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ORIGINAL ARTICLE

Evidence of oligomerization of bovine insulin in solution given by NMR

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Abstract

The protein hormone insulin exists in several forms in nature, and a large number of modified sequences are used in pharmacy. They differ by physicochemical properties and efficiency of biological action. Pancreatic bovine insulin was studied in an acidic solution by nuclear magnetic resonance spectroscopy. ^{1}H and ^{13}C NMR signal assignment of backbone and side chains was made by analysis of a set of 2D spectra obtained on a sample with natural isotope abundance. The presence of certain secondary structure elements was revealed on a qualitative level based on nuclear Overhauser effect spectroscopy, which are similar to those observed in the crystal structure. The C-terminus of the B-chain possessed a remarkable flexibility. The molecule was shown to exist in exchange with oligomers based on its self-diffusion coefficient and correlation time measurements performed at different concentrations. Certain signals in the NOESY and HSQC spectra are consistent with the presence of minor conformers; this is an obstacle in simulating the molecular structure under the conditions used in the experiment.

Keywords Bovine insulin · Secondary structure · NMR · NOESY · DOSY · Oligomerization

Introduction

The family of insulin molecules is known for its role in glucose metabolism. Insulin is an irreplaceable supplement for people suffering from diabetes mellitus (DM). It is classified into four classes: type 1, type 2, gestational DM, and relatively rare cases such as monogenic diabetes syndromes (Classification and Diagnosis 2018). Reduced expression of insulin and insulin receptors in the brain tissue of patients with Alzheimer disease was revealed recently, and this state has been called type 3 DM (Leszek et al. 2017). Type 1 DM is caused by loss of β-cells in pancreas as a result of autoimmune reaction, type 2 DM develops due to insulin resistance and/or a decrease in insulin secretion. All patients with type 1 DM and many suffering from the other types have to take insulin injections on a regular basis throughout life. Some types of the disease, especially type 2, are treated by sulfonylurea; sometimes pramlintide (Ryan et al. 2005), metformin (Viollet et al. 2012) or other compounds are used as an additional drug, but artificial regulation of the blood glucose level by the corresponding hormone—insulin—still remains the only existing way of helping people with deficient insulin secretion.

Depending on the type of the disease, general health and lifestyle of a patient, different types of insulin preparations may be used: rapid-acting, regular, intermediate- and long-acting variants; methods of intake include use of syringe or pen, insulin pump, or less common ways such as inhaling. All these variants lay down certain requirements on the molecular properties of insulin preparations which should provide the best possible efficiency and safety.

Therefore, investigation of its structure and conformation changes which accompany binding to other molecules (stabilizing additives, receptor, zinc ions…) are of fundamental interest. On the other hand, physicochemical properties such as solubility and stability are important in drug preparation. Insulin therapeutic forms usually require the presence of Zn^{2+} ion, which leads to hexamer formation (Kadima 1999; O'Donoghue et al. 2000; Palmieri et al. 2013). Modifying the amino acid sequence which leads to longer and more impressive action of the drug is also an

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