Intramolecular Acyl Transfer between Ester and Amide Groups in 1-Acyloxy-8-acylaminonaphthalene-3,6-disulphonates

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The reversible intramolecular acetyl transfer from 1-acetoxy-8-acetylaminonaphthalene-3,6-disulphonate to give 1-hydroxy-8-bis(acetyl)aminonaphthalene-3,6-disulphonate is promoted by hydroxide ion but catalysis by buffer bases is not observed. In the presence of 0.001 mol dm^{-3} hydroxide ion in aqueous solution at 278 K the half-life for the approach to equilibrium is *ca.* 5 ms. Transfer of a propionyl group occurs at a similar rate. The mechanism involves rapid ionisation of the amide followed by intramolecular nucleophilic attack of the amide anion on the ester carbonyl. By comparison of the rate coefficient for this reaction with that for the intermolecular attack of hydroxide ion on the ester carbonyl, an effective molarity of *ca.* 1×10^5 mol dm^{-3} is calculated for the intramolecular process. In the reverse direction, the reaction involves intramolecular nucleophilic attack by the ionised naphthol on the imide carbonyl and by comparison with the rate of the intermolecular reaction of the imide with hydroxide ion an effective molarity of *ca.* 5×10^5 mol dm^{-3} is estimated.

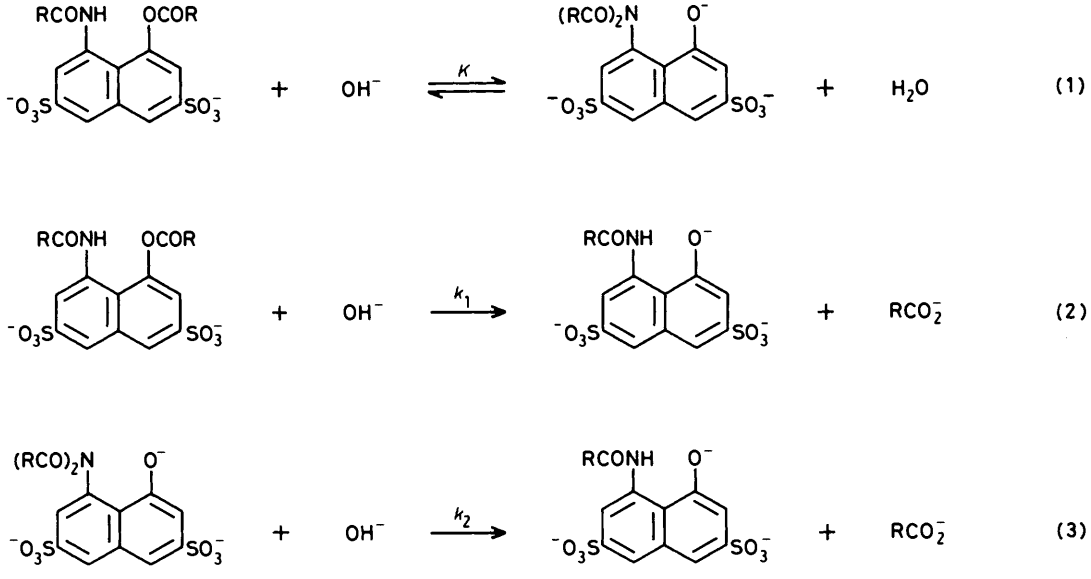
Interest in the mechanism and efficiency of intramolecular reactions arises because of their relevance to the processes occurring within enzyme active sites.[1,2] The rates of intramolecular reactions often exceed the rates of the analogous intermolecular reactions by several orders of magnitude and approach the efficiency of enzyme-catalysed reactions. Various reasons for the enhanced rates of intramolecular and enzyme reactions have been proposed.[1-3]

In studies of the mechanism of hydrolysis of 1-acyloxy-8-acylaminonaphthalene-3,6-disulphonates to 1-hydroxy-8-acylaminonaphthalene-3,6-disulphonates in aqueous sodium hydroxide, see Scheme, the kinetic behaviour suggested[4,5] the occurrence on the reaction pathway of an imide intermediate, 1-hydroxy-8-bis(acyl)aminonaphthalene-3,6-disulphonate, formed by an intramolecular acyl transfer, equation (1). Product formation was considered to occur by intermolecular reaction of the ester and the imide with hydroxide ion as in equations (2) and (3) respectively.

