Polypeptides with supersecondary structures as templates in rational catalyst design. Catalysis of self functionalization by designed helix–loop–helix motifs

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Three designed polypeptides with 42 amino acids each, SA-42, RA-42 and PA-42, were engineered to catalyse acyl-transfer reactions of mono-*p*-nitrophenylfumarate I. The second-order rate constants of the peptides for the formation of *p*-nitrophenol were larger than that of the background reaction with factors of 331, 1750 and 1937, respectively. The background reaction is the reaction between I and trifluoroethoxide ion to form the trifluoroethyl ester. The second-order rate constant for the 4-methylimidazole catalysed reaction between I and trifluoroethoxide ion was 211 times larger than that of the background reaction and the similarity in rate constants suggests that the histidine residues in the polypeptides act as nucleophilic catalysts. The observed rate increases in RA-42 and PA-42 over that of SA-42 identifies the location of the reaction centre. The identity of the reaction products in the RA-42 catalysed reaction was established by NMR spectroscopy and mass spectrometry. The side chain of ornithine-15 was acylated by the fumaryl residue. Thus, in a fast and selective second reaction step the fumaryl group was transferred from histidine-11 to the side chain of ornithine-15. The ability of a designed helix–loop–helix motif, RA-42, to catalyse its own functionalization is thereby demonstrated for the first time.

The understanding of why proteins fold has now reached a level where it is possible, although difficult, to successfully design polypeptides and small proteins.[1,2] Recently a designed four helix bundle, a helix–loop–helix dimer, was shown to have some characteristics of a natural protein for the first time.[3] The design of functionalized proteins is therefore timely. A polypeptide with well-defined and known solution structure is a good template for the introduction of catalytically active sites. Binding residues may be selected from a large number of natural and artificial amino acids and the synthesis of polypeptides with 40–50 amino acids is clearly feasible with automated peptide synthesizers. Designed polypeptides that fold in solution into supersecondary structures therefore have great potential in the design of tailor-made catalysts. So far, only a small number of designed catalytically active polypeptides have been reported in the literature,[4–5] and in only one case is the structural basis for its reactivity at least partially understood.[6]

We recently reported on SA-42, a designed 'template' polypeptide with 42 amino acids that forms an antiparallel helix–loop–helix hairpin dimer in solution[7] (Fig. 1 and Fig. 2). The solution structure was determined by NMR spectroscopy, CD spectroscopy and equilibrium sedimentation ultracentrifugation. SA-42 was designed to catalyse acyl transfer reactions of esters. In order to improve the binding site and to enhance the reactivity of the catalyst, two polypeptides, RA-42 and PA-42, were designed based on the structure of SA-42. Their sequences differ in three and five positions, respectively, from that of SA-42, Fig. 1. The modifications of the amino acid sequences were designed to introduce new functionality close to that of the histidine in the folded peptide. Here we wish to report that the designed polypeptides SA-42, RA-42 and PA-42 catalyse acyl-transfer reactions of mono-*p*-nitrophenylfumarate **I**, the latter two significantly more efficiently than SA-42. In addition, the capacity of RA-42 for catalysing its own functionalization represents a novel way of *in situ* modification of polypeptides with supersecondary and tertiary structures with regeneration of the catalytically active residue.

N-Aib-A-D-Nle-E-A-A-I-K-A-L-A-E-H-Nle-Aib-A-K
1 19
G-P-V-D
20 23
G-Aib-R-A-F-A-E-F-A-K-A-L-Q-E-A-Nle-Q-A-Aib
42 24

SA-42

N-Aib-A-D-Nle-E-A-A-I-K-**H**-L-A-E-**Orn**-Nle-Aib-A-K
1 19
G-P-V-D
20 23
G-Aib-R-A-F-A-E-F-**Orn**-K-A-L-Q-E-A-Nle-Q-A-Aib
42 24

RA-42

N-Aib-A-D-Nle-E-A-A-I-**A**-**Orn**-L-A-E-H-Nle-Aib-A-K
1 19
G-P-V-D
20 23
G-Aib-R-A-F-A-E-F-**Orn**-**A**-A-L-**Orn**-E-A-Nle-Q-A-Aib
42 24

PA-42

Fig. 1 Amino acid sequences of SA-42, RA-42 and PA-42. The residues presented underlined in bold in RA-42 and PA-42 are the only ones that have been changed in comparison with those in SA-42. The one letter code for amino acids is used where A is Ala, D is Asp, E is Glu, F is Phe, G is Gly, H is His, I is Ile, K is Lys, L is Leu, N is Asn, P is Pro, Q is Gln, R is Arg, V is Val. Aib is α-aminoisobutyric acid and Nle is norleucine.

