M. Atakay / Hacettepe J. Biol. & Chem., 2023, 51 (1), 113-124

Research Article

Hacettepe Journal of Biology and Chemistry

journal homepage: www.hjbc.hacettepe.edu.tr

Analysis of Conformational Differences of Copper and Alkali Metal Complexes of Insulin Using Trapped Ion Mobility-Mass Spectrometry Technique

Tuzaklamalı İyon Hareketliliği-Kütle Spektrometrisi Tekniği Kullanılarak İnsülinin Bakır ve Alkali Metal Komplekslerinin Konformasyonel Farklılıklarının Analizi

Mehmet Atakay*

Department of Chemistry, Hacettepe University, 06800, Ankara, Turkey.

ABSTRACT

Molecular recognition, protein folding, and formation of supramolecular structures that occur at the molecular level of biological processes are based on noncovalent interactions. Interactions between metal atoms and proteins are also based on noncovalent interactions that underlie the mechanisms involved in many cellular processes. The activities of enzymes are highly dependent on the interactions of such protein groups with cofactors, substrates, metal ions, and other proteins. The compositions and binding stoichiometry of protein-metal complexes can be determined with high accuracy performing mass spectrometry (MS) analysis. The conformational features of protein-metal complexes can be studied additionally using a mass spectrometer with ion mobility spectrometry (IMS) capability. This study focuses the monitoring the differences in the conformational changes of insulin protein during the formation of its complex with copper and alkali metals using trapped ion mobility spectrometry – time-of-flight (TIMS–TOF) mass spectrometer instrument. The compaction of the insulin structure by the formation of the insulin-copper complexes in the gas phase was determined with TIMS-TOF-MS analyses. However, no change was observed in the insulin structure with the addition of H, Na, and K atoms as adducts at the same analysis conditions.

Key Words

Noncovalent interactions, protein-metal complexes, conformational change, trapped ion mobility-mass spectrometry.

ÖZ

Moleküler tanıma, protein katlanması ve supramoleküler yapıların oluşumu gibi moleküler düzeyde meydana gelen biyolojik süreçler kovalent olmayan etkileşimlere dayanır. Metal atomları ve proteinler arasındaki etkileşimler de birçok hücresel süreçte yer alan mekanizmaların temelini oluşturan kovalent olmayan etkileşimlere dayanmaktadır. Enzimlerin aktiviteleri, bu tür protein gruplarının kofaktörler, substratlar, metal iyonları ve diğer proteinlerle olan etkileşimlerine büyük ölçüde bağlıdır. Protein-metal komplekslerinin bileşimleri ve bağlanma stokiyometrileri, yüksek doğrulukta kütle spektrometrik (MS) analiz ile belirlenebilir. Protein-metal komplekslerinin konformasyonel özellikleri ise iyon hareketliliği spektrometrisi (IMS) özelliğine sahip bir kütle spektrometresi kullanılarak ek olarak incelenebilmektedir. Bu çalışmada tuzaklamalı iyon hareketliliği spektrometrisi - uçuş zamanlı (TIMS-TOF) kütle spektrometresi kullanılarak bakır ve alkali metallerle komplekslerinin oluşumu sırasında insülin proteininin konformasyonel değişimlerindeki farklılıkların izlenmesine odaklanılmaktadır. İnsülin-bakır komplekslerinin oluşmasıyla insülin yapısının gaz fazında daha kompakt hale geldiği TIMS-TOF-MS analizleri ile belirlenmiştir. Ancak aynı analiz koşullarında H, Na ve K atomlarının eklenmesiyle insülin yapısında herhangi bir değişiklik gözlenmemiştir.

Anahtar Kelimeler

Kovalent olmayan etkileşimler , protein-metal kompleksleri, konformasyonel değişim, tuzaklamalı iyon hareketliliği-kütle spektrometrisi.

Article History: Jul 31, 2022; Revised: Oct 7, 2022; Accepted: Oct 11, 2022; Available Online: Oct 15, 2022.

DOI: https://doi.org/10.15671/hjbc.1150033

Correspondence to: M. Atakay, Department of Chemistry, Hacettepe University, 06800, Ankara, TURKEY.

E-Mail: mehmeatakay@hacettepe.edu.tr

INTRODUCTION

Metal ions interact with proteins and form metal-protein complexes in many biological processes. Such processes, which include folding and stabilizing protein structures and acquiring their biological functions, take place in the presence of metal ions [1]. Besides the formation of coordination complexes between metals and proteins, there are also complex formations based on noncovalent interactions such as hydrogen bonding, π-stacking, and hydrophobicity [2]. Protein structures can undergo structural changes specific to the interaction sites for target recognition by forming complexes with metals [3]. Proteins can interact with metals through functional groups such as amine, hydroxyl, phosphate, sulfonate, and carboxylic acid located in their structures. The amine groups at the N-terminus of the protein chains and the carboxylic acid groups at the C-terminus are also potential binding sites for metals [4]. Complexes involving such interactions are characterized by various analytical techniques such as calorimetry, X-ray crystallography, circular dichroism, and nuclear magnetic resonance spectroscopy. In addition to the analytical advantages of these techniques, they also have some shortcomings in the analysis of noncovalent complexes. The necessity for large amounts of samples, long analysis times, complicated interpretation of data due to the interference of signals, the problems encountered in the crystallization of such samples, and the inability to detect complexes using spectroscopic methods are the most prominent shortcomings of these techniques [5-6]. Mass spectrometry (MS) techniques that allow monitoring such interactions with high accuracy and sensitivity can produce ionic species in their both native and gas-phase forms during or after the ionization. In fact, with the development of soft ionization techniques in MS, analyses can be performed to preserve noncovalent interactions in complex structures while transferring from the solution to the gas phase [7-8]. Strong interactions between metal ions and biomolecules in the solution phase are generally not disrupted during electrospray ionization (ESI) [9]. The preservation of the structures of metal-protein complexes based on noncovalent interactions during the soft ionization process makes it possible to analyze these species in their intact forms. The bonding ratios and stoichiometry of the complexes formed due to noncovalent interactions of metals and proteins can be determined by evaluating the mass spectra obtained from the ESI-MS analysis [10-11]. Conformational changes resulting from the binding of metals to proteins can be monitored using the ion mobility-mass spectrometry (IM-MS) technique. The ion mobility units of the IM-MS instruments separate the ions into different ion packets according to their arrival time differences in the gas phase depending on their collision cross section (CCS) values [4]. Such problems in previously mentioned analytical techniques are not encountered in IM-MS analyses. Thus, IM-MS has become prominent among other analytical techniques in the structural analysis of protein-metal complexes.

