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Effects of Transferrin on Enhancing Biological Availability of Iron

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Abstract

In this study, transferrin which is an iron-carrying glycoprotein in plasma was evaluated for its iron binding capacities (TIBC), iron solubilizing abilities, and enhancing effect of biological availability of iron. Results of TIBC showed that 1 mg of transferrin could bind 1.28 μg of iron indicating that one molecule of transferrin can bind about 2 molecules of iron. Also, solubility of iron (7.5 μg Fe/ml) was significantly increased to 96.0% with addition of transferrin (5 mg/ml). When $FeCl_3$ (80 μg Fe/ml) was injected to iron-deficient rats by intestinal segment *in situ* technique, 18.4% of injected iron was absorbed whereas 48.49 and 48.76% of injected iron was absorbed with addition of 10 and 20 mg transferrin/ml, respectively.

Key words: transferrin, total iron binding capacity, iron solubilizing abilities, iron absorption

INTRODUCTION

Iron deficiency anemia is the most prevalent nutritional disorder in the world. Especially, infants under 2 years of age, teenage girls, pregnant women, and elderly are at high risk of iron deficiency anemia. Iron fortification with micro nutrients is one of the least expensive and more effective way of supplying iron to target people. However, the bio logical availability of added iron has shown to be extremely variable. For enhancing biological availability of iron, iron should be in soluble form at duodenum in which most of iron is absorbed. Many factors, such as chemical forms of iron and diet composition, are known to influence the biological availability of iron (1-3). Most of ferric iron (Fe^{3+}) was pre cipitated at the nearly neutral pH encountered in the du odenum, whereas fairly good amount of ferrous iron (Fe^{2+}) still remains soluble (4). Iron absorption can be inhibited to a varying degree by a number of ingredients, including car bonates, oxalates, phosphates, and phytates (5,6). Otherwise, ascorbic acids and certain proteins in foods are known to enhance iron absorption (7,8). Transferrin, which is composed of about 5% of plasma proteins, is a non-heme iron binding glycoprotein. Because of its' strong iron binding abilities, transferrin plays a role in the transfer of iron from storage areas to the erythroblasts (9). Therefore, transferrin can be developed as a functional protein which binds iron to main tain iron in a soluble monomeric form at the alkaline pH of the small intestine. The objectives of this study were to investigate transferrin's iron binding capacities, iron solu bilizing abilities, and effects on enhancing iron absorption through *in vitro* and *in situ* experiments.

MATERIALS AND METHODS

Total iron binding capacity

Transferrin (bovine apo-transferrin) was purchased from Sigma Co. (USA). Total iron binding capacity (TIBC) of transferrin was determined by iron binding capacity kit (Sigma, USA) which measures total iron concentrations (TIC) and unsaturated iron binding capacity (UIBC). TIBC is represented as a sum of TIC and UIBC.

Iron solubilizing ability

To test transferrin's iron solubilizing ability at duodenum condition, 5 ml of $FeCl_3$ (15 μg Fe/ml) was added to 5 ml of 0, 2.5, 5, 10 mg transferrin/ml solution. Then, pH was ad justed to 6 with 1N NaOH and incubated at 37°C for 1hr. After incubation, reaction solution was centrifuged at 4,000× g for 30 min. and supernatant was collected. The pH of su pernatant was readjusted to 2 with 1 N HCl and iron concentrations in supernatant was measured by ferrozine assay (10).

Biological availability of iron (*in situ*)

The effect of transferrin on biological availability of iron was examined by ligated intestinal segment *in situ* technique. Male, weanling rats (Sprague-Dawley) were housed in mesh-bottom plastic cages under a controlled environment. Iron-deficient diet (Table 1) and deionized water were offered *ad libitum* for 8wks to induce iron-deficiency anemia. These rats were then divided into three groups of 5 rats. They were anaesthetized intraperitoneally with sodium pentabarbital. Through a medical laparotomy, the pancreatic-biliary duct was ligated at its drainage into the duodenum. The duodenum was cannulated at its entry and exit, then washed with isotonic solution. Table 2 shows the compositions of the solution injected to the control and transferrin added (10 and 20 mg, respectively) groups. One ml of solution for each group was injected into upper portion of duodenum through a syringe and abdomen was closed using Michel clip. The animals were then maintained in a quiet room at 23°C for 1hr. Then, abdomen was disclosed and both sides of cannulated ends of duodenum segment were cut and separated

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Table 1. Composition of iron deficient diet

