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Original article

Study on the binding interaction between carnitine optical isomer and bovine serum albumin

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Abstract

The reaction between carnitine and bovine serum albumin (BSA) in aqueous solution has been studied by fluorescence spectroscopy and absorbance spectra. The binding interaction between optical isomer, D-carnitine and L-carnitine, with BSA has been compared. Based on the site-binding model and florescence quenching, practical formulas for small molecular ligand binding to bio-macromolecule have been used, and the binding parameters were measured. The binding distance, the energy transfer efficiency between carnitine and BSA was also obtained by virtue of the Förster theory of non-radiative energy transfer. The effect of carnitine on the BSA conformation has been analyzed by using synchronous fluorescence spectroscopy. The influence of Fe^{3+} on the interactions between carnitine optical isomer and bovine serum albumin were also explored in this work. As a conclusion, molecular identification of BSA to carnitine isomer has been suggested preliminary.

Keywords: Carnitine; Bovine serum albumin; Fluorescence spectroscopy; Ultraviolet spectroscopy

1. Introduction

Carnitine (Fig. 1), which was first isolated from meat as a compound, is a vitamin-like substance, and also called vitamin B_T. Carnitine mediates the transport of medium/long-chain fatty acids across mitochondrial membranes, and facilitates their oxidation with subsequent energy production; in addition, it also facilitates the transport of intermediate toxic compounds out of the mitochondria to prevent their accumulation. Carnitine is studied extensively in part because of the important role it plays in fatty acid oxidation and energy production, and it's a well-tolerated and generally safe therapeutic agent [1–4]. Carnitine occurs in two forms, known as D and L; they are mirror images (isomers) of each other with L-carnitine as the biological active form [5–7]. The D-isomer and DL raceme carnitine, which is not biologically active, can compete with the L-isomer so as to potentially increase the risk of L-carnitine deficiency. Therefore, the range of applications of L-carnitine in pharmaceuticals, food and feed industry are widening and the demand for L-carnitine is increasing. Hence carnitine is becoming a hot issue and more researches are undertaken on it than before [8–10].

Both food-derived and liver-synthesized carnitine are transported around the body by the blood stream. Most organic compounds (including carnitine) in the blood stream are mainly carried by transported proteins while only a minor fraction is dissolved in the serum. Serum albumin is the most abundant and most versatile of those transported proteins [11,12]. The exogenous substances and endogenous compounds (ligands) are bound to serum albumin with a high affinity in the processing of transportation, and this interaction results in a stable protein–ligand complex formed. However, the micrometal ions, which are stored in blood plasma, may affect the binding reaction of protein–ligand molecule complex. So it is necessary to investigate the interaction of protein–ligand both in the presence and absence of metal ions. The binding data of serum albumin differ from species to species. Animal experiments are indispensable in providing basic information on the pharmacological actions, bio-transformation, bio-distribution, etc. of a ligand. The BSA is well suited

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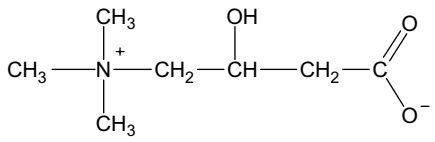

Fig. 1. Chemical structure of carnitine.

to these initial studies since it has been extensively characterized. The study on binding phenomena will also help to explain the relationship between structures and functions of proteins [13–15]. To our knowledge, no attempts have been made on the binding interaction between isomer carnitine and transportation protein.

Fluorescence spectroscopy is an appropriate method to investigate the interaction between small molecular ligands and bio-macromolecules. According to the measurement and analysis of the emission peak, the transfer efficiency of energy, the lifetime, fluorescence polarization etc., a vast amount of information about the structural fluctuations and the microenvironment surrounding the fluorophore in the macromolecules can be given [16,17]. In this paper, the reaction with isomer carnitine binding to BSA was investigated both in the presence and absence of metal ions Fe^{3+}, and the binding parameters and transfer efficiency of energy were measured. Another main purpose of this work was to check the isomer carnitine effect on the conformational changes of BSA. After the characteristics of binding of BSA with different isomer of carnitine were checked, the basic interpretation of biological activity was provided for different isomers of carnitine in the process of transportation.