Results

The design of SA-42 has been described in detail previously.[7] In short, it is made up of two amphiphilic helices connected by a

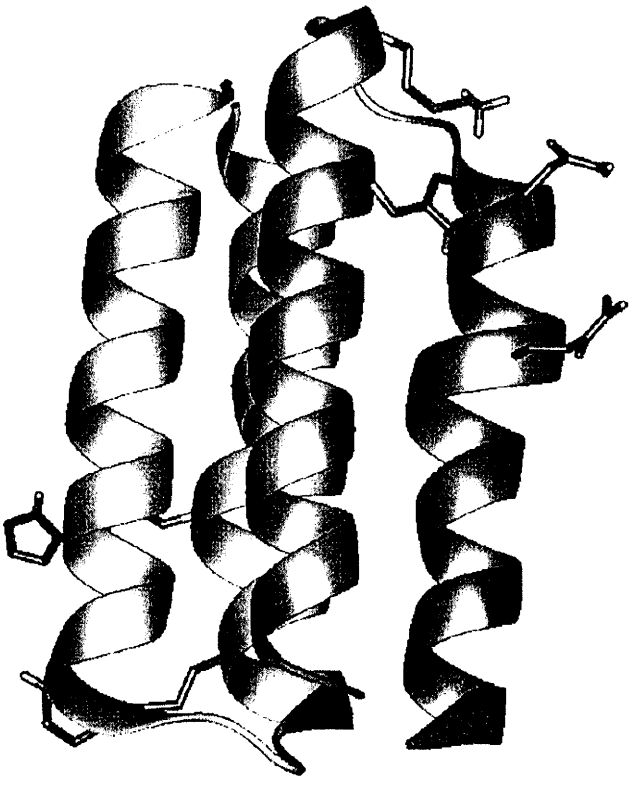
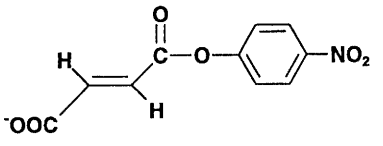


Fig. 2 The solution structure of the designed polypeptide SA-42.[7] The amino acid sequence was designed to fold into two amphiphilic helices connected by a short loop. The hydrophobic interactions provide the main driving force for formation of the hairpin motif and for dimerisation. In the folded polypeptide a binding site is formed involving both helices on the solution exposed sides of the dimer. The reaction centre can therefore interact with the substrate and if it is complementary to the transition state it can catalyse the reaction.



I

short loop. In the design of the helices special attention was paid to helix dipole stabilisation,[8] the helix propensity of the selected amino acid residues,[9] intrahelical salt bridges[10] and N- and C-terminal capping.[11] The design of the hairpin motif was based on the expected hydrophobic interactions between the amphiphilic helices. The hydrophobic interactions were also expected to provide the driving force for dimerisation. The designs of RA-42 and PA-42 were based on the solution structure of SA-42. The ^{1}H NMR NOESY spectrum of SA-42[7] showed resolved cross peaks connecting the ring protons of Phe-35 and Phe-38 with several methyl groups of the residues in the hydrophobic core. It was concluded from these cross peaks that SA-42 formed a hairpin helix–loop–helix motif. In the present investigation corresponding cross peaks were observed in the NOESY spectra of RA-42 and of PA-42 showing that these peptides, too, form the designed motifs.

The substrate **I** was chosen because it is an anion at pH 5.85 and in the activated complex a second charge is developing. The binding properties of positively charged amino acid residues could therefore be tested. The designed binding sites contain a histidine as a nucleophilic catalyst and residues that can provide binding stabilisation in the activated complex, Fig. 3. In SA-42 a protonated lysine and two glutamine residues were designed to bind to the developing oxyanion and to the carboxylate anion, respectively. In order to form stronger interactions ornithine side chains in RA-42 and PA-42 were introduced to bind to the developing oxyanion and to the carboxylate anion. The side chain of ornithine is protonated, and carries a positive charge, at the pH used in the kinetic measurements. In the amino acid sequence of RA-42 only three amino acid residues were changed in comparison with that of SA-42, and in the sequence of PA-42 only five, Fig. 1. The introduced amino acid residues were designed to increase the catalytic activity of RA-42 and PA-42 in comparison with that of SA-42 in acyl-transfer reactions.