Components	Iron deficient diet (g/kg dry weight)
Casein	200
Corn starch	150
Cellulose	50
DL-methionine	3
Mineral mix[1]	35
Vitamin mix[2]	10
Choline bitartarate	2
Sucrose	500
Corn oil	50

[1]The mineral suppliment of diet contained (g/kg): $CaHPO_4$ 500.0, NaCl 74.0, $C_6H_5K_3O_7 \cdot H_2O$ 220.0, K_2SO_4 52.0, MgO 24.0, $MnCO_3 \cdot H_2O$ 3.5, $ZnCO_3 \cdot H_2O$ 1.6, $CuCO_3$ 0.3, KIO_4 0.01, $Na_2SeO_3 \cdot H_2O$ 0.01, $CrK(SO_4)_2 \cdot 12H_2O$ 0.55, finely ground sucrose 124.03

[2]The vitamin suppliment contained (g/kg): thiamin HCl 0.6, riboflavin 0.6, pyridoxine HCl 0.7, Niacin 3.0, calcium pantothenate 1.6, folic acid 0.2, biotin 0.02, vitamin B_{12} 1.0, dry vitamin A palmitate 0.8, dry vitamin E acetate 10.0, vitamin D_3 0.25, menadione sodium bisulfite complex 0.15, sucrose fine powder 981.08

Table 2. Formulation of iron complexes injected into *in situ* ligated segment

Treatment	Control (ml)	Transferrin-10 (ml)	Transferrin-20 (ml)
$FeCl_3$[1]	0.3	0.3	0.3
Transferrin	-	10 mg	20 mg
0.01 mol HCl/L	0.1	0.1	0.1
Demineralized water	0.542	0.542	0.542
0.25 mol/L Tris buffer (pH 8.5)	0.058	0.058	0.058

Final pH: 7.2

[1]80 mg Fe/L in 0.01 mol HCl/L & $^{59}FeCl_3$ (0.5 μCi/ml)

from intestine. The inside of duodenum segment was thoroughly washed with deionized water and washing solution and duodenum segments were collected for determination of ^{59}Fe activities. Concentrations of ^{59}Fe in washing solution and duodenum segment were measured by a gamma counter (Packard Auto- Gamma Model 2000 series). All ^{59}Fe counting data were corrected for decay and counting efficiency. After duodenum segment was removed from the rats, blood was taken and Hb was measured by cyanmethemoglobin method.

RESULTS AND DISCUSSION

Fig. 1 shows the relationship between transferrin concentrations and its' corresponding TIBC. According to the relationship, 1 mg of transferrin can bind 1.28 μg of iron and this result indicated that one molecule of transferrin (MW of ~75,000 dalton) can bind about 2 molecules of iron (MW of 57).

Iron is known to be absorbed from upper intestine (duodenum segment). Solubility may be a prerequisite to iron absorption. Iron forms macromolecules with hydroxyl groups in aqueous solution and ultimately precipitates as pH becomes more alkali. Motzok et al. (11) demonstrated the direct correlation of availability with solubility of iron sources. It

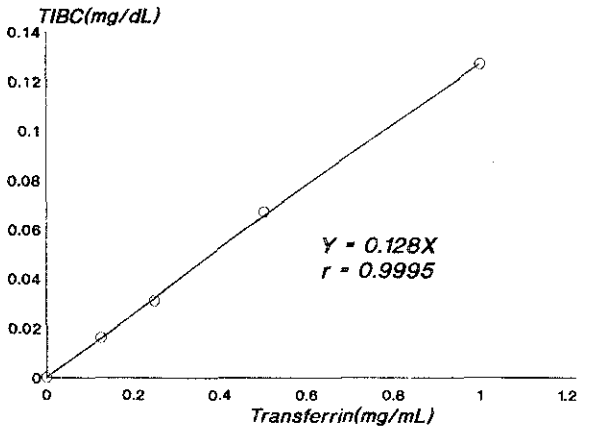

Fig. 1. Total iron binding capacity (TIBC) of transferrin with various concentrations.

has been estimated that some chelating agents can prevent the precipitation of iron in nearly neutral environment of the intestine, thus rendering otherwise insoluble iron available for absorption (12). To study solubility of ferric iron at duodenum condition, ferric iron (7.5 μg/ml) was incubated at pH 6, 37°C for 1hr. After incubation, only 2.2% of added iron was solubilized in supernatant (Fig. 2). However, percentage of soluble iron in supernatant was rapidly increased to 47.0% when 1.25 mg/ml of transferrin was added. With addition of 5 mg transferrin/ml, percentage of soluble iron reached to 96.0%. According to the result of *in vitro* solubility test, it is evident that addition of transferrin increased solubility of iron at duodenum condition.