In this study, the noncovalent complexes of the insulin protein with copper and alkali metals were compared and analyzed in terms of changes in protein's conformational features using the trapped ion mobility spectrometry – time-of-flight – mass spectrometry (TIMS-TOF-MS) technique. Copper is an essential trace element in several redox processes in living organisms. Trace elements which are very important for many biological activities, regulate many physiological reactions by forming complexes with biomolecules [12]. Many living organisms use a certain amount of copper metal to survive and maintain their physiological functions correctly [13]. It is an essential metal ensuring the correct functioning of the proteins and enzymes in the vital biological pathways. It must also be included in metabolic processes to functionalize many enzymes in organisms [12, 14]. Insulin is a hormone that consists of 51 amino acids and is secreted from the pancreas in the body [15]. It regulates glucose, protein, and fat metabolisms in the blood [16]. Proper functioning of insulin metabolism is vital for human health and this metabolism needs to be regulated for patients with diabetes. Insulin commonly forms complexes with metals such as copper, magnesium, sodium, and potassium, in addition to zinc and calcium [17-18]. Pancreatic β-cells within these metabolic activities form insulin-zinc coordination complexes [19]. Group I and II metal ions such as K^+, Na^+, Ca^{2+}, and Mg^{2+} are also involved in insulin secretion from pancreatic β-cells [20].

MATERIALS and METHODS

Chemicals

Insulin human, copper (II) nitrate hydrate (99.999% trace metals basis), methanol, and water (LC-MS grade) were purchased from Sigma-Aldrich (St Louis, MO, USA). The mass and mobility calibration of methods were performed using the standard tune mixture purchased from Agilent Technologies (Santa Clara, CA, USA).

Trapped Ion Mobility-Mass Spectrometry Analyses

Trapped Ion Mobility Spectrometry – Time-of-Flight (TIMS–TOF) mass spectrometer (Bruker Daltonics, Bremen, Germany) was used in mass spectrometry (MS) and ion mobility-mass spectrometry (IM-MS) analyses of insulin and insulin- copper (II) nitrate mixtures. TIMS technique applies an electric field in a funnel to keep ions in a gas flow. Following the ion trapping event, the electric field is gradually reduced by decreasing voltage to allow sequential elution of trapped ions with ascending mobilities [20].

Insulin human protein was dissolved in water at 0.5 mg/mL concentration. The protein solution was diluted with water:methanol mixture (7:3, v/v) to 0.01 mg/mL final concentration prior to TIMS–TOF-MS analyses. Copper (II) nitrate solution was also prepared in water at 1.0 mg/mL concentration. Then, 1.0 µL of the copper (II) nitrate solution was added to the insulin solution before TIMS-TOF-MS analyses. The sample solutions were directly infused into the ESI source at a flow rate of 3.0 µL/min. MS and IM-MS analyses were performed using the following ESI source settings: 4500 V capillary voltage, 0.5 bar nebulizer gas pressure, 4.0 L/min drying gas flow, and 150 °C drying gas temperature. TIMS parameter settings: IMS ramp start: 0.50 V.s/cm^2, ramp end 2.00 V.s/cm^2; ramp time 350 ms; ΔV deflection transfer and capillary exit: 20 V, funnel 1 and deflection transfer: 120 V, ramp start and accumulation exit: 120 V. Nitrogen was used as source gas which is filled in the TIMS unit of the instrument. The mass and mobility calibrations were performed externally using the Agilent ESI-L Low Concentration Tuning Mix [22-23]. DataAnalysis 5.0 software provided by Bruker was used for processing the acquired data.

RESULTS and DISCUSSION

Insulin protein has a molecular weight of approximately 5.7 kDa and contains two chains linked by two disulfide bonds in its structure [24]. The signals of multiply charged ions of the insulin protein are obtained in the mass spectrum as a result of ESI-MS analysis of insulin in the positive ion mode. The m/z values of the signals observed in the mass spectrum generally vary depending on the charge states of the ions formed in the gas phase. The net charges of the ions in the gas phase differ according to the number of protons or metal atoms adducted to the protein structure.