In 10% trifluoroethanol (TFE) (v/v, 1.39 mol dm^{-3}) in 50 mmol dm^{-3} Bis-Tris buffer, pH 5.85, at 290.2 K **I** reacts with the trifluoroethoxide ion to form the trifluoroethyl ester and *p*-nitrophenol. No fumaric acid was observed in the ^{1}H NMR spectrum of the reaction products. The rate of disappearance of **I** in the presence of SA-42, RA-42 and PA-42 was studied at pH 5.85 and 290.2 K in 10% aqueous TFE (v/v). The reaction rates, Table 1, were determined from the rate of formation of *p*-nitrophenol by following the increase in absorbance at 320 nm by UV spectroscopy. Trifluoroethanol was used in order to provide a nucleophile (trifluoroethoxide ion) that could compete for the reactive acyl intermediate. The effect of trifluoroethanol on the structure of RA-42 is to increase the helical content somewhat but the effect on rate enhancements is negligible.

Under conditions of excess peptide over substrate excellent pseudo first-order kinetics were observed, and the reaction rate was proportional to the concentration of peptide in the range from 0.6–1.2 mmol dm^{-3}. Over that concentration range the nature of the reactive species does not change. The reactive species is probably the dimer and it is clearly not the monomer as the reaction rate is proportional to the concentration of peptide and not to the square root of the concentration of peptide. It cannot be excluded (although it is unlikely) that some higher aggregate is the reactive species but the template polypeptide SA-42 has been shown to be a dimer in this concentration range. No sign of saturation kinetics was observed.

In SA-42, Fig. 3, the designed reaction centre is made up of His-15, Gln-26, Gln-30 and Lys-19, although Lys-19 is also in the position to stabilise the helical dipole of helix **I** and may be a poor transition state stabiliser. The second-order rate constant of SA-42 is 5.3 ± 0.2 × 10^{-3} dm^3 mol^{-1} s^{-1} which is comparable to that of 4-methylimidazole, a compound that is structurally similar to the side chain of histidine. The measured second-order rate constant, k_2 (observed rate constant divided by total concentration of 4-methylimidazole), for 4-methylimidazole is 3.38 ± 0.04 × 10^{-3} dm^3 mol^{-3} s^{-1}.

In RA-42, Fig. 3, the reaction centre consists of His-11, Orn-15 (ornithine) and Orn-34, and the second-order rate constant is 2.85 ± 0.09 × 10^{-2} dm^3 mol^{-1} s^{-1}.

For PA-42 the reaction centre is made up of His-15, Orn-11, Orn-30 and Orn-34. The second-order rate constant is 3.16 ± 0.09 × 10^{-2} dm^3 mol^{-1} s^{-1}.

The second-order rate constant for the reaction of **I** in 1.39 mol dm^{-3} TFE to give the TFE ester is 1.57 ± 0.10 × 10^{-5} dm^3 mol^{-1} s^{-1}.

Under conditions of excess substrate over peptide the data could not be fitted to a single exponential function. The ^{1}H NMR spectrum of a solution containing RA-42 and **I** recorded under reaction conditions showed that the substrate reacted with the peptide and formed a covalent bond, Fig. 4. The nature of the reaction products was therefore investigated. The presence of *p*-nitrophenol was identified from the ^{1}H NMR and UV–VIS spectra. The electrospray mass spectrum showed a reaction product with a molecular weight of 4473 which corresponded to the sum of the molecular weights of RA-42 (4375) and fumaric acid (116.07) less the molecular weight of water (18.01). Since RA-42 contains no hydroxy groups that can form esters it was suggested that an amide had been formed. The mass spectrum showed no species with a molecular weight

