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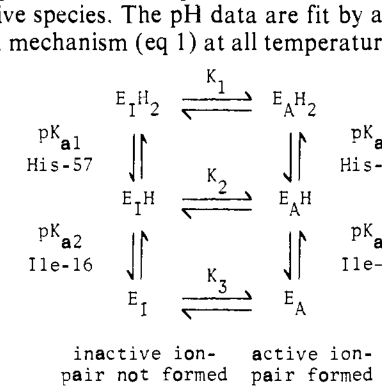
K. J. Shea,* R. Gobeille, J. Bramblett, E. Thompson
Department of Chemistry, University of California
Irvine, California 92717
Received October 3, 1977

Thermal Characteristics of a Refolding Transition. The Alkaline Transition of α-Chymotrypsin[1]

Sir:

The alkaline transition of the serine proteases has already attracted considerable attention and has been studied in some depth with the chymotrypsin members of the family.[2-14] The apparent ubiquitousness of the transition and the participation of the "buried" ion pairs in the transition suggest possible clues to catalytic mechanism. Similarly, charge rearrangements leading to formation of the ILE-16 to ASP-194 ion pair are similar to those in chymotrypsinogen activation, thus providing promise for information of general importance in zymogen activation. Less attention has been given to the transition as a model protein "refolding" process (defined[15] as having large activation enthalpies and entropies but small overall standard enthalpy and entropy changes). Our recent studies have been directed toward the last aspect but produce information of broader applicability.

The stopped-flow, proflavin-binding method described by Fersht and Requena[6] was used to monitor the transition of α-chymotrypsin (Worthington Biochemical Corp.) at 30 to 40 pHs from pH 6 to pH 11, at each of six temperatures from 1 to 31 °C. Four to six determinations were made at each pH and temperature. This extensive data collection was required to achieve the small standard deviations essential for establishing the pH dependence of the equilibrium constant between active and inactive species. The pH data are fit by a minimal two-ionization mechanism (eq 1) at all temperatures.[16] Equation



(1)

1 was used by Fersht[7] to describe this equilibrium in α-CT, which also did not fit a one-ionization mechanism. van't Hoff plots for two of the fitted equilibrium constants are shown in Figure 1 and other "best-fit" values of thermodynamic parameters are given in Table I.

The most striking result is the magnitude of the curvature in the van't Hoff plots for K_1 and K_2 (Figure 1), since this may

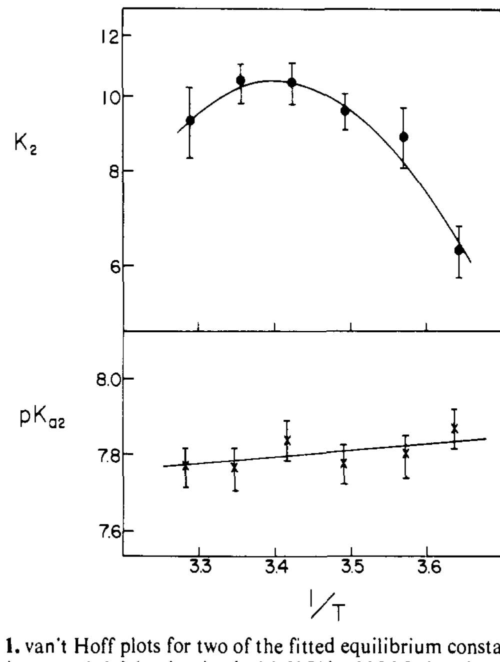


Figure 1. van't Hoff plots for two of the fitted equilibrium constants of eq 1 at ionic strength 0.2 (maintained with KCl in .005 M phosphate buffer). Error bars are estimates determined from the fitting procedure. The lines were drawn using the thermodynamic constants given in Table I: ●, K_2; ×, pK_{a2}. Consult ref 25 for further experimental details.