2. Principle of binding equations for ligand-binding studies

2.1. Binding equation and binding parameters

For experiments carried out at large molar protein/ligand ratios, it was assumed that only strong sites were active in the binding ligand. For simplicity, these strong binding sites were also assumed to be identical and act independently. If these assumptions are valid, the site-binding model could be constructed as follows [18]:

$$\nu = nK[D]/(1 + K[D]), \tag{1}$$

where ν is the average number of ligand molecules bound per protein molecule, n is the number of (strong) binding sites, K is the intrinsic (microscopic) binding (association) constant, and $[D]$ is the concentration of free (unbound) ligand. Eq. (1) can be written in the following form which has been mentioned in some literatures [2,16,19,20] as Scatchard equation:

$$\nu/[D] = K(n - \nu). \tag{2}$$

As to the Scatchard equation, the assay of $[D]$ is a problem in many cases, so a practical formula for small molecular ligand interaction with bio-macromolecule has been derived based on the Scatchard's site-binding model and florescence quenching.

In the case of the fluorescence caused only by protein at the selected wavelength, the relationship between the concentration of protein and the fluorescence intensity can be described as

$$F_0/F = [P_t]/[P]. \tag{3}$$

According to the definition of ν, another equation is also known:

$$\nu = ([D_t] - [D])/[P_t] = n([P_t] - [P])/[P_t] = n(F_0 - F)/F_0 = n\Delta F/F_0, \tag{4}$$

where $[P_t]$ is the total protein concentration, $[D_t]$ is the final ligand concentration, F_0 and F are, respectively, the fluorescence intensity in the absence of a quencher and in its presence at $[D]$ concentration, $\Delta F = F_0 - F$.

The following equation is obtained by combining Eqs. (2) and (4) [14,15,21]:

$$F_0/F = K[D_t]F_0/(F_0 - F) - Kn[P_t]. \tag{5}$$

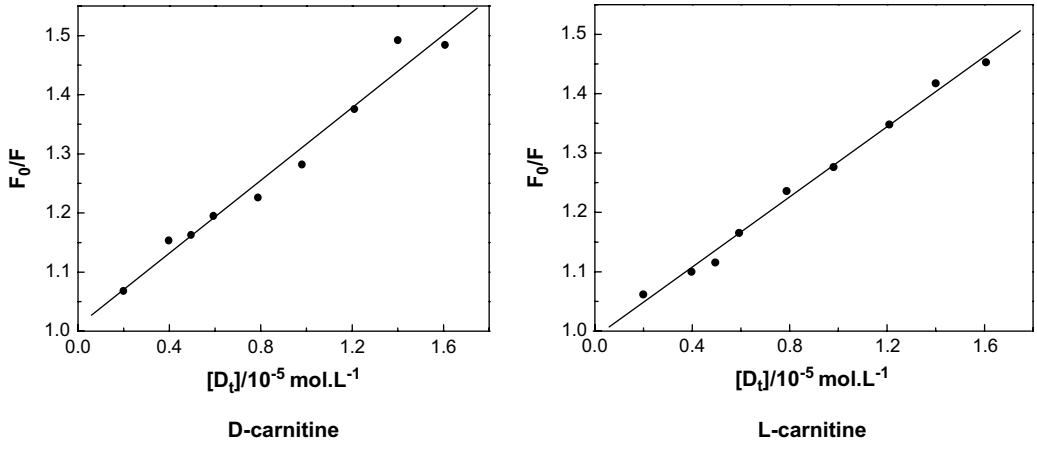

Fig. 2. The Stern–Volmer curve of carnitine binding with BSA. $c_{(BSA)} = 1.0 \times 10^{-5}$ mol L^{-1}, $\lambda_{ex} = 282$ nm.