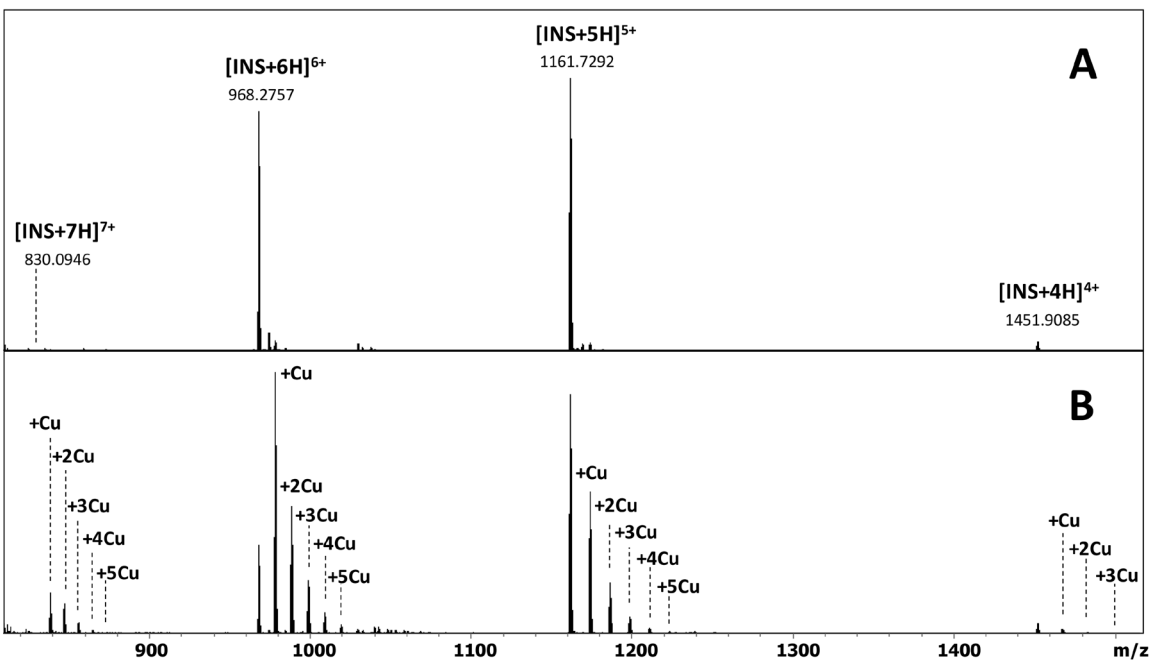

Figure 1. ESI mass spectra of (A) Insulin (B) Insulin with the addition of $Cu(NO_3)_2$. The numbers of attached Cu atoms to insulin are indicated for each peak in the mass spectrum.

Table 1. The m/z list of obtained ions in the TIMS-TOF-MS analyses of insulin and its mixture with $Cu(NO_3)_2$ between 4+ and 7+ charge states.

7+						
INS + 7H	INS + 6H + Na	INS + 6H + K	INS + 5H + Na + K	INS + 5H + 2K	INS + 4H + Na + 2K	INS + 3H + 2Na + 2K
830.0946	833.2323	835.5153	838.6562	840.9356	844.0789	847.214
INS + Cu	INS + 2Cu		INS + 3Cu	INS + 4Cu		INS + 5Cu
838.7953	847.4976		856.3426	865.0437		874.0313
6+						
INS + 6H	INS + 5H + Na	INS + 5H + K	INS + 4H + Na + K	INS + 4H + 2K	INS + 3H + Na + 2K	INS + 3H + 3K
968.2757	971.9408	974.6005	978.2634	980.9256	984.5878	987.2500
INS + Cu	INS + 2Cu		INS + 3Cu	INS + 4Cu		INS + 5Cu
978.4267	988.5789		998.7314	1009.0504		1019.2023
5+						
INS + 5H	INS + 4H + Na	INS + 4H + K	INS + 3H + Na + K	INS + 3H + 2K	INS + 2H + Na + 2K	INS + 2H + 3K
1161.7292	1166.1269	1169.3200	1173.7143	1176.9083	1181.3046	1184.4981
INS + Cu	INS + 2Cu		INS + 3Cu	INS + 4Cu		INS + 5Cu
1173.9100	1186.0929		1198.2752	1210.6593		1223.2418
4+						
INS + 4H	INS + 3H + Na	INS + 3H + K	INS + 2H + Na + K	INS + 2H + 2K	INS + H + Na + 2K	INS + H + 3K
1451.9085	1457.4058	1461.3972	1466.8898	1470.8836	1476.3755	1480.3865
INS + Cu			INS + 2Cu		INS + 3Cu	
1467.1353			1482.6146		1498.0901	

Firstly, human insulin was analyzed in the study in the positive ion mode by the ESI-MS technique without adding any acid or salt. In the mass spectrum obtained from these ESI-MS analyses, signals of the insulin protein ions having charges between 4+ and 7+ were observed (Figure 1). When the signal intensities in the ESI-MS spectrum, which are directly proportional to the relative abundances of the ions, are compared, it is seen that the signal intensities of 5+ (m/z 1161.7292) and 6+ (m/z 968.2757) charged ions of the insulin protein are much higher than the signal intensities of 4+ (m/z 1451.9085) and 7+ (m/z 830.0946) charged ions in the ESI mass spectrum (Figure 1A). In the same mass spectrum, signals of Na+ and K+ adduct ions are also observed at low intensity. Numbers and types of adducted atoms with corresponding m/z ratios of the ions observed at different charge states are given in Table 1.

When the insulin sample was analyzed after mixing with $Cu(NO_3)_2$ salt, it was observed in the obtained ESI-MS spectrum that different numbers of Cu atoms were attached to the insulin protein structure in the 4+ / 7+ charge state range (Figure 1B). These signals observed in the mass spectrum indicate that different numbers of copper atoms can bind to the insulin structure in the gas phase. While up to five copper atoms were bound to insulin at 5+, 6+, and 7+ charge states, maximum of three copper atoms could attach to the same protein in lower charge states (e.g., 4+ charge state). The numbers of attached copper atoms with corresponding m/z ratios of copper adduct ions observed at different charge states are also given in Table 1.

