Vol. 130, No. 1, 1985 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
July 16, 1985 Pages 264-269

ISOLATION OF A PROTEIN FROM HUMAN MILK THAT ENHANCES ZINC ABSORPTION IN HUMANS

Curtis D. Eckhert

Division of Nutritional Sciences
School of Public Health
University of California
Los Angeles, CA 90024

Received June 3, 1985

Summary: Humans absorb zinc more readily from human milk than from the milk of other species. The basis for this species specificity has not been elucidated. This report describes the isolation of a human milk protein which enhances zinc absorption. The consumption of bovine cow's milk supplemented with the human protein resulted in an elevation in human plasma zinc of 101% compared to a 50% rise following the ingestion of cow's milk alone. The identification of this biologically active protein in human milk establishes in part the basis for the species difference in zinc bioavailability. © 1985 Academic Press, Inc.

Children with the once fatal genetic disorder acrodermatitis enteropathica have impaired zinc absorption (1). Even before it was discovered that the plasma of individuals with the disease was abnormally low in zinc, it was known that the prognosis for infants provided human milk was better than for children fed artificial milk formulae (2). The recognition in 1973 that this genetic disease could be managed by zinc supplementation led to the search for differences between human and bovine milk which could account for the enhanced zinc uptake (3-4). The biologically active component(s) from human milk which increase the bioavailability of zinc have not been identified (5). The isolation and identification of a biologically active protein from human milk which enhances the absorption of zinc from bovine milk in humans is presented in this report.

0006-291X/85 $1.50

MATERIALS AND METHODS

Preparation of human milk - Human milk used for the isolation of the protein was stored frozen at -20° C. Frozen milk was allowed to defrost slowly at 22° C. The milk was then thoroughly mixed by swirling, and defatted by centrifuging at 1000 x g for 30 minutes. The fat free milk component was drained and chilled to 4°C.

Chromatographic procedures - Defatted milk (30 ml) was applied to a Sephadex G-50 column (26 mm x 40 cm). Blue dextran (0.6%) and DNP-l-alanine (0.02%) were added (0.1 ml) as markers for void and total volume. The column was eluted with 30 mM Tris buffer, pH 7.1. Five ml fractions were collected and the zinc content determined by flame atomic absorption spectrophotometry. Fractions containing the zinc peak eluting just prior (Kav = 0.8) to the total volume were saved and lyophilized.

Dialysis - Lyophilized zinc containing fractions were redissolved in a 0.45 mM ammonium carbonate buffer adjusted to pH 7.1 with acetic acid. The final solution contained 0.6 µg Zn/ml. The dissolved fractions were placed inside Spectropor dialysis membrane tubing (molecular weight cut off 3,500) and dialysed against 75 times their volume of ammonium carbonate buffer (pH 7.1) at 22° C for 50 hours. The buffer solution was changed once after 20 hours. The zinc concentration of the fractions before and after dialysis were determined by flame atomic absorption spectrophotometry. The fraction remaining in the dialysis tubing was lyophilized and stored at 4°C.

Electrophoretic analysis - The nondialyzable zinc containing fraction was assessed for purity using sodium dodecyl sulfate (SDS) polyacrylamide electrophoresis. Polyacrylamide gels (15%) containing 0.1% SDS were prepared according to the method of Dreyfuss, Adams and Choi (6). Low molecular weight standards (LKB) of myoglobin , myoglobin I, and myoglobin II were used for the estimation of molecular weight. Protein bands were identified using the silver staining technique of Giulian, Moss and Greaser (7).

Biological activity test - The biological activity of the pure protein was assessed by the zinc load procedure of Casey, Walravens, and Hambidge (6). Pasteurized and homogenized grade A bovine cow's milk was used as the carrier. The volume of milk consumed (148 ml) was calculated to contain 5 g bovine milk protein. The zinc load was 25 mg of zinc provided as zinc sulfate. The procedure was initiated following a 12 hour fast. Blood was drawn prior to drinking the test substance (fasting) and then at 30 minute intervals after ingestion for 4 hours. The quantity of human milk protein added was 30 mg which contained 7.4 µg of zinc. The total plasma zinc response was calculated as the area under the curve formed by plotting-the increase in plasma zinc above the fasting concentration against the time interval in minutes following ingestion of the test substance.

