Hyphenated affinity capillary electrophoresis with a high-sensitivity cell for the simultaneous binding study of retinol and retinoic acid in nanomolars with serum albumins

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Abstract

Retinol and retinoic acid are Vitamin A components that are critical for many biological processes. Both of them are strongly complexing with serum albumins giving constants of the order of 10(5) Lmol(-1) or higher. With respect to this fact, affinity capillary electrophoresis (ACE) is not applicable in its commonly used form. Therefore, for the first time, the hyphenated ACE with a high-sensitivity cell was developed and employed to investigate the binding of retinol and retinoic acid in nanomolars with human serum albumin (HSA) and bovine serum albumin (BSA) under physiological conditions. ACE/high-sensitivity coupled cell had contributed to fast the association and dissociation rates of the complexes in nanomolar scale of analytes ensuring the establishment of a dynamic equilibrium within a short electrophoresis time. In addition, this hyphenation led to reduce the concentrations of serum albumins as additives in background electrolyte making a sense beside the proper rinsing protocol for the negligible possibility of their adsorption. The mobility ratio based on nonlinear regression analysis was used to deduce precise binding constants of analytes with serum albumins. The binding constants (K, L mol(-1)) of retinol were 1.28 x 10(5) and 5.25 x 10(6) and retinoic acid were 3.29 x 10(5) and 2.27 x 10(6) with HSA and BSA, respectively. The displacement and reciprocal competitive binding of analytes were investigated and indicated that retinoic acid was able to replace retinol from HSA and vice versa in the case of BSA. (C) 2012 Elsevier B.V. All rights reserved.

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