Ion mobility-mass spectrometry (IM-MS) analyses were also performed for the same samples. In IM-MS analyses, ion mobility diagrams are acquired, in which the mobility ($1/K_0$) and m/z data are located on the y- and x-axis, respectively. In these diagrams, the signal intensities of the ions are expressed by choosing an appropriate color range. Areas appearing in light color on the diagram indicate higher signal intensity. The signals of the 4+ and 7+ charged insulin ions are observed as straight linear lines extending from the corresponding mobility ranges in the ion mobility diagram obtained from the TIMS-TOF-MS analyses of the insulin sample (Figure 2A). It can be noticed that the signals of alkali metal adduct insulin ions form partial lines towards the upper right corners of the linear lines corresponding to each charge

state at higher mobility values. (Figure 2A). This data indicates that the protein structure expands as expected due to the increase in m/z value with the addition of alkali metal atoms to the insulin structure.

Signals of copper adduct protein ions were also observed in the ion mobility diagrams obtained from IM-MS analyses after the addition of $Cu(NO_3)_2$ salt to the insulin sample. (Figure 2B). This diagram shows signals belonging to copper adduct insulin ions in the 4+ and 7+ charge range as straight linear lines extending through their corresponding mobility ranges. It can be seen in Figure 2B that ions with higher numbers of copper atoms in their structure form lines towards the lower right corner with lower mobility values, depending on the change in the number of copper atoms in the copper adduct ions. The m/z values of the total structures increase due to the binding of copper atoms to the insulin protein. The fact that this increase in the m/z values of the ions corresponds to the decrease in their mobility ($1/K_0$) indicates that the structures become more compact in the gas phase due to copper atom binding.

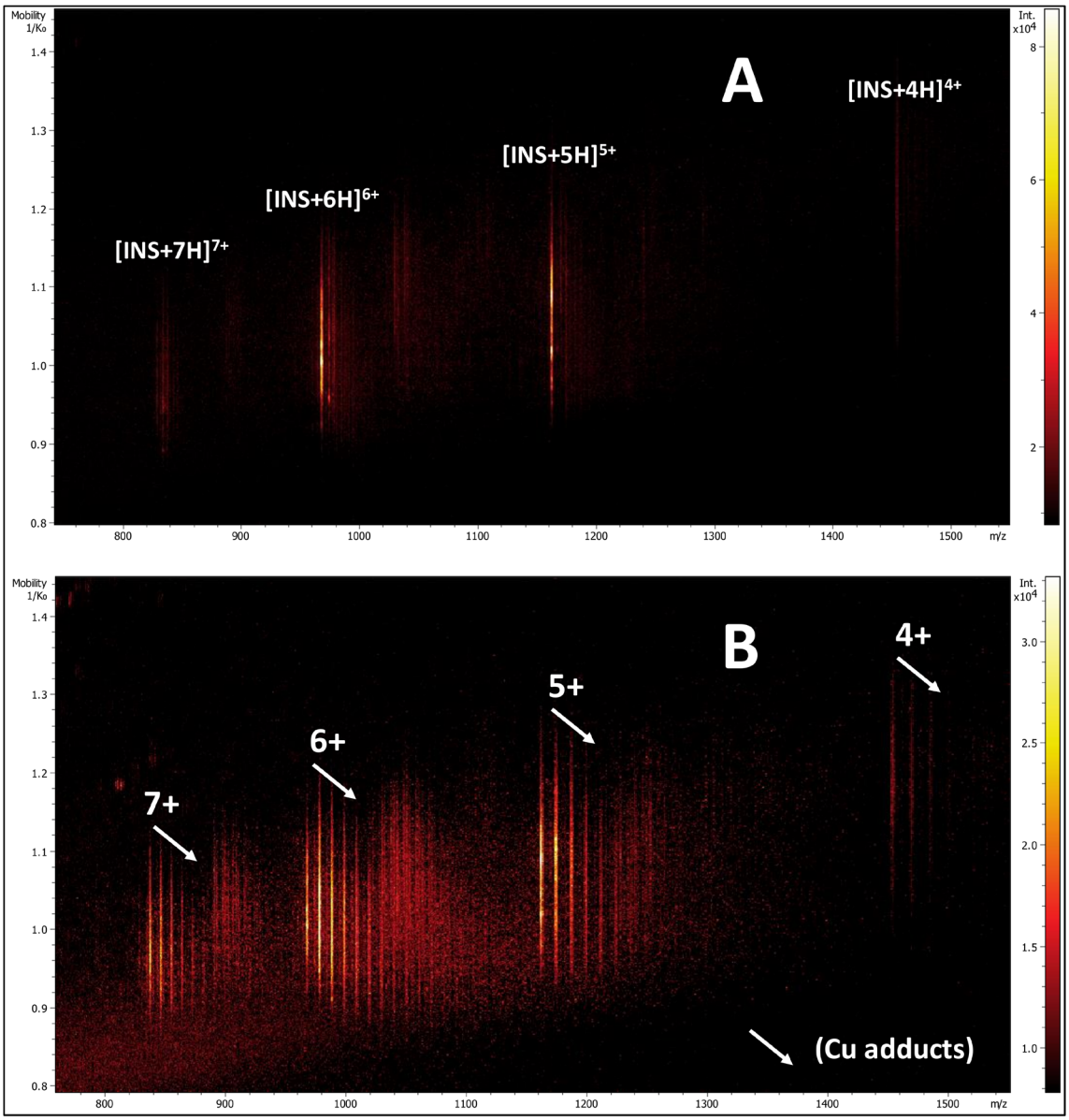

Figure 2. 2D TIMS-MS contour plots of (A) human insulin (B) human insulin with the addition of $Cu(NO_3)_2$. White arrows show the Cu adducts of the insulin ions between 4+ and 7+ charge states.

