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# Evidence for the Intermediacy of *N*-(2-Imino, 1-Oxo-Propyl)-Glycine in the Base-Catalyzed Decomposition of N-halo-Dipeptides.

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*Abstract:* N-(2-imino, 1-oxo-propyl)-glycine are readily formed as intermediates in the base-catalyzed decomposition of (N-X)-Ala-Gly. These compounds suffer a subsequent hydrolysis to the corresponding N-(1,2-dioxo-propyl)-glycine. © 1997 Elsevier Science Ltd.

## Introduction.

Several studies are available on the halogenation of amines and amino acids, as well as on the subsequent reactions of the thus formed (*N*-halo)-compounds.[1,2,3,4,5,6,7] However, despite of their biochemical and environmental relevance,[8,9,10] little attention has been paid to these processes in the case of peptides. Hypochlorous acid has been shown to be enzymatically generated in the body, playing a fundamental rôle in the destruction of pathogens by reaction with important peptides or proteins.[11,12,13] None of the work on these reactions[10,11,12,13,14,15,16] treat them from a mechanistic point of view.

In a previous paper, we have studied the chlorination of dipeptides[17] with aqueous chlorine solutions. Opposite to (*N*-X)-amino acids,[6,18] (*N*-X)-dipeptides are rather stable in solution in the absence of bases. Here, we present the observed behaviour for the base-promoted decomposition of (*N*-Cl)- and (*N*-Br)-Alanyl·Glycine (hereafter quoted as (*N*-X)-Ala·Gly).

## Experimental.

Ala·Gly was purchased from Sigma® and used without further purification. (*N*-Cl)- and (*N*-Br)-Ala·Gly were instantaneously formed by simple mixing of HOCl or HOBr (prepared and titrated as described elsewhere[4]) with 10 % dipeptide excess. The reactions were spectrophotometrically followed both in near neutral and alkaline medium, showing an absorbance maximum at 255 nm for (*N*-Cl)-Ala·Gly, the same value found in the case of (*N*-Cl)-aminoacids.[19] Cary® 1E UV-Vis and a Hi-Tech® SF-61 MX stopped-flow spectrophotometers were used to follow the kinetics of the process. The changes in the absorbance at 237 nm in alkaline medium and 255 nm in neutral medium were used to monitor the process. The first order rate equation was adequately fitted to the kinetic data using the DSC[20] or Marquardt[21] non-linear optimization algorithms. In all cases the temperature was kept to within ±0.1 K and the ionic strength to $I(NaClO_4)$=1.0 mol·dm$^{-3}$.

**Results and discussion.**

The spectrum of (*N*-Cl)-Ala·Gly changes depending on the reaction conditions (see Figure 1). The absorption band centered at 255 nm disappears in basic medium while, simultaneously, a new broad band appears centered at 237 nm until it masks the one at 255 nm. The rate of these changes increases with the basicity of the medium. The absorbance of the second band keeps increasing to reach relatively high values of absorbance and then slowly decreases. Due to the previously mentioned increase in the rate of these changes, only a broad band centered at 237 nm is observed in strong alkaline medium, that decreases with time. Figure 1A shows how maxima at 255 nm and 237 nm change simultaneously. In weak alkaline medium (Figure 1B) only one band increases at 237 nm. Figure 1C shows how the maximum at 237 nm increases and then decreases at different rates. At higher alkalinities (Figure 1D) only the decrease of the 237 nm broad band is observed.