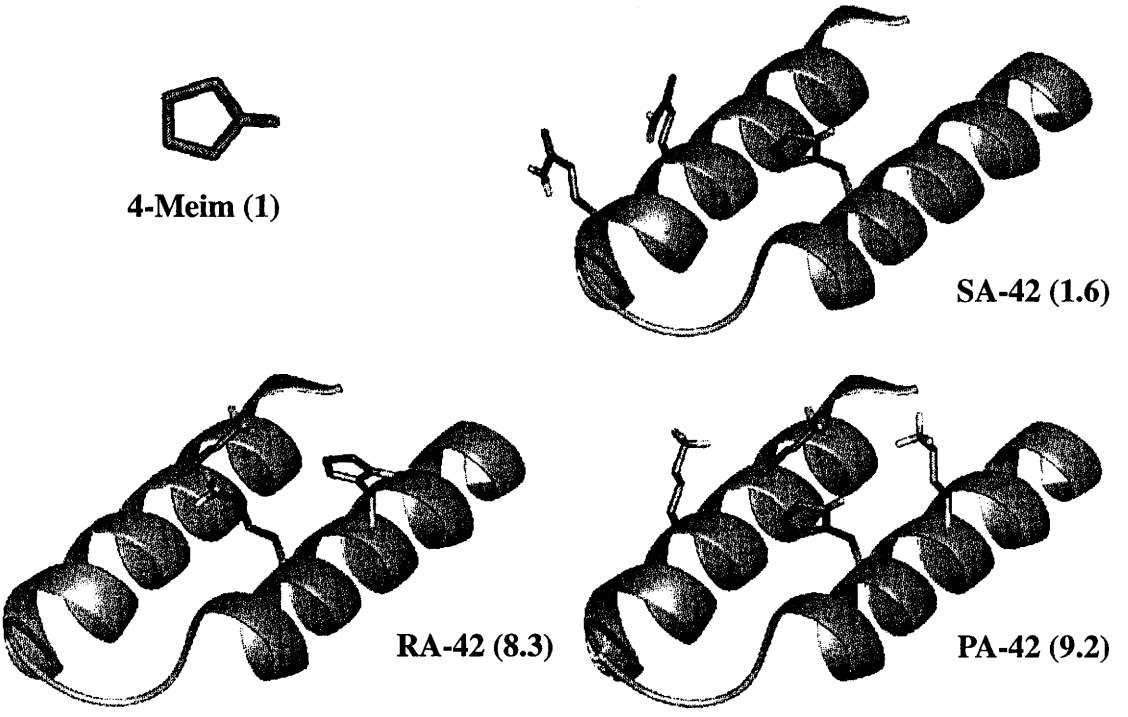


Fig. 3 Schematic representation of the reaction centres of SA-42, RA-42 and PA-42. Dimerisation is not shown for the sake of clarity. The relative rates of reaction are included (within brackets) for comparison with 4-methylimidazole and the measured rate constants are given in the text. The ornithines close to the histidines are positively charged as they are protonated at the pH at which the reaction is carried out. The histidines in SA-42, RA-42 and PA-42 are protonated to different extents depending on the pK_a values. Random coil pK_a values [18] for Orn can be estimated to be close to that of Lys which is 10.4, and that of His is 6.4.

Table 1 Second-order rate constants for the acyl transfer from mono-*p*-nitrophenylfumarate at pH 5.85, 290.2 K in 10 vol% trifluoroethanol in water

Catalyst	$k_2/10^{-3}$ dm^3 mol^{-1} s^{-1}	Relative rate[a]
4-Methylimidazole	3.38	211
SA-42	5.3	331
RA-42	28	1750
PA-42	31	1937

[a] Second-order rate constant relative to that of the reaction of **I** in 1.39 mol dm^{-3} TFE, ion, 1.6 × 10^{-5} dm^3 mol^{-1} s^{-1}.

of RA-42 and all of the peptide had therefore reacted with the substrate. The ^{1}H NMR TOCSY spectrum, Fig. 5, showed that the chemical shifts of the side chain protons of Orn-15 had changed, which was to be expected if a protonated primary amine was transformed into an amide. It was thus concluded that RA-42 had been modified at the side chain of Orn-15, in a highly selective reaction. The side chains of Orn-34 and Lys-10 were not affected. The resonances of the side chains of Lys-19 and Lys-33 overlapped and their intensities could not be separately analysed. No imidazolide-like intermediate was observed in the ^{1}H NMR spectrum of RA-42 during the course of the reaction.

Discussion

I reacts with nucleophiles to produce *p*-nitrophenol. In 10% TFE at pH 5.85 **I** reacts with the trifluoroethoxide ion [12] to form the trifluoroethyl ester. In the presence of SA-42 the reaction rate is enhanced and the second-order rate constant of SA-42 is 331 times larger than that for the reaction of **I** in 1.39 mol dm^{-3} TFE, Table 1. 4-Methylimidazole catalyses the reaction with a 211-fold enhancement of the second-order rate constant which suggests that the imidazole moiety of His-15 is the reactive amino acid residue of SA-42. This has recently been shown to be the case from the pH dependence of the second-order rate constants.[13] The first step of the reaction of SA-42 is therefore the formation of an acylated side chain of histidine, an acyl intermediate. The second-order rate constants for the formation of *p*-nitrophenol in the presence of RA-42 and PA-42 are 1750 and 1937 times faster, respectively, than that of the reaction of **I** in 1.39 mol dm^{-3} TFE. SA-42 contains one histidine and the introduction of ornithine residues in the vicinity of the histidine, Fig. 3, led to more reactive polypeptides. Since the only modifications of the peptide sequences were those in the immediate vicinity of the histidine the excess rate enhancements of RA-42 (a factor of 8.3) and PA-42 (a factor of 9.2) over that of SA-42 provides evidence for the location of the reaction centre.