Next step was done to investigate the effect of transferrin addition on biological availability of iron. The absorption of iron by the body is complex and affected by many factors. It is estimated that only 5 to 15% of the iron in the food is absorbed by adults with normal hemoglobin levels, although absorption can be increased in the presence of an iron deficiency (13,14). In our experiment, test rats were fed

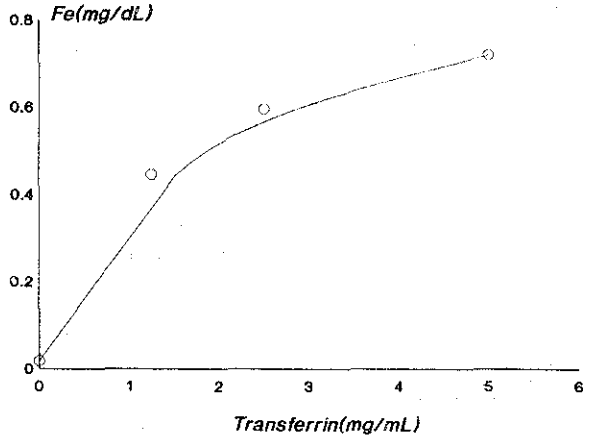

Fig. 2. Iron solubilizing abilities of transferrin with various concentrations at pH 6, 37°C.

iron-deficient diet to induce nearly equal iron-absorption condition. Table 3 shows hemoglobin levels of test rats after taking iron-deficient diet for 8 wks. Since normal hemoglobin level is considered to be around 15 g/dl, all test rats which showed hemoglobin level ranging from 5.5 to 9.4 g/dl were considered as in the state of iron deficiency anemia. Since ligated intestinal segment *in situ* technique gives information on net absorption of minerals from a defined segment of intestine, we chose this technique for testing transferrin's effect on iron absorption. After rats were injected with test solution through duodenum segments, the extrinsically labelled ^{59}Fe activities detected in washing solution and duodenum segment of rats in the control group were 81.6% of total ^{59}Fe activities(Table 4). When transferrin was added at 10 or 20 mg/ml level in injection solution, 51.51 and 51.24% of total ^{59}Fe activities were detected, respectively, in washing solution and duodenum segment. Therefore, truly absorbed iron, which can be estimated by subtracting remaining ^{59}Fe activities detected in washing solution and duodenum segment from 100, through duodenum segment in control, 10, and 20 mg transferrin added groups were 18.4, 48.49, and 48.76%, respectively. From these results, it is evident that transferrin enhances iron absorption through duodenum segment in this *in situ* test. Between 10 and 20 mg transferrin/ml added groups, no significant difference was observed in enhancing absorption of iron in 80 μg Fe/ml level. Although effects of low pH and pepsin in stomach on transferrin's activities was not considered in this *in situ* study, transferrin has good chance to enhance biological availability of iron.

Plasma is a potential source of various proteins. It has been reported that plasma proteins have good rheology-related functional properties such as gelation and emulsification, so their usage in food processing has a lot of advantages (15,16). Like a lactoferrin which is an iron-binding glycoprotein found in externally secreted fluids such as milk to enhance neonate's iron absorption (17,18), transferrin is also an iron carrying glycoprotein in blood. Since both lactoferrin and transferrin have similar molecular weight, amino acid composition, and iron-binding properties, there is a great possibility of transferrin to have various functional properties. Therefore, in terms of utilizing value-added ingredients from waste products, transferrin is a valuable subject for further investigation.

Table 3. Hemoglobin level (g/dl) of SD rat after administering iron deficient diet for 8wks

Control	Transferrin-10	Transferrin-20
	Hb (g/dl)	
6.52±0.78	7.84±1.21	7.58±0.51

Table 4. Absorption of ^{59}Fe from transferrin added solutions injected into ligated segments of small intestine of SD rat

	Control	Transferrin-10	Transferrin 20
	CPM ($\times 10^4$) in washing sol. and duodenum segment		
Avg.(A)	12.77±1.27	8.78±0.48	8.64±2.35
Total(T)	15.65	17.04	16.87
% ^{59}Fe remaining[1]={(A/T)×100}	81.60%	51.51%	51.24%
% ^{59}Fe absorbed ={100 - % ^{59}Fe remaining}	18.40%	48.49%	48.76%

[1]% ^{59}Fe remaining = % ^{59}Fe activies remaining in washing solution and duodenum segment.

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