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Characterization of Vitamin B12 and its binding to monoclonal antibodies in cell culture medium by liquid chromatography- mass spectrometry (LC-MS)

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Vitamin B12 is a group of cobalamins (Cbl) containing cobalt as the central ion in a corrin ring. The cobalt ion can be coordinated to a methyl, 5'-deoxyadenosyl-, hydroxy- or cyano- group referred to as methylcobalamin (MeCbl), 5' -deoxyadenosylcobalamin (AdoCbl), hydroxycobalamin (OH-Cbl), cyanocobalamin (CN-Cbl), respectively. Because CN-Cbl is the most air-stable and inexpensive, it is the most common form of vitamin B12 used in mammalian cell culture medium to produce therapeutic monoclonal antibodies. Following absorption, vitamin B12 is transformed in mammalian cells by coordinating with other ligands into either MeCbl or AdoCbl. The latter two forms are important cofactors of several enzymes and are active in endogenous metabolism of mammalian cells. Vitamin B12 has been reported to bind to the IgM heavy chain in serum of anemia patients. In a certain group of pregnant women, a high vitamin B12 level is associated with elevated levels of IgG, suggesting that unexpected Cbl interactions with immunoglobulins (Ig) may also occur. We have analyzed different forms of vitamin B12 and investigated its binding to a recombinant IgG monoclonal antibody in culture medium. For that purpose, a LC-MS method was developed to separate and quantify different forms of Cbl. The method employed a reversed phase C18 column and a mobile phase containing methanol at a flow rate of 0.325 mL/min. The separated Cbl forms were detected with triple stage quadrupole mass spectrometer. The method demonstrated good precision and accuracy to quantify different forms of Cbl in cell culture medium.

Biography

Jinshu Qiu is a principal scientist at Amgen Inc. and his main focus is to study metabolomics of mammalian cells during the production of therapeutic monoclonal antibody. Pavel V. Bondarenko is a scientific director at Amgen Inc. and he is the leader of the structure and pathway analysis group.