Although compelling mechanistic evidence in favour of the occurrence of the reversible acyl transfer was obtained[4,5] and values of the equilibrium constants for imide formation were deduced, direct kinetic studies of the acyl transfer were not carried out. We now report studies of reaction (1), with R = Me and Et, using the stopped-flow method at reduced temperature. Intramolecular acyl transfers have been implicated in the mechanisms of numerous biological reactions including protein biosynthesis,[6] and in reactions catalysed by proteolytic enzymes[7] but the number of kinetic studies is small.

Experimental and Results

Preparations.—In previous work[5] a pure sample of 1-acetoxy-8-acetylaminonaphthalene was prepared and shown to hydrolyse quantitatively in aqueous sodium hydroxide solutions. The product of hydrolysis, 1-hydroxy-8-acetylaminonaphthalene, was isolated and identified. Concentrated aqueous solutions of 1-acetoxy-8-acetylaminonaphthalene-3,6-disulphonate were also prepared by acylation of 1-hydroxy-8-aminonaphthalene-3,6-disulphonate in aqueous sodium carbonate and on introduction of the concentrated



Scheme.

Table 1. Kinetic and equilibrium results for hydrolysis and acyl transfer[a]

	R = Me	R = Et
Hydrolysis kinetics		
10^{-2} K/dm^3 mol^{-1}	4.82 ± 0.5	1.60 ± 0.2
k_1/dm^3 mol^{-1} s^{-1}	1.27 ± 0.1	1.04 ± 0.1
10^2 k_2/dm^3 mol^{-1} s^{-1}	1.55 ± 0.1	0.68 ± 0.05
Acyl transfer		
10^{-2} K/dm^3 mol^{-1}	4.28 ± 0.3	1.92 ± 0.3
10^{-4} k_f/dm^3 mol^{-1} s^{-1}	4.1 ± 1	3.9 ± 1
10^{-2} k_r/s^{-1}	0.85 ± 0.1	2.1 ± 0.2
10^{-2} k_f/k_r/dm^3 mol^{-1}	4.82 ± 1	1.86 ± 0.6

[a] Conditions: aqueous solution, 278 K, ionic strength 0.2 mol dm^{-3}.

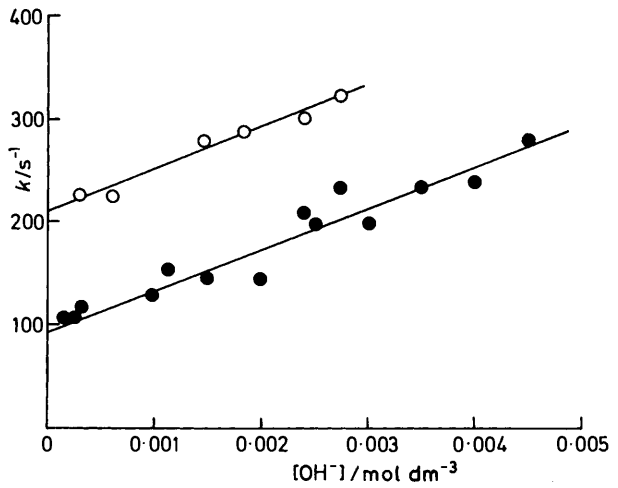


Figure. Rate coefficients (k) for the first-order approach to equilibrium in reaction (1); solid points for acetyl transfer (R = Me) and open points for propionyl transfer (R = Et)

solution into the basic hydrolysis medium it was shown by comparison of the u.v.–visible spectral changes which occurred with those for 1-acetoxy-8-acetylaminonaphthalene that hydrolysis to 1-hydroxy-8-acetylaminonaphthalene-3,6-disulphonate was occurring cleanly. In the present work aqueous solutions of 1-acetoxy-8-acetylamino- and 1-propionyloxy-8-propionylamino-naphthalene-3,6-disulphonate (*ca.* 0.02 mol dm^{-3}) were prepared by reaction of 1-hydroxy-8-amino-naphthalene-3,6-disulphonate with acetic and propionic anhydride in aqueous sodium carbonate. All kinetic and equilibrium measurements were made with these solutions.