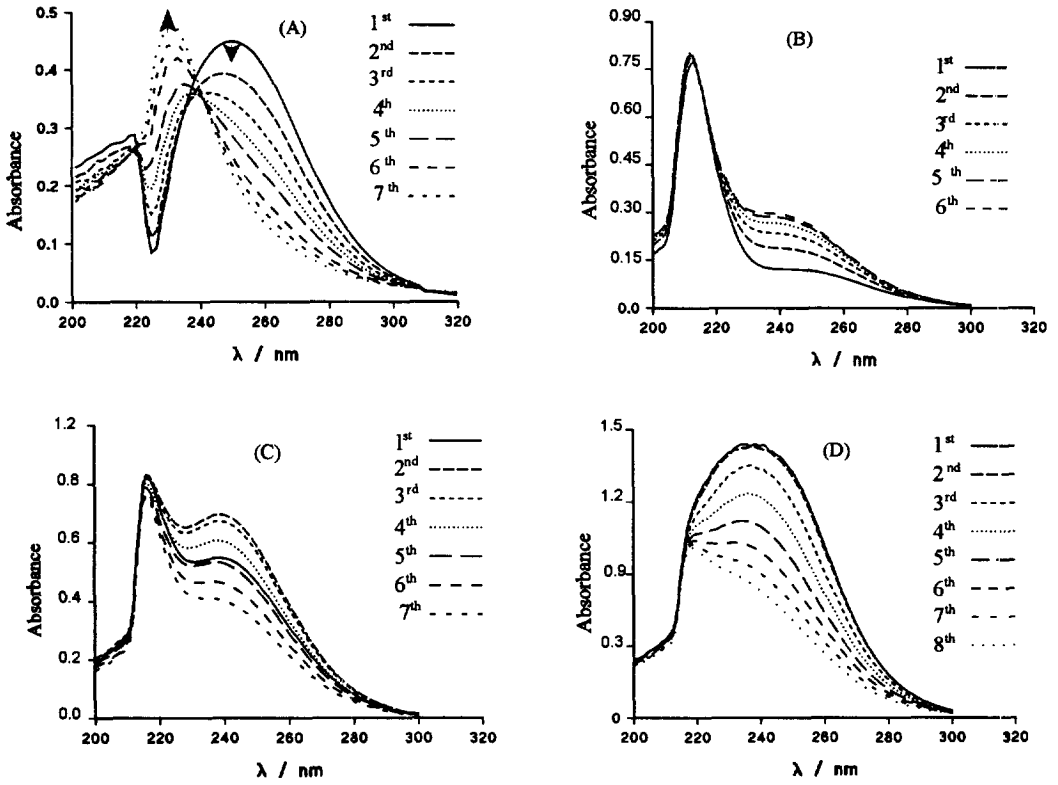



**Figure 1.** Spectral changes observed in the decomposition of (*N*-Cl)-Ala·Gly at different basicities. [(*N*-Cl)-Ala·Gly]=5·10$^{-4}$ mol·dm$^{-3}$, [Gly·Val]=5·10$^{-4}$ mol·dm$^{-3}$, I ($NaClO_4$)=1.00 mol·dm$^{-3}$. (A) pH=10.2, buffer $HCO_3^-/CO_3^{-2}$, 1 scan every 25 min, (B) [NaOH]=0.01 mol·dm$^{-3}$, 1 scan every 0,5 min, (C) [NaOH]=0.06 mol·dm$^{-3}$, 1 scan every 0,5 min, (D) [NaOH]=0.10 mol·dm$^{-3}$, 1 scan every 30 s.

According to these observations two consecutive processes take place in the decomposition of (*N*-X)-Ala·Gly.

The spectral changes observed as basicity increases may suggest the existence of a intermediate involved in an acid-base equilibrium, the apparent change of the extinction coefficient from near neutral to highly alkaline medium is $\Delta\epsilon \sim 1400$ $mol^{-1} \cdot dm^3 \cdot cm^{-1}$. Figure 2 shows a plot of the absorbance at the end of the first process *versus* the pH. From this plot a tentative value of 12.4 could be estimated for the $pK_a$ of the hypothetical acid-base equilibrium.

