De-protonation of glutamic acid in a weak magnetic field

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• pH<3.2 both carboxylic and amine groups are protonated and its ionic charge is -1
• deprotonated species appear increasing pH
• isoelectric point = 3.2 pH, its ionic charge is 0
• above pKₐ=9.7 the amino acid is fully deprotonated and its ionic charge is +2
IR spectra of glutamic acid

(a) COOH

(b) COO⁻

(c) NH₃⁺

(d) NH₂

<table>
<thead>
<tr>
<th></th>
<th>COOH</th>
<th>COO⁻</th>
<th>NH₃⁺</th>
<th>NH₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>ν C=O</td>
<td>1720</td>
<td>1530</td>
<td>1630</td>
<td>1650</td>
</tr>
<tr>
<td>d C-OH</td>
<td>1400</td>
<td>1410</td>
<td></td>
<td></td>
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<tr>
<td>ν C-O</td>
<td>1260</td>
<td></td>
<td>1520</td>
<td></td>
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<tr>
<td>ν antisym.</td>
<td></td>
<td></td>
<td>1530 (ν antisym.)</td>
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<tr>
<th></th>
<th>NH₂</th>
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<td>δ scissoring</td>
<td>1650</td>
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Helmoltz coils used to produce the magnetic field. Samples were placed in the space between the coils on a plane. The distance between the coils was the same as their radius, $\Phi=10$ cm. The diameter of the copper wire was 0.05 cm and the resistance 4.4 Ohm, and an extra 50 Ohm load was added in order to match the output of the waveform generator.

- Magnetic field inside the coils: the simulation was carried out using a finite element code and shows the magnetic field strength $B$ in the half space that was symmetrical with respect to the axis of the coils. $z$-axis: strength of the field in Tesla; $x$-$y$ axes: number of points in the 50X50 mesh used for the calculations.
The amplitude of the field was adjusted to 45 microT in order to match the amplitude of the geomagnetic field as measured.

The maximum power dissipated into the coils was 150 mW.

The coils were kept in the open air and the space within them was maintained at room temperature $T=21\pm 1$ °C, as was the desk where the control samples were stored.

Both the amplitude and the frequency of the magnetic field were monitored continuously during exposure.
In order to test the hypothesis that the effect was related to the cyclotron frequency, a sinusoidal signal was applied to the coils, whose frequency was determined by the cyclotron frequency.

The ionic charge is 0 at the isoelectric point pH=3.2; 1 at $\text{pK}_a < 2.2$ and $4.3 < \text{pK}_a < 9.7$; 2 at $\text{pK}_a > 9.7$

<table>
<thead>
<tr>
<th>Ionic charge</th>
<th>$q/m (\text{Kg/C} \cdot 10^{-6})$</th>
<th>$\omega_c (\text{Hz})$</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>0.656</td>
<td>4.7</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>0.656</td>
<td>4.7</td>
</tr>
<tr>
<td>2</td>
<td>1.312</td>
<td>9.4</td>
</tr>
</tbody>
</table>
Stability of the spectra

In order to establish that our results could be repeated, measurements of consecutive acquisitions of the same native, unexposed, sample were made at 10-minute intervals.

the ratio of the peak heights, is unaffected.

We therefore contend that the effect of the perturbation can be safely evaluated in terms of changes in the peak height ratio, thereby enabling other random effects to be neglected.

IR spectra of pH=1.53 glutamic acid in water. The spectra were measured 10 minutes apart.
At low pH, both acid groups are protonated and contribute to the carbonyl stretches of COOH at (about) 1720 cm\(^{-1}\), however, the presence of an absorption band at (about) 1400 cm\(^{-1}\) indicates that some deprotonated carboxylate groups are present.

The increase in this band after exposure, together with a modification of the band at 1570 cm\(^{-1}\), shows a shift towards the deprotonated form that is induced by the EMF.

Comparison among samples unperturbed (solid line), exposed to the magnetic field for 10 min (dotted) and 20 min (dashed). The major changes in spectra show the absorption for the stretching mode of C=O (1720 cm\(^{-1}\)) and C-O (1260 cm\(^{-1}\)) and for the bending of NH\(^+\) (1520 cm\(^{-1}\));
Effect on the Hydrogen bond

This band reflects the contribution of both the antisymmetric deformation NH$_3^+$ groups and the deprotonated carboxylate groups,

Furthermore, the C-O vibrations are sensitive to H-bondings and the observed shift of the bands may be related to two factors:

- a change in the degree of hydrogen bonding
- the deprotonated state of the carboxylic group

Comparison between unperturbed samples (solid line) and samples exposed to magnetic field for 20 min (dotted line).
**Principal Component Analysis**

The results must be evaluated with respect to many different parameters, all informed by the ratios of the peak heights of the protonated and deprotonated carboxylic groups.

A simple comparison of the spectra of exposed with those of unexposed samples may be misleading.

Principal Component Analysis (PCA) allows the testing of the statistical significance of a hypothesis in a multiparameter space. Here, we projected the two sets of data onto the plane of reduced parameters obtained by the linear combination of the experimental parameters included in the analysis. The reduced parameters were the linear combination that maximised the projections of the experimental parameters on the axis. Two sets of data were taken to be significantly different when they occupied separate parts of the plane.
The glutamic acid should be fully deprotonated; however, the presence of a small band centred around 1650 cm\(^{-1}\) reveals the presence of the contribution of the deformation of NH\(_3^+\) in addition to the deformation of NH\(_2\) and the stretching of C=O. Such a band is severely restricted by exposure.
The observed effect does not depend on the frequency; hence, we find no corroboration of the starting hypothesis that the coupling of the EMF with the structure of the amino acid occurs via the $q/m$ ratio that defines a link between the perturbation and the target. (However, it is possible that the effect observed in our experiments differs totally or in part from that described in the cited literature)

A new hypothesis may therefore be suggested:

- the EMF effect is mediated by the structure of the water

The idea that biological activity is connected with the existence of long-lasting excitations that are mediated by liquid water was first discussed in the 1970s [Fröhlich, Ahmed].

- These long-lived excitations of water structures could be responsible, at least in part, for the electromagnetic properties of living systems.
CONCLUSIONS

- We have herein described the long-lived deprotonation of glutamic acid under exposure to a weak magnetic field.
- The observed effect appeared in the exposed samples within the first 20 min and was reversible within 1 h after exposure.
- No dependence was observed on the frequency of the field in the range 0-7 Khz.
- Our observations cannot be explained by the cyclotron resonance model, and require a wider understanding that takes into account the role of water as a mediator of the interactions involved.
Regular Article

Deprotonation of glutamic acid induced by weak magnetic field:
An FTIR-ATR study

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Article first published online: 1 DEC 2010
DOI: 10.1002/bem.20631
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Keywords:
ELF magnetic fields; FTIR spectroscopy; glutamic acid; H-bonding; long-living excitations