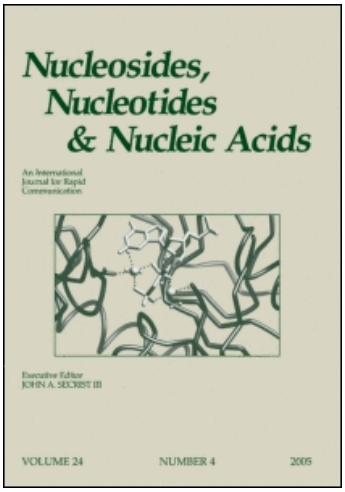

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Lactoferrin Interacts with Nucleotides

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ABSTRACT

Lactoferrin (LF) is an iron-binding glycoprotein found predominantly in milk and in granulocytes. LF is extremely polyfunctional protein some biological functions of which are determined by its capacity to bind iron, but many other functions are iron-independent. In this article we show for the first time that LF interacts with a number of various mononucleotides.

Key Words: Lactoferrin; Nucleotide; Fluorescence analysis.

INTRODUCTION

LF is one of the main glycoproteins of mammal epithelial secretions belonging to the transferrin family and capable of binding two Fe^{3+} ions.[1] LF is known as extremely polyfunctional protein possessing antibacterial and antiviral activity. It is responsible for immunomodulation and cell growth regulation, interacts with polyanions, and possesses RNase activity. LF enters the cell nucleus and activates transcription.[2] In terms of the polyfunctional activity of LF, research of its DNA- and ATP-binding sites and relationship between them would be very useful.

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MATERIALS AND METHODS

Fluorescence was measured at 25°C in the Hitachi MPF-2A spectrofluorimeter. Excitation was at 285 nm and emission, at 325 nm. The reaction mixture contained 20 mM Tris-HCl, pH 7.5, $3.5 \cdot 10^{-6}$ M LF and 10^{-7}–10^{-3} M nucleotide. Circular dichroism spectra were measured at 25°C using the J-600 spectropolarimeter (''Jasko,'' Japan). The reaction mixture contained 20 mM Tris-HCl, pH 7.5, 10^{-6} M LF and 10^{-7}–10^{-5} M ATP.

RESULTS AND DISCUSSION

We carried out detailed analyses of interaction of LF with ATP and a number of other nucleotides by fluorescence analysis, using wide ranges of ligand concentrations

Table 1. Values of K_d for LF binding to various nucleotides.

Ligand	1 site; K_d, M	2 site; K_d, M	3 site; K_d, M
ATP	$8.3 \cdot 10^{-6}$	$2.7 \cdot 10^{-4}$	$1.6 \cdot 10^{-3}$
dATP	$1.1 \cdot 10^{-6}$	$1.3 \cdot 10^{-5}$	$1.4 \cdot 10^{-4}$
ADP	$1.4 \cdot 10^{-5}$	$3.5 \cdot 10^{-4}$	$2.1 \cdot 10^{-3}$
AMP	$9.2 \cdot 10^{-6}$	$3.0 \cdot 10^{-4}$	$2.2 \cdot 10^{-3}$
dAMP	$4.5 \cdot 10^{-6}$	$3.0 \cdot 10^{-4}$	$4.0 \cdot 10^{-3}$
Cyclo-AMP	$3.6 \cdot 10^{-7}$	$7.3 \cdot 10^{-6}$	$2.0 \cdot 10^{-4}$
Adenosine	–	$3.9 \cdot 10^{-4}$	$2.7 \cdot 10^{-3}$
CTP	$8.1 \cdot 10^{-6}$	$4.6 \cdot 10^{-5}$	$2.3 \cdot 10^{-4}$
dCTP	–	$1.6 \cdot 10^{-5}$	$1.9 \cdot 10^{-4}$
CDP	$5.1 \cdot 10^{-6}$	$1.7 \cdot 10^{-5}$	$1.8 \cdot 10^{-4}$
dCDP	$9.8 \cdot 10^{-7}$	$3.4 \cdot 10^{-5}$	$1.4 \cdot 10^{-4}$
CMP	$1.6 \cdot 10^{-6}$	$4.8 \cdot 10^{-5}$	$1.7 \cdot 10^{-4}$
dCMP	$1.1 \cdot 10^{-6}$	$1.4 \cdot 10^{-5}$	$9.2 \cdot 10^{-5}$
Cyclo-CMP	$6.1 \cdot 10^{-7}$	$1.4 \cdot 10^{-5}$	$3.0 \cdot 10^{-4}$
Desoxycytosine	$6.2 \cdot 10^{-6}$	–	$2.7 \cdot 10^{-4}$
GDP	$3.2 \cdot 10^{-6}$	$2.7 \cdot 10^{-5}$	$6.3 \cdot 10^{-4}$
dGDP	$3.7 \cdot 10^{-7}$	$6.1 \cdot 10^{-5}$	$1.6 \cdot 10^{-4}$
GMP	$2.0 \cdot 10^{-6}$	$1.8 \cdot 10^{-5}$	$2.6 \cdot 10^{-4}$
dGMP	$3.3 \cdot 10^{-6}$	$2.9 \cdot 10^{-5}$	$2.0 \cdot 10^{-4}$
Cyclo-GMP	$6.4 \cdot 10^{-6}$	$3.0 \cdot 10^{-5}$	$1.8 \cdot 10^{-4}$
dUTP	$7.6 \cdot 10^{-6}$	$5.5 \cdot 10^{-5}$	$5.2 \cdot 10^{-4}$
UMP	$1.0 \cdot 10^{-6}$	$2.6 \cdot 10^{-5}$	$8.4 \cdot 10^{-4}$
TTP	$9.2 \cdot 10^{-6}$	$1.1 \cdot 10^{-5}$	$3.2 \cdot 10^{-4}$
TMP	$1.5 \cdot 10^{-6}$	$4.8 \cdot 10^{-5}$	$3.0 \cdot 10^{-4}$
ppppA	$2.8 \cdot 10^{-5}$	$1.8 \cdot 10^{-4}$	$2.0 \cdot 10^{-3}$
AppppA	–	$1.3 \cdot 10^{-4}$	$1.5 \cdot 10^{-3}$
AppppA	–	$4.1 \cdot 10^{-4}$	$2.3 \cdot 10^{-3}$
GppppG	$7.2 \cdot 10^{-6}$	$2.8 \cdot 10^{-4}$	–
GpppA	$8.5 \cdot 10^{-6}$	$2.4 \cdot 10^{-5}$	$1.7 \cdot 10^{-4}$
NAD	$1.7 \cdot 10^{-5}$	$3.9 \cdot 10^{-4}$	$1.1 \cdot 10^{-3}$
Desoxyribosophosphate	–	–	–