Table I. Thermodynamic Values for Processes in Eq 1 at Ionic Strength 0.2 at 25 °C[a]

	ΔG^b	ΔH^b	ΔS^c	$\Delta C_p{}^b$
$K_1{}^d$	−1.03 ± 0.04	0.17 ± 0.80	4.02 ± 3.0	−0.43 ± 0.200
$K_2{}^d$	−1.38 ± 0.04	−1.62 ± 0.90	−0.78 ± 3.0	−0.43 ± 0.200
$K_3{}^d$	1.81 ± 0.12	−4.55 ± 1.00	−21.4 ± 3.0	0 ± 0.200
pK_{a1}	9.52 ± 0.13	6.88 ± 1.30	−8.83 ± 3.0	
pK_{a2}	10.61 ± 0.07	1.14 ± 0.50	−31.8 ± 2.0	

[a] Conditions as in Figure 1. [b] In kilocalories/mole. [c] In entropy units. [d] Defined as $K_1 = (H_2E_A)/(H_2E_I)$; etc.

reflect large heat capacity changes. Overall $\Delta G°$, $\Delta H°$, and $\Delta S°$ values for these steps are small, suggesting that the active and inactive forms are very similar, but the large $\Delta C_p°$ values are not a priori consistent with this conclusion. The standard heat capacity change for the transition is ~10% of the $\Delta C_p°$ observed for thermal unfolding of α-CT at similar conditions.[17] It can be argued from protein unfolding studies that heat capacity differences reflect hydrophobic bonding changes in protein conformational changes;[18,19] therefore, one might speculate that the transconformation of HE_A to HE_I (or H_2E_A to H_2E_I) involves a considerable increase in water-polypeptide interaction. According to data from models for such effects, a relatively large negative entropy change should accompany this increased interaction,[15] but we observe very small $\Delta S°$ values (Table I). It is possible that the negative "hydrophobic" entropy change is balanced by a positive configurational entropy change, with a consequent reduction in configurational entropy for the active species. This rationalization agrees with some current proposals for enzymic catalysis, but it is based on the very tentative assumption that the heat capacity changes in a protein conformational transition are primarily a measure of water-polypeptide interaction.

The value of pK_{a1} and the corresponding ionization enthalpy suggest ionization process 1 is due to one of the two histidine residues. Studies of *N*-methyl-HIS-57 α-CT[20] support this assignment, identifying the residue as HIS-57.[21] According to conventional wisdom, pK_{a2} applies to the α-ammonium group of ILE-16[22] when exposed to solvent in form E_I.[2-8] However, although the pK_{a2} value is consistent with pK_a values

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for α-ammonium groups in model compounds, the value for the standard enthalpy of ionization of 1 kcal/mol is not, 10 kcal/mol being a frequently reported value.[23] There are several plausible explanations for our results. (1) The ionization process may be improperly assigned to ILE-16 as has been suggested by several groups of investigators.[21,24] This alternative would contradict considerable apparently sound sets of data and we consider it improbable. (2) The electrostatic environment of the ammonium group in form E_I may be very abnormal. This alternative receives some support from the salt-dependence studies,[25] but as yet such a severe perturbation appears unlikely and needs more extensive support. (3) The process may be linked to an undetected conformational rearrangement with near-zero free-energy change but a large negative enthalpy change. In this interpretation the environment of ILE-16 would be quite different in HE_I and E_I.

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James D. Stoesz
Department of Biochemistry. Brandeis University
Waltham, Massachusetts 02154

Rufus W. Lumry*
Laboratory for Biophysical Chemistry
Department of Chemistry, University of Minnesota
Minneapolis, Minnesota 55455
Received September 12, 1977