The pK_a of His-11 of RA-42 is 6.45 in 10% trifluoroethanol (v/v) at 293 K and the pK_a of the 4-methylimidazolium ion in water at 293 K is 7.95. The pK_a of the latter can therefore be estimated to be close to 8 in 10% trifluoroethanol. Since the Brønsted coefficient β for imidazole-catalysed hydrolysis of *p*-nitrophenyl acetate is 0.8, the observed rate enhancement of RA-42 over that of 4-methylimidazole is mainly due to transition state stabilization and to a lesser degree due to a pK_a depression effect.[13]

The observed rate enhancements upon introduction of ornithines show that the side chains of the ornithine residues provide transition state binding and catalyse the formation of the acyl intermediate. In RA-42 Orn-15 can hydrogen bond to the developing oxyanion and Orn-34 can hydrogen bond to the carboxylate anion. In PA-42 Orn-11 can hydrogen bond to the developing oxyanion and Orn-30 or Orn-34 can hydrogen bond to the carboxylate anion. The contribution from each of the binding residues remains to be elucidated.

It was shown by NMR spectroscopy that the reaction product in the case of RA-42 was a polypeptide with an amide formed at the side chain of Orn-15. The reaction mechanism has recently been determined and it was shown that the acyl group was transferred from the side chain of His-11 to the side chain of Orn-15 in an intramolecular reaction that regenerated the unsubstituted histidine residue.[13] The excess rate enhancement

