DEVELOPMENT AND VALIDATION OF NEW AMPEROMETRIC IMMUNOSENSOR AS BIOMEDICAL DEVICES FOR DIAGNOSIS OF CELIAC DISEASE

M. Giannetto[1,2], E. Umiltà[1],A. Manfredi[1], M. Mattarozzi[1,2], M. Careri[1,2]
[1]Dipartimento di Chimica, Università di Parma, Parco Area delle Scienze 17/A, 43124, Parma
[2]Centro Interdipartimentale SITEIA.PR, Università degli Studi di Parma, Parco Area delle Scienze 17/A, 43124, Parma

The presented study concerns the realization, optimization and validation of an amperometric immunosensor, as biomedical device for the rapid and accurate diagnosis of celiac disease. Particularly, the identification of the enzyme tTG as the main autoantigen involved in the development of celiac disease has attracted great attention on the study of anti-tTG antibodies, considered highly sensitive and specific markers of the disease [1]. In order to assemble the sensor, a glassy carbon electrode is first covered with electrodeposited nanogold. After deposition of a Self-Assembled Monolayer (SAM) of 11-Mercapto-undecanoic acid (MUA), able to interact with the gold surface by means of –SH functionalities, the tTG enzyme was covalently bound through carboxylic units. Our device is the first immunosensor based on the immobilization of the tTG enzyme in its open conformation, which has recently been identified as mainly involved in the pathogenesis of the disease [2]. Considering the clinical relevance of this conformation, particular attention was paid to the identification of the optimal conditions for its maintenance, even after covalent anchoring to the electrodic substrate. Through an experimental design (2 factors and 3 levels), the optimal conditions, in terms of concentration of tTG and HRP-conjugated anti-human reading antibody, have been identified. The immunosensor was validated in human serum for the determination of human IgG anti-tTG, evidencing a linearity range between 0 and 60 AU/mL, a recovery rate of 122 % for the positive control and 104 % for the control negative, less than 8 % of RSD and LOD and LOQ values assessed, respectively, to 2.47 AU/mL and 7.5 AU/mL. Furthermore, the response was not affected by a significant matrix effect. Since the value of LOD is less than the attested threshold value, (3 AU/mL) it is possible to state that the developed device is able to discriminate between the positive and negative samples. On the basis of the obtained results, we can conclude that the immunosensor is reliable as biomedical device for the rapid and accurate diagnosis of celiac disease.

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