Assignment of polymorphic species of insulin analogues in ion mobility mass spectroscopy

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\textbf{A B S T R A C T}

Electrospray ionization – ion mobility spectrometry – mass spectrometry (ESI–IMS–MS) allows the identification of protein polymorphic distribution of protein conformers and oligomers. We report the detailed identification of the species observed with commercially available pharmaceutical preparation of wild-type, regular human insulin.

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\textbf{Specifications Table}

Subject area
Physics, Chemistry, Biology
More specific subject area
Structural Biology
Type of data
Figure (mass spectrometry)

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Mass spectrometry coupled to ion mobility spectrometry (Synapt HDMS G1, Waters Corp)

Data format
Analyzed

Experimental factors
Concentrated sample (human insulin 100 U/mL, 3.5 mg/mL, 600 μM) was diluted to 50 μM in 100 mM ammonium acetate.

Experimental features
Electrospray ionization - mass spectrometry coupled to ion mobility spectrometry measurements of regular-acting human insulin measured in ammonium acetate.

Data source location
Not applicable

Data accessibility
Data is with this article.

Value of the data

- Proteins can populate a broad range of conformation and oligomeric states according to chemical and physical variables.
- Polymorphic distribution is a identity of a formulated biopharmaceutical product.
- Electrospray ionization mass-spectrometry coupled to ion-mobility spectroscopy (ESI–IMS–MS) allows separation of species by cross-sectional area.
- ESI–IMS–MS allowed the assignment of the diversity of oligomeric and conformational distribution of human insulin.
- ESI–IMS–MS may serve for the characterization of the effect of formulation on biopharmaceuticals products.

1. Data

Unidimensional mass spectrometry analysis of proteins, including biopharmaceuticals, does not allow a clear discrimination of polymorphic species with overlapping m/z. Oligomers sometimes can be observed the isotopic distribution, but can be limited in cases of low prevalence or multiple oligomeric states. Moreover, similar oligomeric state with same charged state sometimes may display multiple conformation, which can not be elucidated by unidimensional electrospray ionization mass...
spectrometry (ESI–MS). In such case, the separation of the species, either conformational or oligomeric, by ESI–MS coupled ion mobility spectrometry (ESI–IMS–MS) allow a more in-deep depiction of the complex polymorphic distribution in protein specimens, such as in biological, biopharmaceutical products.

Here we present data concerning the ESI–IMS–MS stripping of the drift-time spectra of commercial regular insulin, and from them we could identify monomers (Figs. 1 and 2), dimers (Figs. 2 and 3), trimers (Figs. 3 and 4), tetramers (Fig. 4), pentamers (Fig. 5) and hexamers (Figs. 3 and 5) and their respective sodium adducts (Fig. 6).
Fig. 4. ESI–IMS–MS spectra of regular insulin – trimer and tetramer. The dt.m/z spectra of the (A) T+8, (B) T+7, (C) T+4 and (D) Q+9 charged states and their respective sodium adducts.

Fig. 5. ESI–IMS–MS spectra of regular insulin – pentamer and hexamer. The dt.m/z spectra of zinc complexes of (A) P+13, (B) P+9, (C) P+8, (D) H+10, (E) H+11 and (F) H+13, and their respective sodium adducts.
2. Experimental design, materials and methods

2.1. Chemicals

The pharmaceutical product regular human insulin (100 U/mL = 600 μM; Humulin R®; lot #C020193, #A560347A), was purchased from local pharmacy and stored at 4 °C – 8 °C until use. All other reagents were of analytical grade.

2.2. Electrospray ionization–Ion mobility spectrometry–Mass spectrometry (ESI–IMS–MS) measurements

ESI–IMS–MS measurements were conducted in a Synapt G1 (Waters, USA) high definition mass spectrometer (HDMS) quadrupole-travelling wave mass spectrometer. The insulin samples were prepared from the formulated stock solution (600 μM = 100 U/mL) by diluting 5 times (120 μM = 20 U/mL) directly with the 100 mM ammonium acetate buffers pH 7.4. Measurements were performed immediately after sample preparation. The insulin samples were measured in a positive ESI mode, with a capillary voltage of 2.8 kV and N2g at 0.4 bar. The samples were injected at a rate of 10 μL/min. Data were accumulated for 20 min with a 3 s acquisition time per point, over the range of m/z 500–4000. Other typical experimental settings involved sampling and extraction cone set respectively at 30 V/5.0 V, source temperature of 70 °C, and nanoflow gas pressure of 400 mbar. The trap (before IM cell) and transfer (after IM cell) cells voltage were set to 6 and 4 V, respectively. The cell pressures were controlled by argon gas, while IM separations were performed by using N2g, and transfer wave velocity of 248 m/s and transfer wave amplitude set at 3.0 V. The mass calibration was performed on a dynamic mode by using H3PO4. All data were analyzed by using MassLynx 4.1 (Waters Corporation, Brazil) and DriftScope 2.4 (Williams, Lough 2009) (Waters Corporation, Brazil).

Fig. 6. ESI–MS spectra of insulin at pH 7.4 and pH 5.6. S (Sigma-Aldrich, no formulation components), (H) regular Humulin R, (N) regular Novolin N, (I) regular (Insunorm R), (A) aspart (Novorapid), (L) lispro (Humalog), (D) detemir (Levemir), (G) glargine (Lantus). The list and relative intensity of the identified ions are depicted in Supporting Table S1.
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Transparency document. Supporting information

Transparency data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.dib.2016.12.020.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.dib.2016.12.020.

Further Reading

[1] Favero-Retto, et al., Polymorphic distribution of proteins in solution by mass spectrometry: the analysis of insulin analogues, Biologicals. (2016). http://dx.doi.org/10.1016/j.biologicals.2016.09.011.