THE ISOLATION OF THE FOLATE BINDING PROTEIN FROM COMMERCIALLY PURIFIED BOVINE BETA LACTOGLOBULIN*

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1. Introduction

Commercially prepared bovine beta lactoglobulin contains binding determinants for both reduced and oxidized mono and polyglutamates [2] and therefore has been utilized as a stable binder in the radioisotopic assays for serum and whole blood folates [3,4]. However, because of the comparatively large amount of the crystalline product needed to bind 50% of a tracer amount of tritiated pteroylglutamic acid ([^{3}H]PGA), (0.25 ng [^{3}H]PGA bound/0.1 mg) it was felt that the folate binding moiety was not the beta lactoglobulin per se but rather a separate protein purifying very closely to the beta lactoglobulin itself. As part of a study to identify the folate binding protein (FABP) of cow's milk we fractionated mixed beta lactoglobulin into its phenotypic alleles and in so doing effectively separated the folate binder from the composite whole. The present report describes the isolation of FABP from the beta lactoglobulin fraction of cow's milk in sufficient quantity to enable its preliminary characterization.

2. Materials and methods

[^{3}H]PGA, 26 Ci/mM was purchased from Amersham/Searle, Des Plaines, Illinois. Crystalline bovine beta lactoglobulin is a product of Sigma Chemicals, St. Louis, Missouri and Miles Laboratories, Elkhart, Illinois. DEAE cellulose was purchased from Schleicher and Schuell, Keene, New Hampshire. Radioactivity of the samples was determined as previously described [2].

2.1. *Chromatographic separation of FABP from beta lactoglobulin*

Crystalline bovine beta lactoglobulin from two different commercial sources was dissolved in 0.05 M Na-K PO_4 buffer, pH 5.8 and subjected to DEAE cellulose chromatography using a linear salt gradient [5]. Protein was monitored with a Uvicord II at 280 nm.

2.2. *Determination of folate binding capacity*

Aliquots of each fraction were assayed for their ability to bind [^{3}H]PGA by the method of Waxman, et al. [3]. Hemoglobin coated charcoal was used to separate the bound from free folate and protein concentrations were determined by the method of Lowry et al. [6].

2.3. *Electrophoretic analysis*

Protein solutions were subjected to polyarcylamide disc gel electrophoresis in pH 8.3 Tris–glycine buffer with the standard 7.5% gel [7]. Gels were stained for protein with Coomassie brilliant blue. Unstained gels were fractionated in a Savant Autogel Divider.

Isoelectric focusing was accomplished in an LKB 110 column using 1% ampholytes with a pH range of 3–10 on a sucrose support and an initial output of 3.2 W.

2.4. *Gel filtration analysis*

Mol. wts were estimated using a 2.2 × 85 cm

* Some of these studies were presented at the National Meeting of the American Society of Hematology, Atlanta, Georgia, December 1974 [1].

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Table 1
Folic acid binding capacity of mixed bovine beta lactoglobulin fractions after passage through DEAE cellulose

Source	Milligrams protein assayed	Nanograms folic acid/bound milligram
Mixed beta lactoglobulin (Sigma)*	0.1	2.83
Mixed beta lactoglobulin (Miles)*	0.1	2.89
A Allele	0.1	0.015
B Allele	0.1	0.05
A + B Allele	0.2	0.07
Folate Binding Protein	0.001	350 00

* Prior to DEAE cellulose chromatography

column of Sephadex G-200 equilibrated with 0.1 M PO_4 buffer pH 7.2 containing 0.5 M NaCl. The columns were marked and calibrated according to the method of Laurent and Killander [8].

2.5. *Immunological studies*

Rabbit anti-bovine beta lactoglobulin (Antibodies Inc., Davis, California) was dialysed overnight against 0.9% saline at 4°C prior to use in order to remove any free folate from the serum carrier. Standard Ouchterlony techniques were used throughout [9].

2.6. *Uptake studies in HeLa cell cultures*

The uptake of [^{3}H]PGA and the effect of FABP on this process was studied as previously described [10].

3. Results

Folate binding protein was clearly separated from beta lactoglobulin and purified 150-fold by elution from the DEAE column with the early effluent at a near zero salt concentration (table 1). No binding of [^{3}H]PGA was observed with the protein fractions associated with the A or B alleles of the beta lactoglobulin. Acrylamide gel electrophoresis revealed two protein bands in the upper 1/3 of the gel both of which bound [^{3}H]PGA, whereas beta lactoglobulin was found in the region of the tracker dye without a corresponding radioactive peak. Passage of [^{3}H]PGA labeled FABP through the G-200 Sephadex column revealed a mol. wt of 36 000. Isoelectric focusing revealed three peaks of folate binding protein with a pI of 7.2, 7.8 and 8.6, all of which were immunologically distinct from beta lactoglobulin. Antibody to beta lactoglobulin did not block [^{3}H]PGA binding or increase the size of [^{3}H]PGA bound to impure beta lactoglobulin as determined by G-200 Sephadex filtration. [^{3}H]PGA bound to FABP was not available to the HeLa cell monolayer. Table 2 summarizes the characteristics of beta lactoglobulin and the folate binding protein isolated from the crystalline preparation.

4. Discussion

A method has been utilized which separates folate binding protein from previously crystallized preparations of bovine beta lactoglobulin, thus providing a convenient source of folate binder for study. Our findings were not dissimilar from those

Table 2
Characteristics of beta lactoglobulin as compared to the partially purified folic acid binding protein

Characteristic	Beta lactoglobulin	FABP
Molecular size	35–42 000	36 000
pI	5.1–5.2	7.2–7.8–8.6
Precipitation by anti-BLG serum	+	–
Delivery of folate to HeLa monolayer	+	–
Electrophoretic mobility	cationic	anionic

obtained by Ford et al. when bulk milk was used as the starting preparation [11]. Preliminary characterization reveals similarities between the folate binding protein of cow's milk and the FABP obtained from the milks of goat and human [12]. It is clear that folates bound to this protein are unavailable for cellular uptake and could significantly affect a folate dependent cell or organism [10]. Further studies are underway to determine the role of folate binding protein in the transport of folates across the cell membrane and its effect on DNA synthesis.

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