A Virtually Completely Asymmetric Synthesis

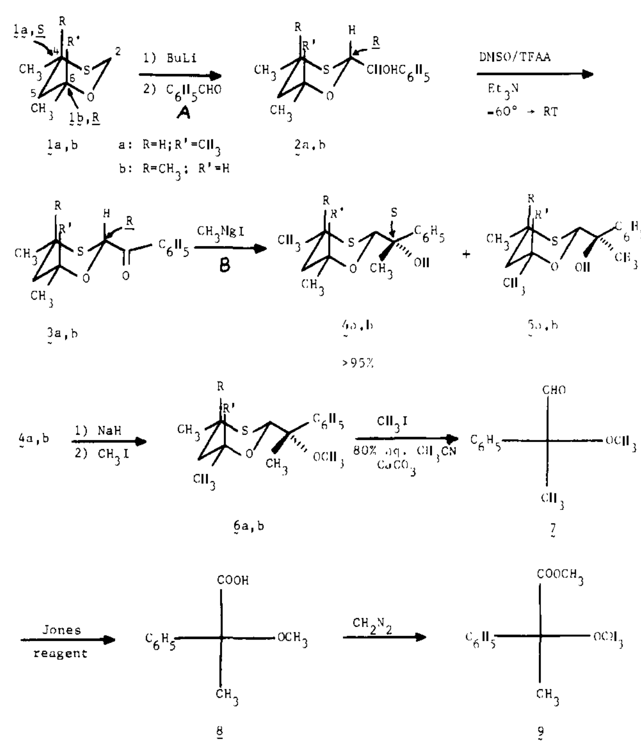
Sir:

We wish to report an asymmetric synthesis of (*S*)-(+)-atrolactic acid methyl ether which proceeds in extremely high (~100%) optical yield, effects the separation of the chiral product from the original inducing chiral center, and allows, in principle, for the recovery of the chiral auxiliary reagent.

The reaction sequence includes two highly stereoselective steps. The first makes use of the observation[1] that electrophilic attack on 2-lithio salts of conformationally locked 1,3-oxathianes—like that on 2-lithio-1,3-dithianes[2]—leads exclusively to equatorially substituted products. The second step is an extremely diastereoselective reaction of a Grignard reagent with a ketone (Cram's rule). Scheme I outlines the reactions involved.

Metalation of **1a** ($[\alpha]^{25}{}_D$ −30.4° ($CHCl_3$), 44% e.e. (enantiomer excess)) was effected in THF by addition of BuLi at −78 °C, followed by stirring of the reaction mixture for 15 min at ambient temperature. Addition of 1 equiv of C_6H_5CHO after recooling to −78 °C gave, after workup, **2a** (yield 95%, $[\alpha]^{25}{}_D$ −42.3° ($CHCl_3$)) as a mixture of diastereomers which were exclusively equatorially substituted at C-2. Oxidation of **2a** with Me_2SO in the presence of trifluoroacetic anhydride and triethylamine[4] gave, in 75% yield, **3a** ($[\alpha]^{25}{}_D$ −27.4° ($CHCl_3$)), 44% e.e. as determined by ^1H NMR using the optically active shift reagent $Eu(hfc)_3$. Ketone **3a** in ether/THF[5] was added to an excess[6] of methylmagnesium iodide in ether which afforded diastereomer **4a** ($[\alpha]^{25}{}_D$ −40.9° ($CHCl_3$)) in 95% yield (no **5a** could be detected[7] by either ^1H NMR or ^{13}C NMR[8]). Methylation of **4a** with sodium hydride/methyl iodide produced **6a** (96%, $[\alpha]^{25}{}_D$ −23.1° ($CHCl_3$)) which was cleaved[9] in a refluxing mixture of excess methyl iodide and 80% aqueous acetonitrile in the presence of $CaCO_3$ to give the aldehyde **7** (90%, $[\alpha]^{25}{}_D$ −44.5° ($CHCl_3$)). Compound **7** was oxidized with Jones reagent to atrolactic acid methyl ether (**8**) (68%, $[\alpha]^{25}{}_D$ +13.9° (MeOH)) which was (by CH_2N_2), converted to its methyl ester **9** (96%, $[\alpha]^{25}{}_D$ +6.4° (MeOH)). The e.e. in this product was again 44%[10] as determined by ^1H NMR using $Eu(hfc)_3$ and its physical and spectral properties

Scheme I



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