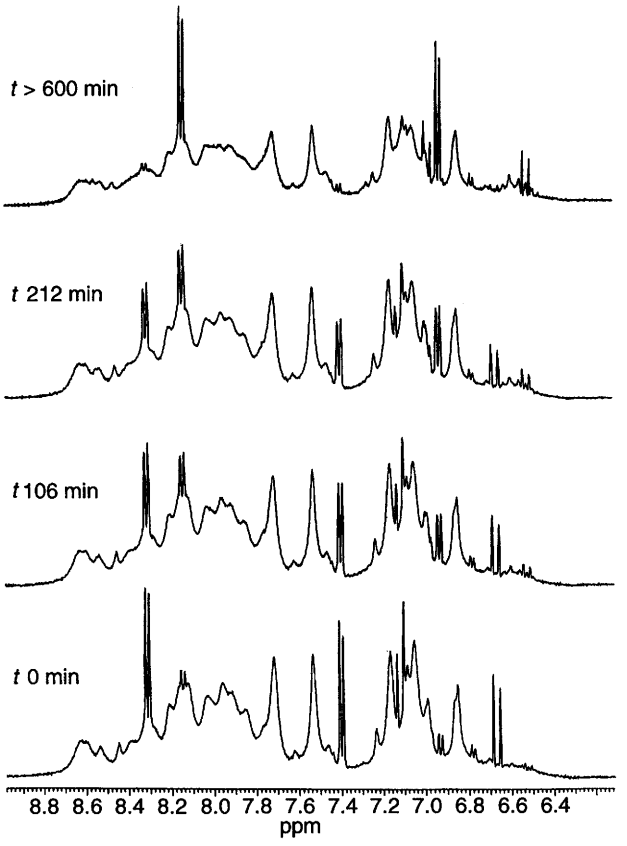


Fig. 4 Part of the ^{1}H NMR spectrum of RA-42 and **I** in 10% TFE 50 mmol dm^{-3} Bis-Tris buffer in H_2O–D_2O 90 : 10 (v/v) at pH 5.85 at 0, 106, 212 and >600 min of reaction. Under the reaction conditions the reaction of the trifluoroethoxide ion with **I** competes with the reaction of RA-42 with **I**. In the spectrum recorded at the start of reaction (t = 0) the resonances of the protons of the *p*-nitrophenyl moiety of **I** are clearly visible at 7.40 and 8.31 ppm and those of the ethylenic protons appear at 6.67 and 7.12 ppm. Note the large *trans* coupling constant of 16 Hz. As the reaction proceeds **I** is consumed and *p*-nitrophenol is produced (6.94 and 8.13 ppm) as well as the trifluoroethyl ester (6.53 and 6.97 ppm) of fumaric acid. The TFE ester is the product of the reaction of the trifluoroethoxide ion and **I**. The observation of a new spin system with a *trans* ethylenic coupling constant is evidence for the appearance of a new monosubstituted fumaric acid derivative. The spectrum of fumaric acid is a single peak for symmetry reasons. However, the intensities of the resonances at 6.53 and 6.97 ppm are less than half of those of *p*-nitrophenol and another product has to be formed. A broadened doublet appears at 6.58 ppm due to the amide formed at the side chain of Orn-15.

of RA-42 over that of SA-42 in the reaction with **I** was lost upon transformation of the ornithine side chain to an amide. The loss of excess rate enhancement when the positively charged hydrogen bond donor (side chain of Orn-15) was transformed into a weaker and neutral hydrogen bond donor (the amide formed at the side chain of Orn-15) was in agreement with the proposed role of the ornithine. However, it cannot be ruled out at this point that the introduction of a fumaryl group has a more subtle effect.

The intramolecular acyl-transfer reaction is much faster than the initial attack by histidine on **I**, and no direct evidence for the formation of an acyl intermediate was obtained from the ^{1}H NMR spectrum. From the reported reactivity of acetylimidazole towards nucleophiles[12] one would not expect to observe an acyl intermediate since acetylimidazole is more reactive than *p*-nitrophenylesters.[14] However, in 1.39 mol dm^{-3} TFE at pH 5.85, the trifluoroethoxide ion would have been expected, from the rates of the intermolecular reactions, to compete successfully for the acyl intermediate with the amine side chains of lysines and ornithines in the polypeptides and form exclusively the trifluoroethyl ester.[12] Instead, as far as can be estimated from the ^{1}H NMR spectrum, Fig. 4, no trifluoroethyl ester is formed *via* the acyl intermediate, only the amide, and the intramolecular reaction therefore has to be very fast. The effective molarity of amine is high although its magnitude cannot be determined since the reaction rate for the second step cannot be directly measured. Competition from a more reactive nucleophile than the trifluoroethoxide ion would have to be used to determine the rate of reaction in the acyl transfer from His-11 to Orn-15. The formation of amide from **I** and the side chain of Orn-15 is therefore catalysed by His-15.

The rate enhancement of the overall reaction at pH 5.85 may be estimated from the rate of aminolysis of *p*-nitrophenyl acetate by an aliphatic primary amine.[15] The second-order rate constant for the reaction of ethylamine (pK_a 10.97) with *p*-nitrophenyl acetate is 5.74 dm^3 mol^{-1} s^{-1} at 308 K. At pH 5.85 the amine is predominantly protonated and only a small fraction is reactive. The expected second-order rate constant at that pH would be 4.35 × 10^{-5} dm^3 mol^{-1} s^{-1}, approximately a factor of 1000 smaller than that observed for the reaction of RA-42 and **I** (2.8 × 10^{-2} dm^3 mol^{-1} s^{-1}). Temperature effects on reaction rates and possible differences in reactivity between acetic acid and fumaric acid esters have been neglected and the rate enhancement is probably larger. The origin of the observed rate enhancement is to be found in the unique organization of a histidine close to an ornithine in space. The other ornithine in RA-42, Orn-34, has no neighbouring histidine and does not form an amide. Lys-10 is next to His-11 in the amino acid sequence but it does not react either and the origin of the selectivity is therefore of great interest. Catalysis of self functionalization provides a novel way for post synthetic modifications of polypeptides to introduce new functionality or to build novel polypeptide structures. It also implies the possibility of autocatalytic reactions in peptides as pathways in primitive biosynthesis of proteins.

The formation of a covalent bond between the polypeptide and the substrate can be avoided without losing catalytic activity by replacing Orn-15 with a residue that can hydrogen bond and that carries a positive charge but does not form long-lived covalent bonds with acyl groups, *e.g.* a histidine or an arginine. This hypothesis has been tested and shown to hold in the case of a polypeptide containing neighbouring histidines instead of an ornithine next to a histidine.[16] The design principles therefore appear to have been successful.

