Studies on interferon-gamma – Cibacron blue F3GA interaction

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Interaction between Cibacron blue F3GA and interferon-gamma in the presence of Tween 20 has been investigated by difference spectroscopy. Titration curves show the existence of different kinds of dye-binding sites. Apparently electrostatic interaction is the primary driving force for Cibacron blue F3GA – interferon-gamma binding.

Key words: interferon-gamma, Cibacron blue F3GA, dye–protein binding

INTRODUCTION

Dye-ligand chromatography is a powerful technique for protein purification. The triazinyl-based reactive dye Cibacron blue F3GA is widely used as a ligand in affinity chromatography systems. The dye is commercially available, inexpensive and can be easily immobilized [1]. Cibacron blue F3GA as a ligand is able to bind most types of proteins [2–4]. In spite of the extensive application, the binding mechanisms between Cibacron blue F3GA and proteins remain poorly understood. The interaction can proceed by a complex combination of electrostatic, hydrophobic, hydrogen bonding [1, 5].

We report here results of an investigation of the interaction between recombinant human interferon-gamma and Cibacron blue F3GA.

MATERIALS AND METHODS

Cibacron blue F3GA was obtained from Serva and recombinant human interferon-gamma was provided by Biofa company (Lithuania). Buffer salts were of the highest purity (Reachim). The difference spectra were registered on Ultrospec 4000 spectrophotometer (Pharmacia Biotech) provided with SWIFT II software in the wave-length region 400–800 nm using the path length of 1 cm. Difference spectral titrations were performed in 0.025 M Na-phosphate buffer pH 6.86 at 25 °C. The sample cuvette contained 1.97 μM interferon-gamma and 0.01 mg/ml Tween 20, while the dye concentration in both the sample and the reference cuvette ranged from 2 to 14 μM. At a higher concentration of interferon-gamma and Tween 20 (7.89 μM and 0.04 mg/ml, respectively) the dye concentration was 4–50 μM.

RESULTS AND DISCUSSION

Spectral methods are used for studying protein–dye binding [6]. Interaction between Cibacron blue F3GA and interferon-gamma, containing Tween 20 as a protein stabilizing agent, has been investigated by difference spectroscopy. The absorption spectrum of Cibacron blue F3GA shows a broad band with its maximum at 610–615 nm (Fig. 1A, curve 2). In order to check the linearity in the Beer–Lambert law plot spectra of Cibacron blue F3GA were taken in 0.025 M Na-phosphate buffer pH 6.86 up to dye concentration of 50 μM. The difference spectrum (Fig. 1A, curve 3) indicates that a comparable shift to the long-wave side occurs when Tween 20 is ad-

ded to the dye solution. A shift becomes still more apparent in the presence of Tween 20 and interferon-gamma (Fig.1A, curve 1). For characterization of the dye binding process, interferon-gamma in the presence of Tween 20 was titrated with increasing the amount of dye, and the intensity of the 690 nm peak of each difference spectrum was plotted *vs*. the dye concentration as shown in Fig. 2. A form of difference spectrum (Fig. 1B) and titration curves (in both interferon-gamma concentration 1.97 and 7.89 μM; Fig. 2) show the existence of different kinds of dye binding sites. Titration curves similar to those shown in Fig. 2 were obtained for interactions of phospholipase A_2 with Cibacron blue F3GA [7] and of alcohol dehydrogenase with active Orange 5K [8].

The interaction of Cibacron blue F3GA with interferon-gamma in the presence of Tween 20 is com-