Kinetic and equilibrium studies in this work were carried out in aqueous solution at 278 K and ionic strength maintained at 0.2 mol dm^{-3} by addition of sodium chloride. The 1-acyloxy-8-acylaminonaphthalene-3,6-disulphonates were always present in low concentration, typically *ca.* 1 × 10^{-5} to 10^{-4} mol dm^{-3}.

Kinetic Studies of Hydrolysis.—The hydrolysis of 1-acetoxy-8-acetylamino- and 1-propionyloxy-8-propionylamino-naphthalene-3,6-disulphonate to the corresponding 1-hydroxy-8-acylaminonaphthalene-3,6-disulphonates was observed spectrophotometrically over the range 250—400 nm. The reactions were studied at hydroxide ion concentrations from 0.001—0.20 mol dm^{-3}. As observed previously [5] for 1-acetoxy-8-acetylaminonaphthalene-3,6-disulphonate, the dependence of the first-order rate coefficient (k_h) on hydroxide ion concentration is complex, with a curved dependence at low concentrations which becomes linear at high hydroxide ion concentrations. The experimental results were fitted by equation (4) which is derived from the mechanism in the Scheme

$$k_h = k_1[OH^-]/(1 + K[OH^-]) + k_2K[OH^-]^2/(1 + K[OH^-]) \quad (4)$$

on the assumption that formation of the imide intermediate occurs rapidly. The best-fit values of K, k_1, and k_2 are given in Table 1. The results for 1-acetoxy-8-acetylaminonaphthalene-3,6-disulphonate are compatible with those previously obtained [5] at 298 K.

Equilibrium Studies of Imide Formation.—The equilibrium between 1-acetoxy-8-acetylaminonaphthalene-3,6-disulphonate and 1-hydroxy-8-bis(acetyl)aminonaphthalene-3,6-disulphonate and the corresponding equilibrium for 1-propionyloxy-8-propionylaminonaphthalene-3,6-disulphonate, equation (1), were studied spectrophotometrically in aqueous solutions of sodium hydroxide (0.001—0.02 mol dm^{-3}). Measurements were made before significant hydrolysis could occur by mixing a stock solution of the 1-acyloxy-8-acylaminonaphthalene-3,6-disulphonates with aqueous sodium hydroxide using a rapid mixing device (Hi-Tech SFA-11) in the cell compartment of the spectrophotometer (Hewlett Packard Diode Array 8451) and the spectrum (250—500 nm) was recorded within 1 s. Equilibrium constants (K) for reaction (1) were calculated from absorbance readings at a chosen wavelength, 360 nm, and the results are given in Table 1. The values are in satisfactory agreement with the values obtained from the kinetic analysis of the hydrolysis reaction on the assumption that the mechanism in equation (1) applies, see Table 1.

Kinetic Studies of Imide Formation.—Kinetic studies of the establishment of the equilibrium between 1-acetoxy-8-acetylaminonaphthalene-3,6-disulphonate and 1-hydroxy-8-bis(acetyl)aminonaphthalene-3,6-disulphonate, equation (1) with R = Me, were made in the presence of sodium hydroxide using stopped-flow spectrophotometry (Hi-Tech SF51). The reaction of 1-propionyloxy-8-propionylaminonaphthalene-3,6-disulphonate was studied similarly. Measurements of the rapid change in absorbance on mixing stock solutions of the 1-acyloxy-8- acylaminonaphthalene-3,6-disulphonates with aqueous sodium hydroxide were made at *ca.* 360 nm. Measurements were also made in the presence of phosphate and carbonate buffer solutions in order that the reaction could be studied at low concentrations of hydroxide ion. First-order rate coefficients (k) for the approach to equilibrium for the acetyl and propionyl derivatives over a range of sodium hydroxide concentrations are given in the Figure. The reproducibility of observed rate coefficients was usually within ±5%. The scatter of the rate coefficients in the Figure arises because the amplitude of the relaxation is limited by the need to maintain pseudo-first-order conditions. The total change in absorbance was compatible with that predicted from equilibrium measurements and the molar absorptivities of the imide and the 1-acyloxy-8-acylaminonaphthalene-3,6-disulphonates.