In conclusion, it is possible to catalyse acyl transfer reactions using functionalized helix–loop–helix motifs designed from simple principles of transition state binding. Positively charged hydrogen bond donors can be introduced that interact with negatively charged substrates in a predictable way. Furthermore it has been demonstrated for the first time that fast intramolecular reactions can be used to introduce new functionality in designed polypeptides. Further work is now necessary to enhance the reactivity and selectivity of functionalized polypeptides, in particular towards stereoselective reactions.

Experimental

The synthesis of SA-42 has been described in detail, previously.[7] The syntheses of RA-42 and PA-42 follow that of SA-42, with the exception of the amino acid derivatives used at the modified positions. The side-chain protection group of ornithine was 2-Cl-CBZ (2-chlorocarbobenzoxycarbonyl). The polypeptides were synthesized on an automated peptide synthesizer (Biosearch 9600), using *t*-BOC protection groups and phenylacetamidomethyl-(PAM) linked resins.[17] They were cleaved from the resin by anhydrous HF on a Teflon vacuum-line (Peptide Institute Inc.) and purified by size-exclusion chromatography and reversed-phase and ion-exchange HPLC. Electrospray mass spectrometry (VG Analytical, ZabSpec) and amino acid analysis were used to establish the identity of the peptides and the purity was checked by HPLC.

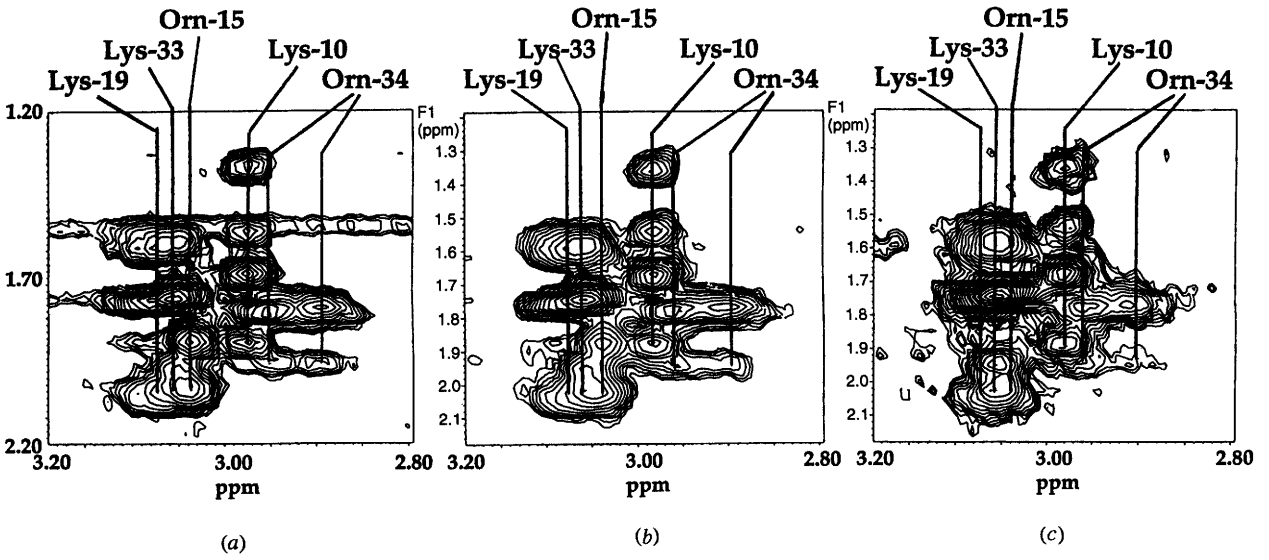


Fig. 5 Part of the TOCSY spectrum of RA-42 at different degrees of reaction with **I**, (*a*) before the reaction, (*b*) after the consumption by RA-42 of 0.5 equiv. of **I** and (*c*) after the consumption by RA-42 of 1.5 equiv. of **I**. The part of the TOCSY spectrum that is shown contains the cross peaks of the side chains of lysines and ornithines that are spin coupled to the ε protons of the lysine side chains and to the δ protons of the ornithine side chains. These protons resonate close to 3 ppm. In the TOCSY spectrum all protons of a spin system will show connectivities to every other proton in that spin system. All protons of the side chains of Orn and Lys will therefore appear on a straight line originating from the chemical shift of the δ and ε protons. The spin systems of the side chains are indicated in the spectrum. The spin system of Orn-15 is partly hidden due to spectral overlap, but the cross peak at 1.86 ppm is clearly visible. It is significantly reduced after the reaction of RA-42 with 0.5 equivs. of substrate which shows that the side chain is transformed and not only shifted in the spectrum. After the consumption of an excess of substrate the spin system is no longer visible. In the case of side chains of lysine and ornithine the heteroatom is protonated and carries a positive charge at the pH at which the reaction is carried out. When that primary amine is transformed into an amide the chemical shift becomes very different and the spin system is shifted.