The mobility values in the data obtained from the ion mobility diagrams were converted to CCS values according to the Mason-Schamp equation [25-26]. The y-axis is proportional to the relative abundances of the ions, while the x-axis corresponds to the CCS (Å^2) values in the plots obtained after the conversion. The shifts of the curves in these plots on the CCS axis (x-axis) make it possible to determine the extent to which the analyzed species contains compact or extended forms in the gas phase and to monitor the formation of these conformers. Extracted ion mobilograms of various copper adduct insulin ions between 4+ and 7+ charged states obtained from TIMS-TOF-MS analyzes are given in Figure 3A-D. The conformational change in each charge state is evaluated individually. At the highest charge state (7+ charge state), it is seen that the CCS value of the structure decreases significantly as the number of copper atoms attached to the insulin structure increases (Figure 3A). This plot shows that the green-colored curve (INS+5Cu) has a higher intensity signal in the lower CCS region compared to the other curves. It is seen that the curve intensity increases in this compact CCS region from the black-colored curve (INS+Cu) to the green-colored curve (INS+5Cu). This data shows that with the addition of copper atoms to the insulin structure, the abundance of the compact form of the structure incre-

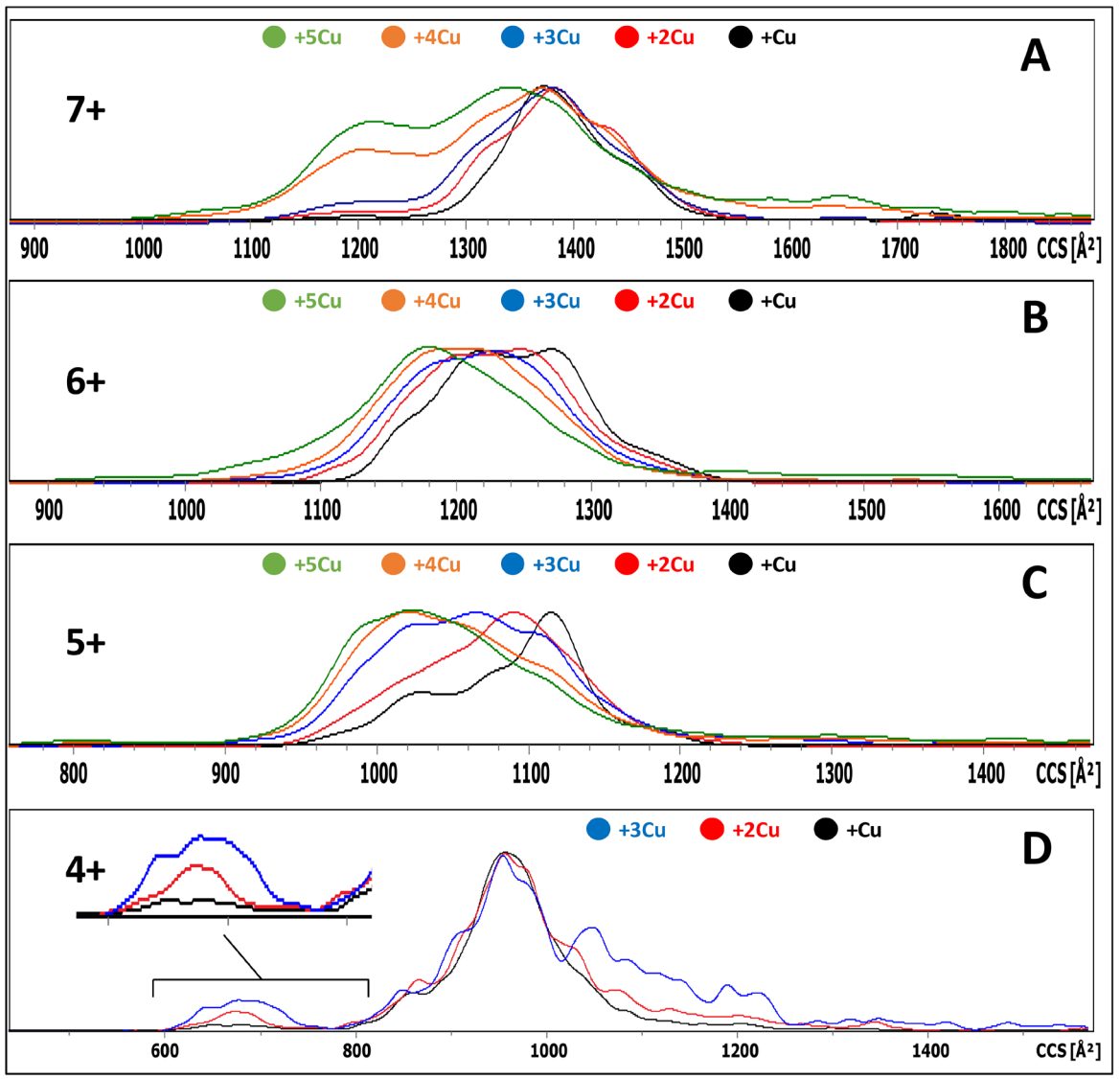

Figure 3. Extracted ion mobilograms of various Cu adducts of insulin ions between 4+ and 7+ charged states obtained from TIMS-TOF-MS analyses (A-D). The numbers of attached Cu atoms to insulin protein are shown at each set of mobilograms with their correlated colors.

ases significantly at the 7+ charge state. These nonspecific interactions between metals and proteins usually cause the compaction of the proteins in the gas phase [27-28]. Similarly, in the 6+ and 5+ charge states, the curves shifted to lower CCS values as the copper atom binds to the insulin protein, indicating that the addition of the copper atom makes the protein more compact in the gas phase (Figures 3B and 3C).

