNase S (△), and RNase-S-(21–124) (□). Activity was measured and units were calculated as in the legend to Figure 1.

trichloroacetic acid (5% v/v final concentration) to the complex solution. Bio-Rad P-4 chromatography of the supernatant, shown in Figure 2, yielded two main peaks. The first eluted peak was identified as product 1–15 and the second as 1–10, in both cases by amino acid composition (see Table I). A 50% recovery of formed 1–15 was obtained in the combined precipitation–chromatography procedure based on the amount of peptide expected from the enzymatic activity exhibited by the final condensation reaction mixture. The product behaved identically with synthetic-(1–15) derived from total solid-phase synthesis (Dunn et al., 1974) when subjected to high-voltage paper electrophoresis on Whatman 3MM paper using a Gilson Model D electrophorator at 2 kV for 90 min in pyridine–acetate buffer, pH 6.5, and when fractionated by Bio-Gel P-4 filtration.

Proteolysis of Reaction Components. It was expected that complications in the fragment restitching reaction could arise from proteolysis of the trap RNase-S-(21–124), either alone or as part of the trapped complex (1–15)·(21–124). The data in Figure 3 show the effect of clostripain on RNase-S-(21–124), RNase A, and RNase S (which should have essentially the same proteolytic susceptibility as the complex of synthetic-(1–15) with RNase-S-(21–124). Results with RNases A and S were observed directly by assay of aliquots with ribonuclease substrate cytidine 2′,3′-phosphate. The effect on RNase-S-(1–124) was determined by adding a 3-fold molar

excess of RNase-S-(1–20) to aliquots of the RNase-S-(21–124) hydrolysis mixture and assaying the resultant mixture for RNase S activity against cytidine 2′,3′-phosphate. Note that although RNase-S-(21–124) is hydrolyzed very rapidly under these conditions, the hydrolysis decreases to a final activity of 20% of initial activity. This activity was maintained for at least 7 days, during which clostripain was still active. Three cycles of manual dansyl–Edman N-terminal sequence analysis of clostripain-treated RNase-S-(21–124) provided evidence that inactivation resulted from cleavage at Arg-33. The profiles of Figure 3 also show that RNase S is hydrolyzed much more slowly than RNase-S-(21–124), although eventually to the same 20% residual activity. This stabilization to proteolysis observed for RNase-S-(21–124) due to binding of the amino-terminal fragment apparently is sufficient to allow accumulation of a complex of synthetic-(1–15) and RNase-S-(21–124) in the resynthesis reactions of Figure 1.

Aside from stability of the molecular trap itself, a second concern was the susceptibility of the synthetic peptide fragment to hydrolysis. With the synthetic-(1–10) fragment used in this work, lysine is present at positions 1 and 7. The protonated α-amino group on Lys-1 would make the Lys-1–Glu-2 bond a poor substrate. The Lys-7 bond may be hydrolyzed by clostripain, but only at about 1% the rate of typical arginyl bond hydrolysis (Schenkein et al., 1977; Gilles et al., 1979). However, control experiments showed that when 20 mM synthetic-(1–10) and 0.2 mg/mL clostripain were incubated for up to 10 h (condition in Figure 1), no new peptide forms were detected by high-voltage paper electrophoresis.

Discussion

A general problem in the chemical synthesis of polypeptides is stereospecific fragment condensation. Usually, such condensations need to be directed by blocking all or most side chain and main chain functional groups which are not desired for reaction, leaving only the α-amino of the potential carboxyl-terminal component and the α-carboxyl of the potential amino-terminal component free for peptide bond formation. While such a process usually is facile for small peptides, many fragment condensations, especially for large peptides, are impeded by problems of solubility of protected fragments, complexity of protection and deprotection steps, and low yields in peptide bond formation between protected peptides. Within this context, methods of fragment condensation which avoid side chain protection can be of special appeal in synthesizing large polypeptides and proteins. It is with this in mind that the present work was undertaken to examine the use of enzymatic peptide bond synthesis using proteases to condense nonassociating fragments, the case representative of the most common peptide synthesis demand.