The synthesis of mono-*p*-nitrophenylfumarate

Freshly-distilled fumaryl chloride (0.9 g, 5.9 mmol) was dissolved in 100 cm^3 of acetone and allowed to react with the residual water of the solvent for 30 min. A small portion (0.2 cm^3) was transferred to a dry round bottom flask and the solvent evaporated. The residual mixture was dissolved in $CDCl_3$ and transferred to an NMR tube. The ^1H NMR spectrum showed a mixture of fumaryl chloride (7.10 ppm, s) and partly hydrolysed fumaryl chloride (6.98 and 7.07 ppm, dd, $J = 16$ Hz). Fumaric acid is only sparingly soluble in chloroform. Water was then added to the acetone solution and the NMR analysis was repeated. A total of 54 mm^3 (3 mmol) was added in small portions with a syringe, and after each addition the degree of reaction was analysed by NMR spectroscopy. When the solution contained no more fumaryl chloride, the acetone was evaporated and the remaining oil was dissolved in 50 cm^3 ethanol-free chloroform and centrifuged to remove fumaric acid. The supernatant was transferred to a round bottom flask and 1.8 g freshly-prepared sodium *p*-nitrophenolate, dried under heating *in vacuo* until it turned bright orange, was added. The slurry was allowed to react with stirring overnight and was then centrifuged. The solid material was extracted with 3 × 50 cm^3 water and the combined aqueous phase was titrated to pH 6 with 0.3 mol dm^{-3} acetic acid and extracted with 5 × 30 cm^3 of CH_2Cl_2 to remove the *p*-nitrophenol. The aqueous phase was titrated to pH 4.3 with 0.3 mol dm^{-3} acetic acid and extracted with 5 × 30 cm^3 of CH_2Cl_2. The combined organic phase was allowed to stand overnight at 255 K. A small crop of crystals (40 mg) was collected and after partial evaporation of the solvent in a stream of dry nitrogen a second crop was collected (60 mg) consisting of the desired product, mono-*p*-nitrophenylfumarate. No attempts were made to optimize the yield. The product was identified from NMR spectroscopy and from mass spectrometry.

NMR

^1H NMR spectra were recorded on a Varian Unity 500 NMR spectrometer equipped with a matrix shim system MHU 383 (Resonance Research Inc.). The TOCSY experiments were run at 313 K in 10% [$^2H_3$]trifluoroethanol in H_2O–D_2O 10:90 (v/v) and a 90° pulse of 8.5 μs was used in connection with a mixing time of 80 ms. The 90° pulses for the spin lock was 21 μs. The spin systems of RA-42 and PA-42 were assigned from the TOCSY spectra and from the amide region of the NOESY spectra. The assignment of the ^1H NMR spectrum of SA-42 has been described in detail previously.[7]

Kinetic measurements

These were carried out at 290.2 K in 10% TFE in 50 mmol dm^{-3} Bis-Tris buffer at pH 5.85 by following the increase in absorbance at λ 320 nm using a Cary 4 spectrophotometer equipped with a Cary temperature controller. The peptide was dissolved in 300 mm^3 of buffer solution and titrated to pH 5.85 by 0.1 mol dm^{-3} NaOH in 10% TFE solution and centrifuged. 300 mm^3 of clear peptide solution was transferred to a 1 mm UV cuvette and placed in the thermostatted compartment of the UV spectrometer. The substrate was weighed and dissolved in buffer solution and 20 mm^3 was transferred to the cuvette by pipette and the reaction started. The concentrations of the peptides were determined by quantitative amino acid analysis and each rate constant was the average of two runs. Each kinetic run was followed for at least two half-lives and a single exponential function of the form $A + A_1e^{-kt}$ was fitted to the data. Under conditions of excess peptide each peptide solution was used for two runs and to start the second reaction a second portion of 20 mm^3 was added to the cuvette. In the case of RA-42 where the excess rate enhancement was lost upon reaction with the substrate the concentration of peptide in the second run was corrected for the loss of reactive peptide. The total concentration of substrate was 0.13 mmol dm^{-3} and the corrections of the second-order rate constants were small, less than 10%. The same correction was applied to the rate constants of PA-42.

Acknowledgements

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