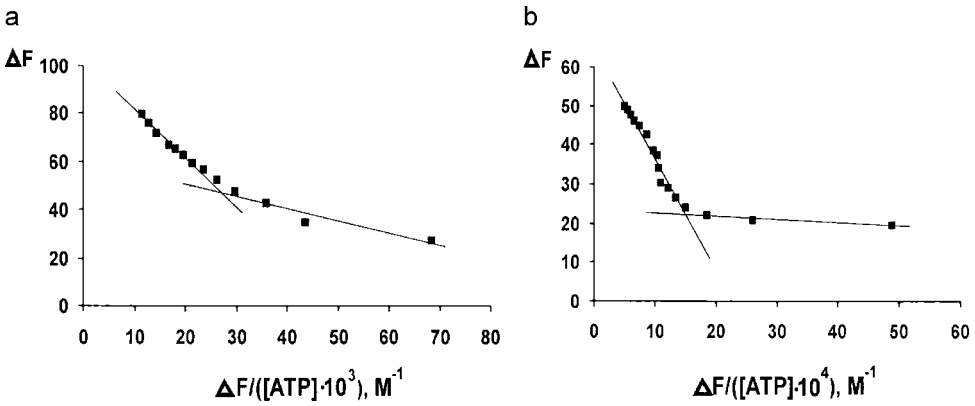

Figure 1. A Scatchard plot for ATP binding to LF as measured by fluorescence spectroscopy under concentration ATP 10^{-3} M (a) and 10^{-4} M (b). ΔF is alteration of LF fluorescence; [ATP] is concentration of free ATP in reaction mixture.

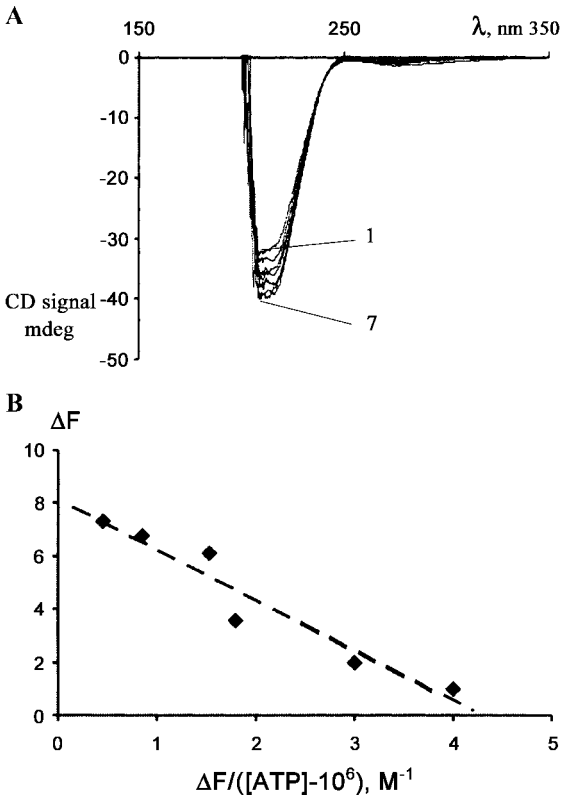

Figure 2. (A) The circular dichroism (CD) spectrum of LF before (curve 1) and after addition of ATP at different concentrations (0.5–16 μM, curve 7 corresponds to 16 μM ATP). Stepwise change of the amplitude of CD spectrum was used to estimate the K_d value of the complex between LF and ATP using Scatchard plot (B).

(Table 1). We found that with low nucleotides concentrations (10^{-6}–10^{-5} M) fluorescence extinguishing is weak ($\leq$5%). Greater extinguishing was observed with concentration about 10^{-4}–$5 \cdot 10^{-3}$ M (~40%). Additional extinguishing occurred with nucleotides concentration in excess of $5 \cdot 10^{-3}$ M. The fluorescence analysis data were linearized by Scatchard graphical method (Ref. [3]; $\Delta F = \Delta F_{max} - K_d$ ($\Delta F/[L]$), where [L] is a concentration of free nucleotide) and revealed three major linear regions (Fig. 1). Thus, LF has three general nucleotide-binding sites, which are characterized by three dissociation constants (K_d) (Table 1).

Circular dichroism spectra were measured for LF, ATP and mixtures of LF with ATP (10^{-7}–10^{-5} M) (Fig. 2). The K_d of the LF-ATP complex ($1.8 \cdot 10^{-6}$ M) estimated by this method was comparable with one derived above from the fluorescence data, which confirm an existence of LF center with high affinity for ATP. Thus, LF possesses three binding sites interacting with different nucleotides. Complex formation LF with nucleotides leads to an alteration in the intrinsic LF fluorescence, likely indicating conformational changes in the protein caused by these nucleotides.

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