RESULTS AND DISCUSSION

The distribution of human milk zinc on Sephadex G-50: The defatted human milk was separated on a Sephadex G-50 column to isolate low molecular weight zinc containing fractions. Zinc

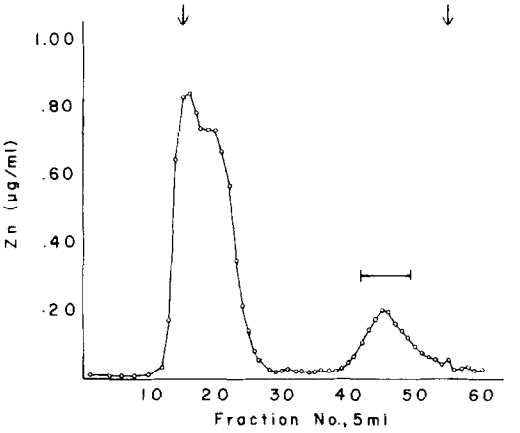
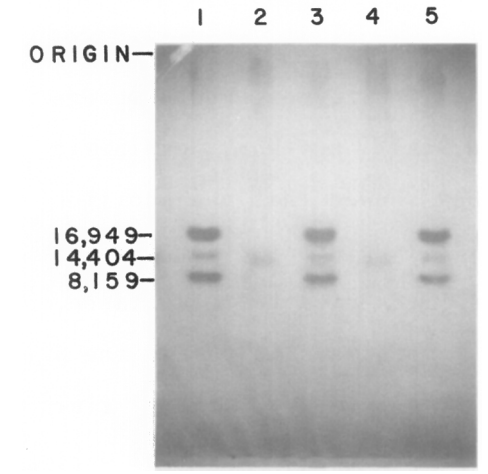


Fig. 1. Elution of defatted human milk separated by gel filtration as a function of zinc concentration. Thirty ml of defatted milk were applied to a Sephadex G-50 column (26 mm x 40 cm) and eluted with 30 mM Tris buffer pH 7.1. Arrow on left indicates elution of blue dextran (void volume) and arrow on right indicates elution of DNP-l-alanine (total volume). Bar denotes low molecular weight zinc containing fractions (Kav = 0.8) saved for further purification.

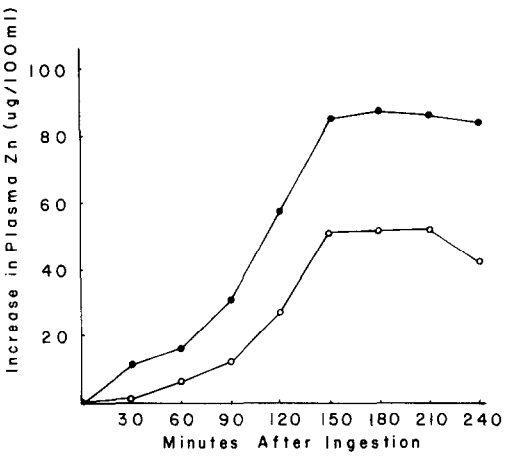
containing low molecular weight fractions, Kav = 0.80, (fig. 1) were pooled, lyophilized and stored at 4°C.

Dialysis of zinc containing fractions: Dialysis of the low molecular weight zinc containing fractions resulted in a loss of some zinc. The zinc concentration (µg/ml) within the dialysis membrane (molecular weight cut off 3,500) following 50 hours of dialysis was 61.1 ± 0.8% of initial values. The volume of fluid within the membrane increased by 30% during the dialysis procedure.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the zinc-binding nondialyzable fraction: The purity of the nondialyzable zinc containing fraction was assessed by sodium dodecyl sulfate polyacrylamide electrophoresis. Only one band could be demonstrated by silver staining gels containing increasing concentrations (2, 3, 4, 5, and 6 µg/ml) of the lyophilized unknown. The molecular weight was estimated as 12,500 by comparison with the migration of myoglobin, myoglobin I, myoglobin II and myoglobin I + II (fig. 2).