It is expected that the addition of copper atoms carrying extra charge to the protein will be less tolerated by the protein at the lowest charge state (4+ charge state). For this reason, the mobilograms obtained in this charge state, where only three copper atoms can be added to the insulin protein, are given in Figure 3D. The signals having lower intensity could be obtained at this lower charge state compared to the others due to the lower stability of the copper adduct protein ions. Therefore, it is more difficult to notice conformational changes from the signals in the mobilograms obtained at this charge state. It is seen that the signal intensities at lower CCS values increase due to the increase in the number of copper atoms attached to the protein from the black-colored curve (INS+Cu) to the blue-colored curve (INS+3Cu) (Figure 3D). This data also supports the inference that copper atom bonding increases the relative abundance of the compact form of the insulin protein in the gas phase.

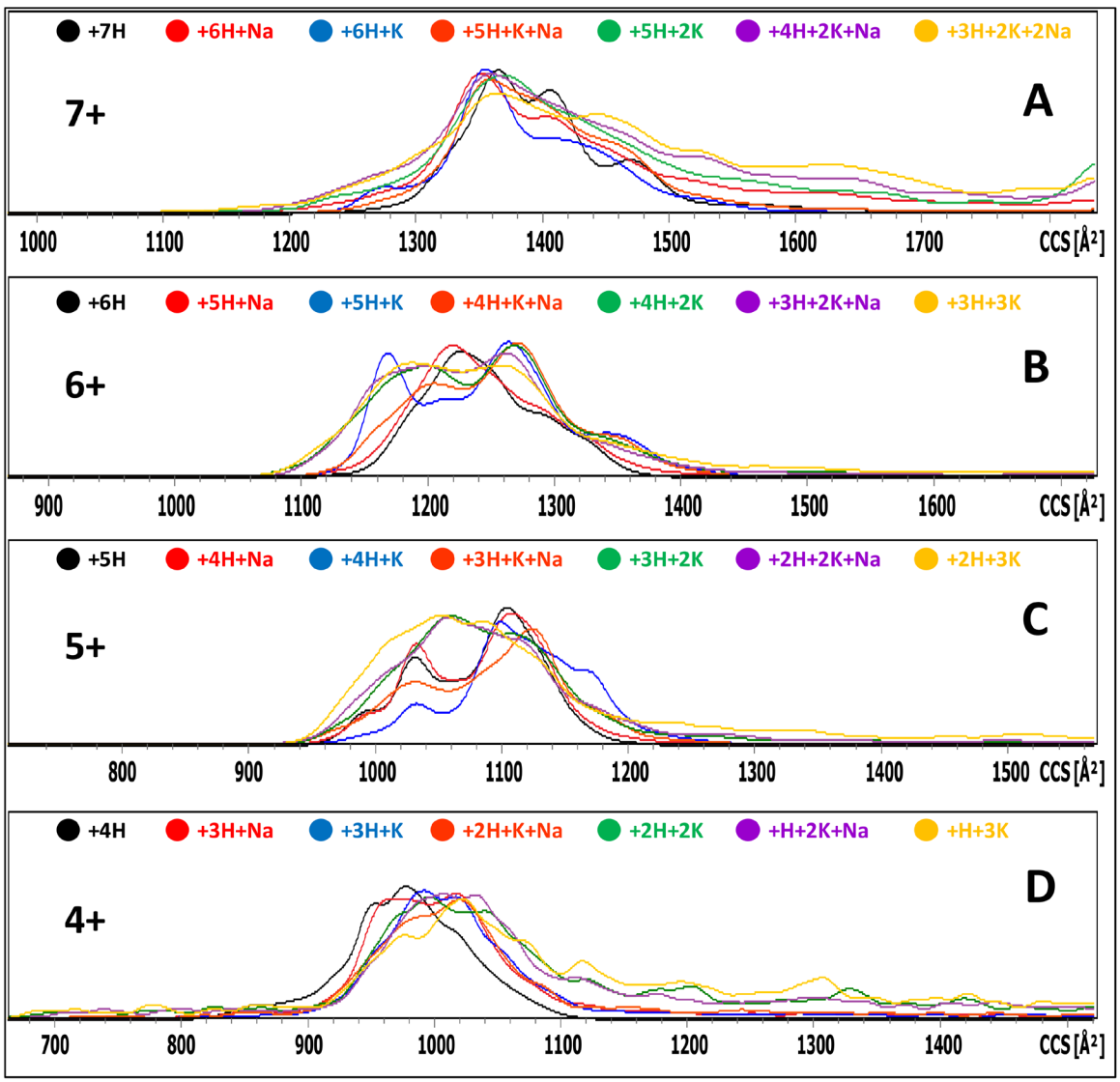

Figure 4. Extracted ion mobilograms of various H, Na, and K adducts of insulin ions between 4+ and 7+ charged states obtained from TIMS-TOF-MS analyses (A-D). The numbers of attached H, Na, and K atoms to insulin protein are shown at each set of mobilograms with their correlated colors.

MS analyses performed in positive ion mode have a high probability of binding sodium and potassium alkali metals to the analytes. The alkali metal adduction can be observed even at very low salt levels. In contrast, at higher salt concentrations, it can cause the presence of dominant signals of adducted ions in the mass spectrum. Especially in ESI-MS analyses that provide multiply charged ion formation, it is highly probable that more than one sodium and potassium ions bind to a single species even in a very low amount of salt. The formation of ions by binding alkali metals to peptides and proteins can also cause changes in the conformation of these species in the gas phase [29]. The insulin protein was analyzed by the IM-MS technique without adding any metal salt to the sample. Thus, the signals of H, Na, and K adduct protein ions with different combinations of these atoms were also evaluated for conformational analysis. The mobilograms obtained for these ions at different charge states are given in Figure 4A-D. The different colored extracted ion mobilograms of various H, Na, and K adduct insulin ions between 4+ and 7+ charged states obtained from TIMS-TOF-MS analyses are evaluated on the CCS axis (x-axis) of the mobilograms depending on different numbers and types of adduct atoms. It is seen that they do not show apparent shifts in the corresponding x-axis (Figure 4 A-D). In addition, no significant change was detected in the intensities of the curves in the mobilograms at both low and high CCS values corresponding to the compact and extended forms of the protein, respectively. The IM-MS data containing these signals with random distributions show that no obvious conformational change in the insulin protein structure occurs with the addition of H, Na, K atoms, and their different combinations. In some cases, the addition of such atoms increases the CCS value slightly with the increase in molecular mass, but it does not even cause a net change in the CCS value.

