P-04

Study of the Degradation of Human Insulin

MELINDA ANDRÁSI[1]; BLERTA PAJAZITI[2]; BETTINA SÍPOS[1]; CYNTHIA NAGY[1]; NARMIN HAMIDLI[1]; ATTILA GÁSPÁR[1]

[1]*Department of Inorganic and Analytical Chemistry, University of Debrecen, Hungary, Egyetem tér 1, H-4032 Debrecen, Hungary*
[2] *University of Prishtina, Bulevardi I Deshmoreve, 10000 Prishtina, Kosovo*

Correspondence: andrasi.melinda@science.unideb.hu

Keywords: *Protein deamidation, biopharmaceutical, capillary electrophoresis, mass spectrometry, biologicals*

1. Introduction

Insulin is an important peptide hormone regulating glucose metabolism. Currently the majority of insulin used for medicinal purposes is produced by recombinant DNA technology. The biotechnologically engineered product can undergo several post-translational modifications (PTM). Deamidation is one of the most prominent PTM, which occurs as a result of the removal of amide groups in asparagine (N) and glutamine (Q) residues by hydrolysis resulting in free carboxylate groups. Asparagin is converted to aspartic acid and iso-aspartic acid through the formation of a succinimide intermediate. PTMs cause alterations in biological activity, immune response and stability, therefore their characterization during the manufacturing and storage is essential [1].

2. Results

Capillary zone electrophoresis (CZE) with UV and MS detection was tested to determine the deamidation isoforms of human insulin (Humulin R). For the degradation studies insulin pharmaceutical formulations were subjected to acidic condition (pH=1) at room temperature. Samples were analyzed several times but at different incubation times. For the CZE measurements uncoated fused silica capillary was used. Ammonium-acetate buffer of pH 9 (a higher pH than the pI value of insulin) was selected to minimize interactions of insulin with the deprotonated silanol groups of the inner surface. In order to further enhance selectivity, the effect of adding organic solvent (methanol, acetonitrile and isopropanol 10% (v/v)) to the buffer electrolyte was investigated. Resolution improved with buffers containing methanol or acetonitrile. Fastest separation was observed when acetonitrile was employed. Isopropanol provided better separation of degradation products but at longer analysis time (45 min). Increasing the isopropanol content of the background electrolyte to 20 % led to further separation of the degradation products, although at the expense of longer migration time. At least five isoforms can be seen with UV detection in the acidified sample stored at room temperature for 12 hours. Additional ten components appeared in the sample stored for one month.

3. Conclusion

CE coupled with mass spectrometry was used for the determination of the molecular mass of each deamidated isoform. The deamidation of one ami-

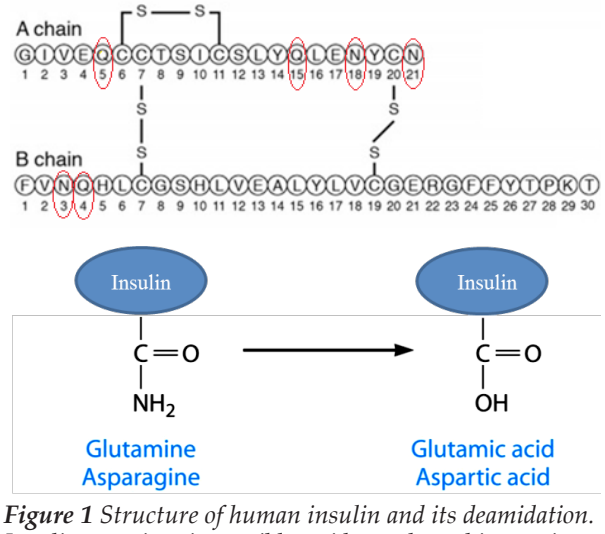

Figure 1 *Structure of human insulin and its deamidation. Insulin contains six possible residues where this reaction could take place: Q(A5), Q(A15), N(A18), N(A21), N(B3), Q(B4)*

 DOI: 10.33892/aph.2020.91.169-170

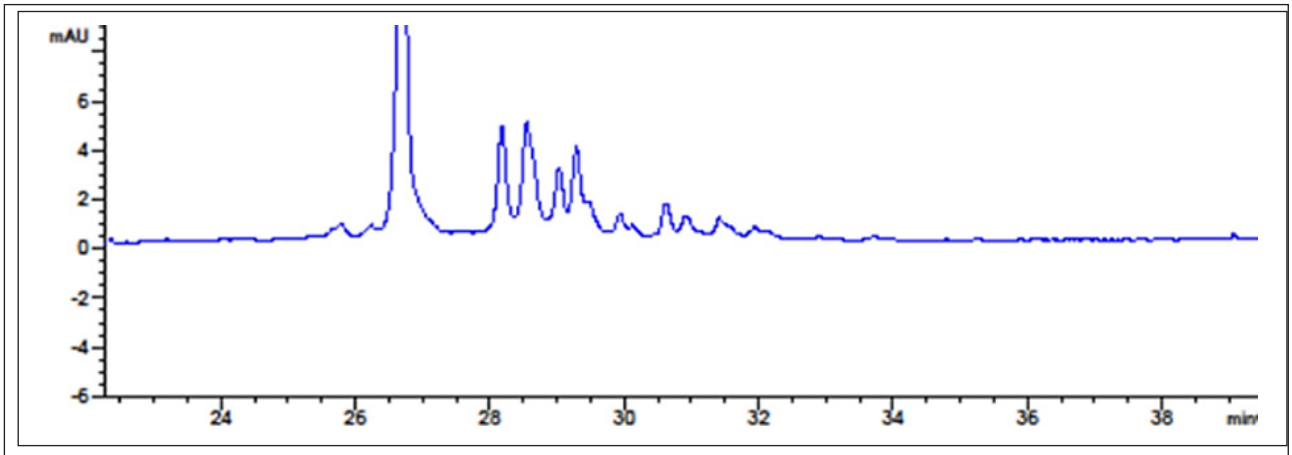

Figure 2 *CZE separation of insulin and its degradation products, using running buffer of 50 mM ammonium-acetate, 20% isopropanol, pH 9, uncoated silica capillary: 85 cm x 50μm i.d., sample was injected by 100 mbar·s, 25 kV, λ= 200 nm The 3.5 mg/ml pharmaceutical product of insulin (Humulin) was acidified with HCl solution (0.1 M)*

no acid leads to a 1 Da mass increase. CZE was suitable for the separation of isoforms possessing different charge to mass ratio and MS detection enabled the identification of the degradation products.

Acknowledgement

The research was supported by The National Research, Development and Innovation Office, Hungary (K127931).

References

1. *Gervais, D., Protein deamidation in biopharmaceutical manufacture: understanding, control and impact, J. Chem Technol Biotechnol, 91: 569-575 (2016).*
2. *Mane, K., Srivatsa, K., Naik, A., Kodali, P., Separation and characterization of deamidated isoforms in insulin analogue and its underlying mechanism, J. Pharmaceutical and Biological Sciences, 5: 258-264 (2017).*
3. *Moritz, B., Locatelli, V., Niess, M., Bathke, A., Kiessig, S., Entler, B., Finkler, C., Wegele, H., Stracke, J.,Optimization of capillary zone electrophoresis for charge heterogeneity testing of biopharmaceuticals using enhanced method development principles, Electrophoresis,38:3136-3146(2017).*