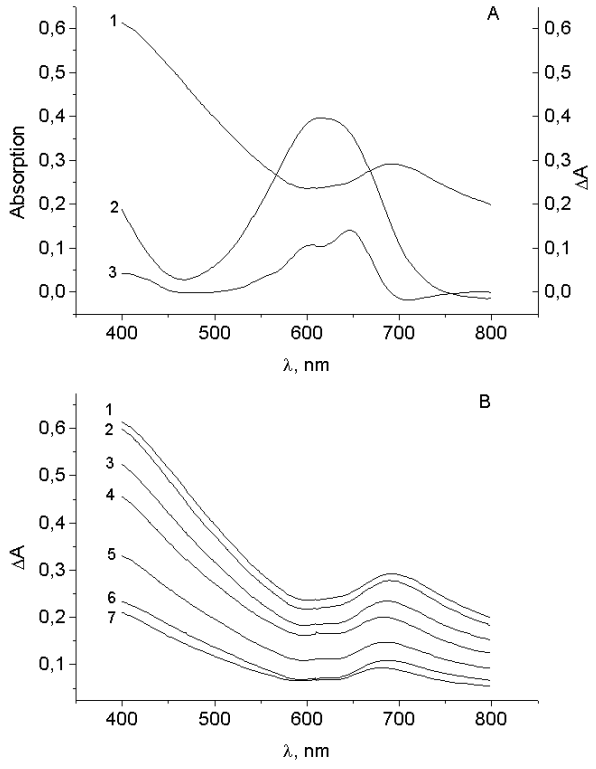

Fig. 1. **A –** absorption spectrum of Cibacron blue F3GA in 0.025 M Na-phosphate buffer pH 6.86 (2); difference spectra of Cibacron blue F3GA in the same buffer in the presence of Tween 20 (3) and in the presence of Tween 20 and interferon-gamma (1). **B** – typical difference spectra of Cibacron blue F3GA binding to interferon-gamma in the presence of Tween 20. Both the sample and the reference cuvettes contained 0.025 M Na phosphate buffer pH 6.86 and the following dye concentrations: 1 – 48; 2 – 40; 3 – 24; 4 – 20; 5 – 18; 6 – 16; 7 – 12 μM. The sample cuvette also contained 7.89 μM interferon-gamma and 0.04 mg/ml Tween 20

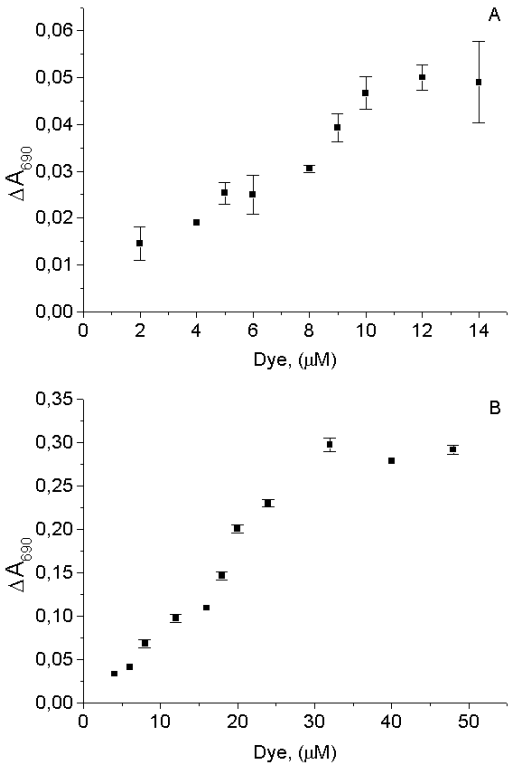

Fig. 2. Titration of interferon-gamma by Cibacron Blue F3GA at λ = 690 nm in the presence of Tween 20. The sample cuvette contained 1.97 μM (**A**) and 7.89 μM (**B**) interferon-gamma and the following Tween 20 concentrations: 0.01 mg/ml (**A**) and 0.04 mg/ml (**B**). Standard deviations for three identical measurements are given

plicated. It has been shown that interferon-gamma binds Tween [9]. Very likely Tween binding is driven by hydrophobic interaction [10]. Hydrophobic regions of the protein globule probably are not accessible to dye. Apparently electrostatic interactions play the main role in the interferon-gamma – Cibacron blue F3GA binding.

Our consideration is in good agreement with the results of Cibacron blue F3GA – protein complex investigation by MALDI mass spectrometry [4]. By using a range of proteins melittin, insulin, ubiquitin and cytochrome c, B. Salih and co-worker proved that the interaction is predominantly electrostatic.

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INTERFERONO-GAMMA SĄVEIKOS SU CIBAKRONU MĖLYNUOJU F3GA TYRIMAS

S a n t r a u k a

Tirta Cibakrono mėlynojo F3GA sąveika su interferonu-gamma, kai yra tvinas 20. Iš gautų titravimo kreivių galima spręsti, kad egzistuoja keli skirtingi dažo sujungimo centrai. Tikriausiai elektrostatinės jėgos ir nulemia dažo ir baltymo sąveiką.