The data in the mobilograms given in Figures 3 and 4 are obtained under the same analysis conditions. According to these mobilograms, it was determined that the protein-metal complexes formed as a result of the nonspecific binding of copper atoms (monoisotopic mass of Cu: 62.9296 Da) to the insulin protein became more compact than the single insulin protein in the gas phase. However, there is no change in the protein structure such as compaction as a result of the addition of atoms such as H (1.0078 Da), Na (22.9898 Da), and K (38.9637 Da), which are smaller than the copper atom. These results showed that copper metal and alkali metals interact with insulin protein in different ways in the gas phase and this difference can be monitored through conformational changes with IM-MS analysis [30-31].

Conclusion

Mass spectrometry techniques that allow monitoring noncovalent interactions with high accuracy and sensitivity can produce ionic species in their intact forms during or after ionization. Various types of ionic species of the same sample can be transferred to the mass analyzer by performing ionization, where many parameters are effective. As a result of these ionization processes, protonation or metal ion adduction may occur according to the chemical environment of the analyte. In MS analyses, metal complex structures are detected due to ion-ion/ion-neutral interactions occurring in the gas phase. Therefore, analyzing the formation and conformational features of such dynamic metal inclusion complexes requires reliable analytical methods with high sensitivity. The ESI-MS technique provides many advantages over other biophysical techniques, especially high sensitivity and speed, in the characterization of biological complexes containing noncovalent interactions. In addition, mass spectrometry enables direct determination of m/z values with high accuracy, making it possible to determine the compositions and stoichiometry of complexes. Besides determining the chemical structures of the noncovalent complexes, conformational features of the complexes and changes in their shape during the complex formation can also be monitored dynamically using the IM-MS technique. The IM-MS instruments provide conformational data by performing the characterization of the analytes according to their masses, charge states, and shapes simultaneously in the gas phase. The signals of protonated ions were dominant with the presence of Na+ and K+ adduct ion signals in the ESI mass spectrum obtained in this study from the IM-MS analysis of human insulin in the positive ion mode. The IM-MS data indicated no noticeable conformational change in the structure of the insulin protein with the binding of H, Na, K atoms and their different combinations. Signals of copper adduct insulin ions from 4+ to 7+ charge states were observed in the ESI mass spectrum obtained from IM-MS analysis after the addition of $Cu(NO_3)_2$ salt to the insulin sample. The shift of extracted ion mobilograms of copper adduct insulin ions to lower CCS values with the addition of copper atoms to the insulin structure proves that the abundance of the compact form of the insulin increases in the gas phase. The data obtained from the

IM-MS analyses of insulin-metal complexes show that alkali metals and copper bind to insulin by different mechanisms. The changes in the protein conformation caused by the attachment of various metals to the insulin protein can be distinguished with high sensitivity and accuracy by performing IM-MS analyses.

Acknowledgments

I would like to acknowledge Prof. Dr. Bekir Salih for his helpful discussions. The mass spectrometry laboratory is financially supported by the Presidency of the Republic of Turkey, Presidency of Strategy and Budget under project number 2016 K 121230.