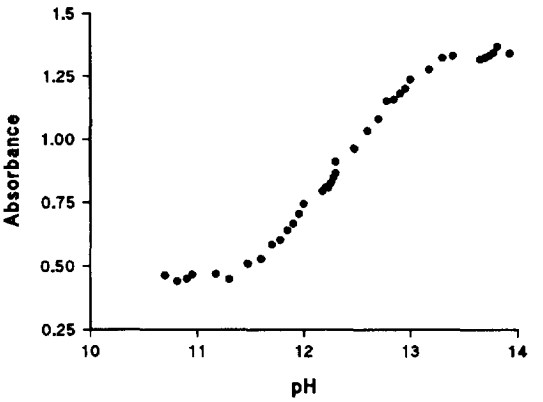



**Figure 2.** Plot of the absorbance of the intermediate *versus* the pH.

However, we believe this change in the absorbance of the intermediate to be an artifact due to the relative change in the rates of the two consecutive processes as the pH of the medium changes, *i.e.:* the faster the first process is relative to the second (or what is equivalent, the more basic the medium is), the higher the absorbance of the intermediate gets to. This can be easily theoretically simulated, obtaining exactly the same effect.

$NH_3$ and $X^-$ ions were found as final products of the overall process. This is in agreement with previous observations in neutral[22] and alkaline[11] medium finding ammonia, chloride ion and the diketonic compound (*N*-1,2-dioxopropyl)-Glycine.

The production of ammonia and chloride ion was followed with appropriate Ingold® selective electrodes. In this way it has been proved that $Cl^-$ is produced in the first process in which no $NH_3$ is generated.

The reaction was also followed by $^1H$ NMR in order to confirm the abstraction of the proton by the base. The peak corresponding to the proton on the $C_\alpha$ to both the (*N*-X)-amino group and the peptidic bond appears at $\delta$=3.9 ppm as a quartet.

Owing to the complexity of the overall process, up-till-now we have kept our interest to the first process, to which we will refer in what follows unless otherwise indicated.

This process is first order relative to the concentration of the (*N*-X)-Ala·Gly. Consistent values are obtained using NMR. When [NaOH]=0.1 mol·dm$^{-3}$ is added, the signal at δ= 3.9 ppm disappears in two minutes, which is consistent with the spectroscopically observed rate constant ($k_{obs}$=0.0209 s$^{-1}$, $t_{½}$ =33.3 s).

From the kinetic runs using $Cl^-$ ion selective electrode, the rate constant thus obtained ($k_{obs}$=3.1·10$^{-4}$ s$^{-1}$) fully agree with those obtained spectrophotometrically ($k_{obs}$=2.5·10$^{-4}$ ) in the same acidity conditions. In the case of (*N*-Br)-dipeptides the first process is *ca.* 2 orders of magnitude faster, while the rate of the second process is not altered.

No influence of the concentration of Ala·Gly was observed on the rate constant, nor of the ionic strength. Conversely, $k_{obs}$ increases linearly with the concentration of base as shown in Figure 3.

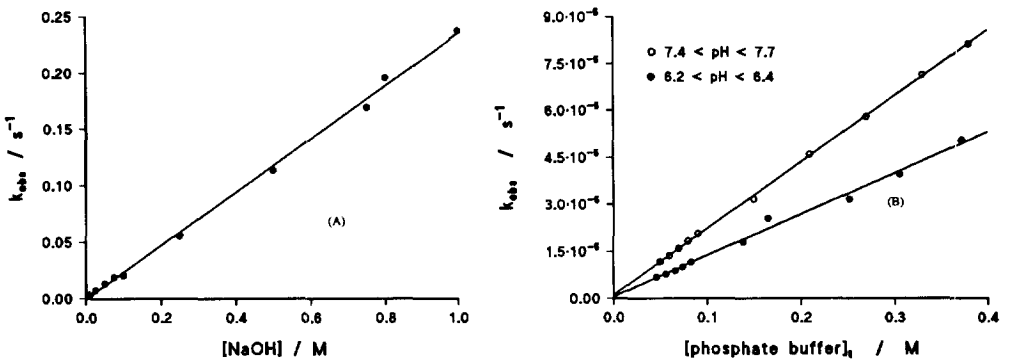



**Figure 3.** Influence of base concentration on the rate of the first process in the decomposition of (*N*-Cl)-Ala·Gly. [(*N*-Cl)-Ala·Gly]=1·10$^{-3}$ mol·dm$^{-3}$, [Ala·Gly]=2·10$^{-4}$ mol·dm$^{-3}$, I ($NaClO_4$) = 1.00 mol·dm$^{-3}$. (A) Influence of [NaOH]. (B) Influence of $[Phosphate\ buffer]_{total}$.