The data in the Figure are compatible with equation (5) which applies for the approach to equilibrium in a reaction of the type in equation (1).

$$k = k_f[OH^-] + k_r \quad (5)$$

The values of k_f and k_r calculated from the gradient and the intercept of the plots are given in Table 1. The ratio of gradient to intercept is compatible, within experimental error, with the values of the equilibrium constants of the reactions measured directly and determined from the kinetic studies of the overall hydrolysis (see Table 1).

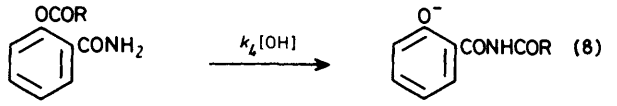
Table 2. Kinetics of acetyl transfer for 1-acetoxy-8-acetylaminonaphthalene-3,6-disulphonate in phosphate buffers

10^2 [HPO_4^{2-}]	10^2 [PO_4^{3-}]	10^4 [OH^-]	k/s^{-1}
2.06	1.94	6.07	136.7 ± 20
1.06	0.94	5.75	124.2 ± 14
0.450	0.350	5.02	120.3 ± 13
0.242	0.158	4.21	124.7 ± 13

To investigate the effect of a change in buffer concentration on the rate of the acyl transfer, the reaction of 1-acetoxy-8-acetylaminonaphthalene-3,6-disulphonate was studied in the presence of phosphate buffers at a fixed buffer ratio over a range of buffer concentrations. The values of the first-order rate coefficients (k) are given in Table 2. Because of the relatively high hydroxide ion concentrations in phosphate buffers, the buffer ratio changes as the buffer is diluted and the change in the values of k in Table 2 is due to this effect. The data show that the reaction is not susceptible to catalysis by buffer. In the previous studies[5] of the kinetics of the hydrolysis of 1-acyloxy-8-acylaminonaphthalene-3,6-disulphonates, a series of substituted benzoyl derivatives was investigated. In the present work we attempted to study the kinetics of the transfer of a substituted benzoyl group, equation (1) with R = XC_6H_4. Although formation of the corresponding imides is observed to occur from 1-aroyloxy-8-aroylaminonaphthalene-3,6-disulphonates it was found that the reactions occurred rapidly and equilibrium with the imide was reached within the mixing time of our stopped-flow equipment (*ca.* 2 ms).

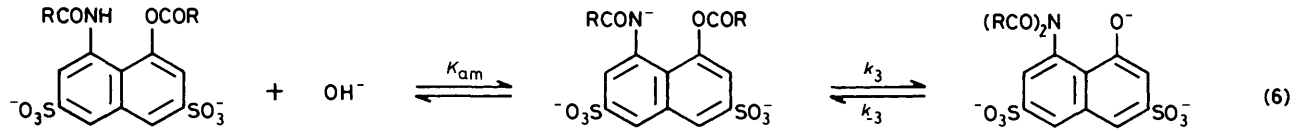
of the equilibrium constant for formation of the imide from 1-propionyloxy-8-propionylaminonaphthalene-3,6-disulphonate.

The values of k_f in Table 1 are similar to the value k_4 2 × 10^4 dm^3 mol^{-1} s^{-1} observed for acetyl transfer for *O*-acetylsalicylamide,[8,9] equation (8), and similar to the value (3 × 10^3 dm^3 mol^{-1} s^{-1}) observed for acetyl transfer for



