Role of Lysine in catalysing Ester Hydrolysis

Recently, Gero and Withrow[1] have cited the catalysis of alkaline triglyceride hydrolysis by lysine as a chemical model of a biological reaction. They postulated a bifunctional[2] attack by lysine upon the carbonyl O and C of the ester linkage as the mechanism of this hydrolysis.

We believe that some objections can be made to their arguments for this mechanism. They have stated, "It stands to reason that the hydrogen bonds which the ε-ammonium group is to form with the carboxyl group of lysine and the O of the ester should be strongest when the N does not hold its hydrogens too tightly, because that is when they will be most readily donated for a hydrogen bond. Such would be the case just below the *p*H at which the ε-ammonium group of lysine loses a hydrogen and its positive charge. That happens at *p*H 9·5, so that lysine should be maximally active as a catalyst for ester hydrolysis around *p*H 8–9". The authors are clearly implying that N—H bond-strength is a function of *p*H. This, of course, is not the case. Instead, since they are describing the competition of several bases for protons, one would expect the usual arguments for acid-base equilibria to be applicable. In this case they are :

$$K_1 = \frac{[\epsilon NH_2]\ [R-\overset{\overset{\oplus OH}{\|}}{C}-OR']}{[-NH_3{}^{\oplus}]\ [R-\overset{\overset{O}{\|}}{C}-OR']}$$

$$K_2 = \frac{[R-\overset{\overset{\oplus OH}{\|}}{C}-OR']}{[H^{\oplus}]\ [R-\overset{\overset{O}{\|}}{C}-OR']}$$

$$K_3 = \frac{[\epsilon NH_2]\ [H^{\oplus}]}{[\epsilon NH_3{}^{\oplus}]}$$

Since K_3 has a value of $10^{-10.53}$ for lysine[3] and K_2 should be about $10^{6.0}$, depending upon the nature of the substituents of the carbonyl group[4], the concencentration of the reacting species, $-NH_3{}^+$ and $R-\overset{\overset{O}{\|}}{C}-OR'$, in the first equilibrium should be maximal and independent of *p*H over at least the *p*H range 4·0–9·5. Below the former *p*H the rate of ordinary acid hydrolysis and the protonation of the lysine carboxyl group would have to be taken into consideration. Consequently, the rate of the hydrolysis catalysed by lysine should be maximal and constant over this *p*H range. Inspection of their results does not show this to be the case. Instead the decrease of the hydrolysis rate with decreasing alkalinity below *p*H 8·6 is seen to follow closely the expected protonation of the α-amino group.

A further objection can be made to their choice of *p*H 8–9 as the range of maximal catalysis. The ε-ammonium group would not be expected to be appreciably dissociated below *p*H 9·5 since its pK_A is 10·53. Catalysis then should not fall off below *p*H 9·0. Again, their results do not show this to be true.

Therefore it seems more likely to us that this is an example of general base catalysis similar to that

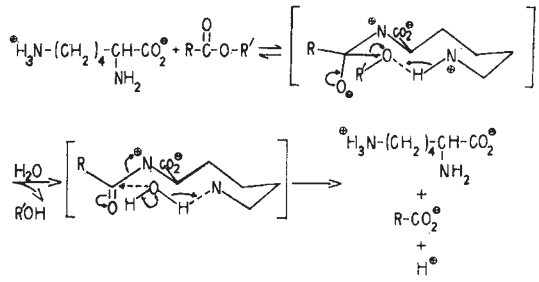

Fig. 1

reported for the imidazole group[5]. That is, the picture of bifunctional catalysis shown in Fig. 1 would be predicted.

An alternative to the second intermediate would be the co-ordination of a hydroxyl group by the ε-amino group into a position favouring its attack upon the carbonyl C. However, there is little to choose between the two possibilities.

Lysine as a ring structure as postulated by Gero and Withrow is sterically less suitable as a catalyst in this reaction sequence than it is in an open-chain form. Assumption of such a ring structure would be possible only with dissociation and loss of positive charge by the α-amino group, since it would otherwise require apposition of the two positive poles in the molecule. Consequently, the catalytic ability of lysine should decrease as the possibility for formation of ring structure increases above *p*H 8·95, the pK_A of the α-amino group[3]. This is precisely what Gero and Withrow observed.

Consideration of the reaction sequence postulated above suggests that lysine should be very reactive with aldoses (in the hemiacetal configuration) since it is in good agreement with the mechanism deduced[6] for the Maillard reaction of amino-acids with aldoses. Inspection of the literature[7–9] shows that lysine is indeed particularly reactive with aldoses over the expected *p*H range, 7·0–9·5.

William R. Chesbro
Leslie R. Hedrick

Biology Department,
Illinois Institute of Technology,
Chicago 16.
Oct. 27.

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In their criticism of our paper[1], Chesbro and Hedrick make some points that are indeed well taken. Their objection to the implication that bond-strength is a function of *p*H is undoubtedly valid. However, the statement they quote from our paper does not imply such a functional relationship. Rather, we had in mind the stretching of the N—H bond which may be expected to occur before the bond breaks (more so the more alkaline the environment) and which certainly will allow the proton to function more effectively as an acidic catalyst. It is regretted