The present data show how the two RNase fragments 1–10 and 11–15, peptides which would not be expected to be condensed noncatalytically in high yield (DiBello & Tonellato, 1981; DiBello et al., 1978), were driven to condense with clostripain catalysis by trapping the product with RNase-S-(21–124). The initial concentration of both the trap and substrates was found to be very important. At 0.1 mM 21–124 and up to 1.0 mM synthetic peptides, no condensation was observed. Nonetheless, the yield effected at higher concentrations of reactants (Figure 1) is high enough to be practical. Further, the substrates 1–10 and 11–15 can be reused (during the incubation period, there was no detectable hydrolysis of the Lys-7–Phe-8 bond in 1–10). It should be noted that only active 1–15 can be formed in this system, since any inactive peptide, if formed at all, is not pulled out of the equilibrium by forming a stable RNase S complex but instead would be rehydrolyzed. The present study denotes a basic limitation in using polypeptide kinetic traps for protease-catalyzed fragment condensation. The efficiency of RNase-S-(21–124) was reduced by clostripain hydrolysis (about 80%) of 21–124 at the Arg-33 bond.[2] The hydrolyzed 21–124 product was found to be inactive. The failure of this inactive species to bind S-peptide likely is responsible for the less than quantitative resynthesis of 1–15 observed.

The present study also emphasizes the need in protease-catalyzed fragment condensation to use enzymes with substantially limited specificity. For the (1–10)–(11–15) condensation, use of a relatively arginyl-specific proteinase was a virtual necessity. Interestingly, the arginine-specific protease from mouse submaxillary gland was found to catalyze the condensation of peptides much more slowly than the other arginine-specific protease, clostripain. Had trypsin been used, the Lys-7–Phe-8 bond in S-peptide and many bonds in S-protein eventually could have been hydrolyzed. Many of the highly specific proteases being defined from a variety of cascade, precursor processing, and other biological systems offer tools of potential interest in future enzymatic resyntheses.

The use of a molecular trap in the RNase S system to drive the unfavorable enzymatic condensation of two unassociated peptide fragments can be compared with previously described fragment condensations achieved by protease catalysis.

case I

$$\text{RNase S} \rightleftharpoons \text{RNase A}$$

K_{syn} = [RNase A]/[RNase S] = 0.4 in water (% synthesis in 90% glycerol is 50%)

case II

$$\text{Cbz-Trp} + \text{GlyNH}_2 \rightleftharpoons \text{Cbz-TrpGlyNH}_2$$

$K_{syn} = [\text{Cbz-TrpGlyNH}_2]/([\text{Cbz-Trp}][\text{GlyNH}_2]) = 0.45$ M^{-1} in water (% synthesis in 85% 1,4-butanediol is 80%)

Cases I and II illustrate organic cosolvent induced condensation of associated and nonassociated fragments, respectively. Both systems rely on the ability of the organic cosolvent to elevate the pK of the carboxylate derived from the hydrolyzed peptide bond and hence to raise the free energy of peptide bond hydrolysis. Thereby, the equilibrium is shifted toward peptide bond synthesis. The case I system requires a compromise: the cosolvent must lower the dielectric constant enough to elevate the pK but not enough to dissociate the complex and change the equilibrium to that of case II. Thus, while 1,4-butanediol can effect a lower dielectric constant than glycerol, glycerol effects greater synthesis in case I because it is not as disruptive to the RNase S complex. In case I, synthesis will be independent of protein concentration, assuming that the concentration is above the fragment dissociation constant of RNase