(8)

methyl phthalamate.[8,10] The reactions of *O*-acetylsalicylamide and methyl phthalamate occur to completion but in the present systems the acyl transfer is reversible and the reverse reactions have been observed. Approximate values for the effective molarities of the acyl transfer (1) can be estimated by comparison with data for related intermolecular reactions and since rate coefficients for acyl transfer in the forward and reverse directions have been measured it is possible to calculate effective molarities in both directions. For acetyl transfer from the ester to give the imide, assuming that the mechanism in equation (6) operates, the rate of intramolecular attack of the ionised amide can be calculated from equation (7) providing the equilibrium constant (K_{am}) for ionisation of the amide can be estimated. Data for the ionisation of amides are rather scarce.[11] A value pK 15.1 is found[11] for acetamide and it is likely that the pK of 1-acetoxy-8-acetylaminonaphthalene-3,6-disulphonate will be somewhat lower than this. Values of pK 25.5 and 21.45 have been determined[12] for acetamide and acetanilide in Me_2SO whereas thiobenzamide and thiobenzanilide have aqueous pK



(6)

$k_f = K_{am}k_3$ $k_r = k_{-3}$ (7)

Discussion

The observation of a first-order dependence of the rate of acyl transfer on hydroxide ion and the absence of catalysis by buffer species identifies the mechanism of reaction given in equation (6). Assuming that the ionised amide is present in low concentration and is formed rapidly, equation (7) is obtained for the forward and reverse rate coefficients for acyl transfer. The values of k_f and k_r and the values of the equilibrium constants for transfer of acetyl and propionyl groups are given in Table 1. The overall equilibrium constant (K) for formation of the imide from 1-propionyloxy-8-propionylaminonaphthalene-3,6-disulphonate is *ca.* three-fold lower than the value for imide formation from 1-acetoxy-8-acetylaminonaphthalene-3,6-disulphonate. It was previously observed[5] that electron-withdrawing substituents in the aroyl group increased the equilibrium constant for imide formation from 1-aroyloxy-8-aroylaminonaphthalene-3,6-disulphonates and so the effect for acetyl and propionyl transfer is in the same direction. The rate coefficient for acyl transfer (k_f) is similar for the propionyl and acetyl derivatives. According to the mechanism in equation (6), the ionised amide will be destabilised by the additional methylene group but the nucleophilicity of the amide anion will be increased. Thus for the propionyl transfer, the value of K_{am} will be decreased but k_3 will be increased. The higher value of k_r for 1-propionyloxy-8-propionylaminonaphthalene-3,6-disulphonate almost precisely reflects the decrease in the value values of 12.8 and 10.6, respectively.[11] If a value of pK 14.0 is assumed for 1-acetoxy-8-acetylaminonaphthalene-3,6-disulphonate the result k_3 8 × 10^4 s^{-1} is calculated from equation (7). A reasonable intermolecular analogue for this process is the reaction of the ester group in 1-acetoxy-8-acetylaminonaphthalene-3,6-disulphonate with hydroxide ion for which a rate coefficient under the present conditions of 1.27 dm^3 mol^{-1} s^{-1} was obtained from the hydrolysis data (see Table 1). Assuming that the rate coefficients for intermolecular nucleophilic attack by an amide anion of pK 14 and by hydroxide ion are similar, an effective molarity for the intramolecular acyl transfer of *ca.* 10^5 mol dm^{-3} is calculated. For acetyl transfer in the reverse direction, the rate coefficient k_r 85 s^{-1} is found for intramolecular attack of the naphthol anion on the imide carbonyl. The required intermolecular analogue of this reaction is nucleophilic attack by a naphthol anion on the imide carbonyl. A rate coefficient for the intermolecular reaction of hydroxide ion with the imide carbonyl (k_2 0.0155 dm^3 mol^{-1} s^{-1}) has been obtained from the kinetics of the overall hydrolysis. The rate coefficient for the reaction of a more weakly nucleophilic naphthol anion will be much lower than this. For example the rate coefficients for reaction of hydroxide ion and phenolate ion with phenylacetate differ[13] by a factor of *ca.* 50. If it is assumed that the rate coefficients for the intermolecular reactions of hydroxide ion and a naphthol anion with the imide carbonyl differ by a factor of 100, an effective molarity of

5×10^5 mol dm^{-3} is calculated for intramlecular nucleophilic attack of the naphthol anion. The result is roughly comparable with the value estimated for acetyl transfer in the forward direction.

Acknowledgements

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