The rate equation for the process of decomposition corresponds to a overall second order, order one relative to both [(*N*-X)-Ala·Gly] and [Base], *i.e.:*

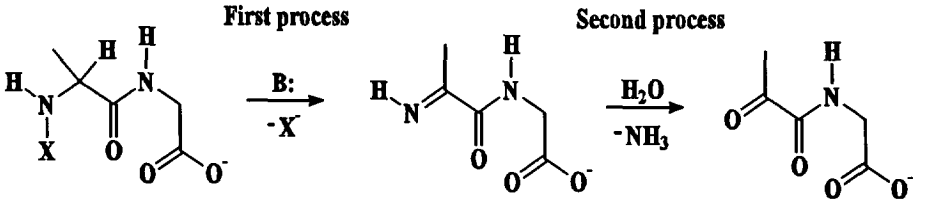
$$r\ k_{Base}\cdot[Base]\cdot[(N\text{-}X)\text{-}Ala\cdot Gly]$$

The catalytic rate constants obtained with different bases are compiled in Table 1. It is worth noting that the stronger the base, the bigger the rate constant.

**Table 1.** Catalytic rate constants for the base-promoted decomposition of (*N*-X)-Ala·Gly. [(*N*-X)-Ala·Gly]=1.0·10$^{-3}$ mol·dm$^{-3}$, [Ala·Gly]=1.2.·10$^{-3}$ mol·dm$^{-3}$, I ($NaClO_4$)=1.00 mol·dm$^{-3}$.

| k / mol$^{-1}$·dm$^3$·s$^{-1}$ | N-Cl | N-Br |
|---|---|---|
| k(OH$^-$) | 0.236 | 22 |
| k (2,2,2-Tri-Fluor-Ethanoxide) | 0.060 | -- |
| k (1,1,1,3,3,3-Hexa-Fluor-2-Propanoxide) | 0.00367 | -- |
| k ($H_2PO_4^-$) | 2.42·10$^{-4}$ | -- |
| k ($HPO_4^{-2}$) | 1.04·10$^{-4}$ | -- |

Taking into account all these facts, we propose for the first process of the decomposition of (*N*-X)-Ala·Gly the reaction mechanism shown in the Scheme. This mechanism takes place in two consecutives steps *via* an intermediate formed by elimination of a proton and a halide from the (*N*-X)-Ala·Gly.




**Scheme:** mechanism proposed for the base-promoted elimination of (*N*-X)-Ala·Gly.

Considering the $pK_a$ values for the different bases used, the Brønsted β can be estimated as β=0.27±0.05. Similarly, using the $pK_a$ values for HCl and HBr,[23] a $\beta_{lg}$ value for the leaving group (using $OH^-$ as base) can be roughly estimated as $\beta_{lg}$=0.33. According to the β and $\beta_{lg}$ values thus obtained, the elimination process can be described as a concerted $A_{xh}D_HD_N$ elimination with a reactant-like transition structure. Thus, a 6% of imbalance[24] takes place in the TS, with the N-X bond-breaking process being ahead of the C-H bond-breaking. No information is available for the C=N bond-making process. In principle, there is no reason to expect significant changes in the mechanisms for similar dipeptides, although the position of the transition structure on the free energy hypersurface may be different.

Further research is in course in order to confirm the proposed mechanism and to extend this study to other compounds, as well as to the second process.

**Conclusion.**

(*N*-X)-Ala·Gly decomposes in the presence of bases in two consecutive steps, the rather unstable intermediate *N*-(2-imino, 1-oxopropyl)-Glycine is generated in a first process, and subsequently hydrolyses to *N*-(1,2-dioxopropyl)-Glycine in a second process. The first step is an $A_{xh}D_HD_N$ elimination process, with a reactant-

like transition structure.

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