[2] The hydrolysis of 21–124 at the Arg-33 bond during the inactivation of RNase-S-(21–124) is interesting in itself. Partial characterization of products of clostripain hydrolysis of RNase-S-(21–124) suggests that, during proteolysis, an equilibrium exists between two forms, active (intact) RNase-S-(21–124) and an inactivated form, with a ratio of these two being about 1:4. The fact that hydrolysis is limited to 80% (Figure 3) suggests (but does not prove) that an equilibrium exists between hydrolyzed and intact 21–124. If an equilibrium does exist, the relatively low hydrolysis equilibrium constants could be due to the disulfide bonds on either side of Arg-33 (Cys-26–Cys-86 and Cys-40–Cys-95), which could stabilize the region against hydrolysis and therein prevent entropy gaining processes. This can be compared to the case of protein proteinase inhibitors, the latter of which retain their rigidity when hydrolyzed and have hydrolysis equilibrium constants near unity.

S. A 50% formation of ribonuclease A has been achieved (Homandberg & Laskowski, 1979) by equilibrating the system with subtilisin in 90% glycerol. In case II, there is a dependence of synthesis on reactant concentration. The cosolvent 1,4-butanediol was used to lower the dielectric constant as much as possible to effect synthesis. Use of this solvent and the effect of mass action have provided an increase in K_{syn} of nearly 100-fold. Thus, in 85% 1,4-butanediol at 1 mM Cbz-Trp and 100 mM $GlyNH_2$, chymotrypsin can catalyze the synthesis to nearly 80% Cbz-Trp$GlyNH_2$ (Homandberg et al., 1978). While large concentrations are reasonable for Cbz-Trp and $GlyNH_2$, such concentrations for large synthetic peptides and protein fragments are quite impractical if not impossible to achieve. Thus, mass action generally is not an avenue open for fragment condensation. If the concentration of both substrates in the Cbz-Trp$GlyNH_2$ system in 1,4-butanediol were 1 mM, a concentration typically used in systems such as case I, only 3.7% synthesis would occur. At the fragment concentration used here in the (1–10)–(11–15) case, only 0.05% synthesis would be expected if the resynthesis were done in water. The 100-fold increase in the product achieved here in water was attributable directly to introduction of the 21–124 trap.

In a related study, carboxypeptidase-catalyzed addition of single amino acids to modified protease inhibitor has been assisted by coupling this reaction to complexation of reconstituted inhibitor with a protease which recognizes the intact inhibitor (Sealock & Laskowski, 1969). The protease acts as a trap, making this type of process of amino acid–peptide condensation quite analogous to that of peptide–peptide condensation achieved in the present work.

The use of molecular traps for condensation of unassociated peptides may well prove to have at least somewhat general application. Syntheses such as that achieved in the present study can be expected to be achievable for other noncovalently associated fragment complexes. In such cases, one of the component fragments can be used as a trap for condensation of subfragments of the other component of the complex. Noncovalent fragment complexes have been derived from a number of proteins, including, in addition to bovine pancreatic RNase (Richards & Vithayathil, 1959; Lin et al., 1970), staphylococcal nuclease (Taniuchi et al., 1967; Taniuchi & Anfinsen, 1969), cytochrome *c* (Harris & Offord, 1977; Parr et al., 1978; Juillerat et al., 1980), immunoglobulin G (Hochman et al., 1976), somatotropin (Li & Bewley, 1976), barnase (Hartley, 1977), and *Escherichia coli* β-galactosidase (Langley & Zabin, 1979), to name a few. However, proteins of the above class represent a somewhat special case, not typical for all proteins and protein fragments. A more general potential tactic for nonassociating fragment condensation could be the use of antibodies as traps. Subpopulations of antibodies to polypeptides could be obtained which are specific for the product of subfragment condensation but not for the reactant subfragments alone. The use of nonprotein traps (metals or other ligands), when these can interact with the products of condensation, also could be helpful. It remains to be shown experimentally how valuable enzymatic fragment condensation will be as a general tool in the chemical synthesis of polypeptides and proteins. Nonetheless, the present study clearly indicates its feasibility for many peptides of native protein sequence.

References

Allende, J. E., & Richards, F. M. (1962) *Biochemistry 1*, 295–304.