References

1. C.M. Crittenden, E.T. Novelli, M.R. Mehaffey, G.N. Xu, D.H. Giles, W.A. Fies, K.N. Dalby, L.J. Webb, J.S. Brodbelt, Structural Evaluation of Protein/Metal Complexes via Native Electrospray Ultraviolet Photodissociation Mass Spectrometry, J Am Soc Mass Spectrom 31(5) (2020) 1140-1150.
2. E. Meggers, From conventional to unusual enzyme inhibitor scaffolds: the quest for target specificity, Angewandte Chemie International Edition 50(11) (2011) 2442-2448.
3. S.J. Archibald, R. Smith, Protein-Binding Metal Complexes: Noncovalent and Coordinative Interactions, Comprehensive Inorganic Chemistry II2013, pp. 661-682.
4. L.A. Angel, Study of metal ion labeling of the conformational and charge states of lysozyme by ion mobility mass spectrometry, Eur J Mass Spectrom (Chichester) 17(3) (2011) 207-15.
5. N. Potier, H. Rogniaux, G. Chevreux, A. Van Dorsselaer, Ligand–Metal Ion Binding to Proteins: Investigation by ESI Mass Spectrometry, Biological Mass Spectrometry2005, pp. 361-389.
6. A.B. Ilesanmi, T.C. Moore, L.A. Angel, pH dependent chelation study of Zn(II) and Ni(II) by a series of hexapeptides using electrospray ionization – Ion mobility – Mass spectrometry, International Journal of Mass Spectrometry 455 (2020).
7. J.A. Loo, R.R.O. Loo, H.R. Udseth, C.G. Edmonds, R.D. Smith, Solvent-induced conformational changes of polypeptides probed by electrospray-ionization mass spectrometry, Rapid Communications in Mass Spectrometry 5(3) (1991) 101-105.
8. S.K. Chowdhury, V. Katta, B.T. Chait, An electrospray-ionization mass spectrometer with new features, Rapid Communications in Mass Spectrometry 4(3) (1990) 81-87.
9. E.N. Yousef, L.A. Angel, Comparison of the pH-dependent formation of His and Cys heptapeptide complexes of nickel(II), copper(II), and zinc(II) as determined by ion mobility-mass spectrometry, J Mass Spectrom 55(3) (2020) e4489.
10. J.A. Loo, Studying noncovalent protein complexes by electrospray ionization mass spectrometry, Mass spectrometry reviews 16(1) (1997) 1-23.
11. S. Banerjee, S. Mazumdar, Electrospray ionization mass spectrometry: a technique to access the information beyond the molecular weight of the analyte, International journal of analytical chemistry 2012 (2012).
12. R. Mukherjee, The bioinorganic chemistry of copper, (2003).
13. Z. Zhang, H. Wang, M. Yan, H. Wang, C. Zhang, Novel copper complexes as potential proteasome inhibitors for cancer treatment (Review), Mol Med Rep 15(1) (2017) 3-11.
14. H. Tapiero, D.á. Townsend, K. Tew, Trace elements in human physiology and pathology. Copper, Biomedicine & pharmacotherapy 57(9) (2003) 386-398.
15. Z. Fu, E. R Gilbert, D. Liu, Regulation of insulin synthesis and secretion and pancreatic Beta-cell dysfunction in diabetes, Current diabetes reviews 9(1) (2013) 25-53.
16. A.R. Saltiel, C.R. Kahn, Insulin signalling and the regulation of glucose and lipid metabolism, Nature 414(6865) (2001) 799-806.
17. M. Gülfen, A. Özdemir, J.L. Lin, C.H. Chen, Investigation of non-covalent complexations of Ca (II) and Mg (II) ions with insulin by using electrospray ionization mass spectrometry, Rapid Communications in Mass Spectrometry 30(19) (2016) 2171-2182.
18. M. Gülfen, A. Özdemir, J.-L. Lin, C.-H. Chen, Quantifying Na (I)-insulin and K (I)-insulin non-covalent complexes by ESI–MS method and calculation of their equilibrium constants, International Journal of Biological Macromolecules 103 (2017) 910-918.
19. M.F. Dunn, Zinc–ligand interactions modulate assembly and stability of the insulin hexamer–a review, Biometals 18(4) (2005) 295-303.
20. M. Gulfen, A. Ozdemir, C.H. Chen, Monitoring Silver(I)-Insulin Complexes with Electrospray Ionization Quadrupole Ion Trap Mass Spectrometry, J Am Soc Mass Spectrom (2021).
21. J.M. Spraggins, K.V. Djambazova, E.S. Rivera, L.G. Migas, E.K. Neumann, A. Fuetterer, J. Suetering, N. Goedecke, A. Ly, R. Van de Plas, High-performance molecular imaging with MALDI trapped ion-mobility time-of-flight (timsTOF) mass spectrometry, Analytical chemistry 91(22) (2019) 14552-14560.
22. C.G. Vasilopoulou, K. Sulek, A.-D. Brunner, N.S. Meitei, U. Schweiger-Hufnagel, S.W. Meyer, A. Barsch, M. Mann, F. Meier, Trapped ion mobility spectrometry and PASEF enable in-depth lipidomics from minimal sample amounts, Nature communications 11(1) (2020) 1-11.
23. L.V. Tose, P. Benigni, D. Leyva, A. Sundberg, C.E. Ramirez, M.E. Ridgeway, M.A. Park, W. Romao, R. Jaffe, F. Fernandez-Lima, Coupling trapped ion mobility spectrometry to mass spectrometry: trapped ion mobility spectrometry-time-of-flight mass spectrometry versus trapped ion mobility spectrometry-Fourier transform ion cyclotron resonance mass spectrometry, Rapid Commun Mass Spectrom 32(15) (2018) 1287-1295.
24. M. Weiss, D.F. Steiner, L.H. Philipson, Insulin biosynthesis, secretion, structure, and structure-activity relationships, (2015).
25. M.E. Ridgeway, M. Lubeck, J. Jordens, M. Mann, M.A. Park, Trapped ion mobility spectrometry: A short review, International Journal of Mass Spectrometry 425 (2018) 22-35.
26. E.A. Mason, E.W. McDaniel, Transport properties of ions in gases, Wiley Online Library1988.
27. T. Wyttenbach, M. Grabenauer, K. Thalassinos, J.H. Scrivens, M.T. Bowers, The effect of calcium ions and peptide ligands on the relative stabilities of the calmodulin dumbbell and compact structures, The Journal of Physical Chemistry B 114(1) (2010) 437-447.

28. T.G. Flick, S.I. Merenbloom, E.R. Williams, Effects of metal ion adduction on the gas-phase conformations of protein ions, Journal of the American Society for Mass Spectrometry 24(11) (2013) 1654-1662.
29. T. Solouki, R. Fort, A. Alomary, A. Fattahi, Gas phase hydrogen deuterium exchange reactions of a model peptide: FT-ICR and computational analyses of metal induced conformational mutations, Journal of the American Society for Mass Spectrometry 12(12) (2001) 1272-1285.
30. E.M. Martin, F.D. Kondrat, A.J. Stewart, J.H. Scrivens, P.J. Sadler, C.A. Blindauer, Native electrospray mass spectrometry approaches to probe the interaction between zinc and an anti-angiogenic peptide from histidine-rich glycoprotein, Scientific reports 8(1) (2018) 1-13.
31. A. Noor, S. Qayyum, F. Jabeen, A.U. Rehman, Mononuclear Tricoordinate Copper (I) and Silver (I) Halide Complexes of a Sterically Bulky Thiourea Ligand and a Computational Insight of Their Interaction with Human Insulin, Molecules 27(13) (2022) 4231.