Chaiken, I. M., & Taylor, H. C. (1976) *J. Biol. Chem. 251*, 2044–2048.

DiBello, C., & Tonellato, M. (1981) in *Peptides 1980* (Brunfeldt, K., Ed.) pp 355–358, Scriptor, Copenhagen.

DiBello, C., Marigo, A., & Pandin, M. (1978) in *Semisynthetic Peptides and Proteins* (Offord, R. E., & DiBello, C., Eds.) pp 373–379, Academic Press, London.

Dunn, B. M., DiBello, C., Kirk, K. L., Cohen, L. A., & Chaiken, I. M. (1974) *J. Biol. Chem. 244*, 6295–6301.

Finn, F. M., & Hofmann, K. (1965) *J. Am. Chem. Soc. 87*, 645–651.

Gilles, A., Imhoff, J., & Keil, B. (1979) *J. Biol. Chem. 254*, 1462–1468.

Harris, D. E., & Offord, R. E. (1977) *Biochem. J. 161*, 21–25.

Hartley, R. W. (1977) *J. Biol. Chem. 252*, 3252–3254.

Hochman, J., Gavish, M., Inbar, D., & Givol, D. (1976) *Biochemistry 15*, 2706–2710.

Hofmann, K., Finn, F. M., Limetti, M., Montibeller, J., & Zanetti, G. (1966) *J. Am. Chem. Soc. 88*, 3633–3639.

Homandberg, G. A., & Laskowski, M., Jr. (1979) *Biochemistry 18*, 586–592.

Homandberg, G. A., & Chaiken, I. M. (1980) *J. Biol. Chem. 255*, 4903–4909.

Homandberg, G. A., Mattis, J. A., & Laskowski, M., Jr. (1978) *Biochemistry 17*, 5220–5227.

Homandberg, G. A., Komoriya, A., Juillerat, M., & Chaiken, I. M. (1980) in *Peptides, Structure and Biological Function* (Gross, E., & Meinhofer, J., Eds.) pp 597–600, Pierce Chemical Co., Rockford, IL.

Juillerat, M., & Homandberg, G. A. (1981) *Int. J. Pept. Protein Res.* (in press).

Juillerat, M., Parr, G. R., & Taniuchi, H. (1980) *J. Biol. Chem. 255*, 845–853.

Kent, S. B. H., Mitchell, A. R., Engelhard, M., & Merrifield, R. B. (1974) *Proc. Natl. Acad. Sci. U.S.A. 76*, 2180–2184.

Komoriya, A., Homandberg, G. A., & Chaiken, I. M. (1980) *Int. J. Pept. Protein Res. 16*, 433–439.

Langley, K. E., & Zabin, I. (1976) *Biochemistry 15*, 4866–4875.

Li, C. H., & Bewley, T. A. (1976) *Proc. Natl. Acad. Sci. U.S.A. 73*, 1476–1479.

Lin, M. C., Gutte, B., Moore, S., & Merrifield, R. B. (1970) *J. Biol. Chem. 245*, 5169–5170.

Parr, G. R., Hantgan, R. R., & Taniuchi, H. (1978) *J. Biol. Chem. 253*, 5381–5388.

Potts, J. T., Jr., Young, D. M., & Anfinsen, C. B. (1963) *J. Biol. Chem. 238*, 2593–2594.

Richards, F. M., & Vithayathil, P. J. (1959) *J. Biol. Chem. 234*, 1459–1465.

Schenkein, I., Levy, M., Franklin, E. C., & Frangione, B. (1977) *Arch. Biochem. Biophys. 182*, 64–70.

Sealock, R. W., & Laskowski, M., Jr. (1969) *Biochemistry 8*, 3703–3710.

Taniuchi, H., & Anfinsen, C. B. (1969) *J. Biol. Chem. 244*, 3864–3875.

Taniuchi, H., Anfinsen, C. B., & Sodja, A. (1967) *Proc. Natl. Acad. Sci. U.S.A. 58*, 1235–1242.