(2)



(3)

Fig. 2. Sodium dodecyl sulfate polyacrylamide analysis of human milk zinc binding protein prepared according to Dreyfuss et al. (6). Lyophilized fractions of the dialyzed human milk protein and protein standards were applied to adjacent lanes of a 15% gel containing 0.1% SDS. Protein standards (LKB) consisting of myoglobin (16,949 MW), myoglobin I + II (14,404 MW) and myoglobin I (8,159 MW) were applied to lanes 1 (5 µg), 3 (4 µg) and 5 (3 µg). Lanes 2 and 4 contain 5 and 4 µg by weight of the lyophilized human milk protein. Protein bands were identified by the silver staining precedure of Giulian et al. (7).

Fig. 3. Biological activity of the 12,500 MW human milk protein was assessed by determining the increase in plasma zinc concentration over the 3 hour period following the ingestion of the protein dissolved in 148 ml of bovine cow's milk containing 5 g of protein and loaded with 25 mg of zinc (5). The 3 hour plasma zinc concentration rose by 51 µg/100 ml for cow's milk (○) and 87 µg/100 ml for cow's milk + human milk protein (30 mg) (●). The area under the cow's milk + human milk protein curve at 3 hours was 1.7 times the area of the curve for cow's milk alone demonstrating the 12,500 MW protein was biologically active.

Biological activity: A comparison between the response of plasma zinc following the ingestion of cow's milk alone or cow's milk supplemented with the 12,500 MW human milk protein is given in figure 3. The concentration of plasma zinc rose to 50% above fasting levels 3 hours following ingestion of the zinc loaded cow's milk. The addition of the human milk protein to the zinc loaded cow's milk doubled the plasma zinc response to a level of 101% above fasting. The 3 hour total plasma zinc response cow's milk + human protein was 1.7 times the response obtained for cow's milk alone.

Both citrate and picolinic acid have been suggested as the biologically active compounds in human milk responsible for the enhanced zinc absorption (8-10). Casey, Walravens, and Hambidge tested these substances but found that neither exhibited biological activity (5). In fact, they showed that the addition of these agents resulted in a decrease in plasma zinc. Their index of biological activity was the ability of a test substance, ingested with 25 mg of zinc, to increase plasma zinc during the 3 hour period following consumption. The relative plasma zinc responses were: human milk (250 ml), 100%; cow's milk (152 ml), 45%; cow's milk + 148 mg citric acid, 28%; and cow's milk + 94 mg picolinic acid, 38%. The absorption of zinc from human milk was 2.2 times the value obtained for cow's milk. By comparison, in the present study the addition of the human 12,500 MW milk protein to cow's milk (148 ml) resulted in an increase in plasma zinc which was 1.7 times greater than the value for cow's milk alone. This value is less than the 2.2 times difference reported by Casey, Walravens and Hambidge and may reflect differences in the amount of the human milk protein ingested. Sandstrom, Cederblad and Lonnerdal (11) used radioactive extrinsic zinc labeling to measure bioavailability and reported that the zinc absorption from human milk was 1.5 times that of bovine cow's milk.

In this report the biological assay applied by Casey, Walravens, and Hambidge to screen substances for their ability to enhance zinc absorption has been used to test a 12,500 MW zinc binding protein isolated from human milk. A positive plasma zinc response was achieved when bovine cow's milk was supplemented with the human milk protein. These results suggest that the greater absorption of zinc from human milk by humans is due in part to the presence of this biologically active protein.

REFERENCES

1. Polanco, I., Nistal, M., Guerrero, J. and Vazquez, C. (1976) Lancet I, 430.

2. Brandt, T. (1937) Acta Derm. Venereol. 17, 513-546.

3. Barnes, P. M. and Moynahan, E. J. (1973) Lancet I, 327-329.

4. Eckhert, C. D., Sloan, M. V., Duncan, J. R., and Hurley. (1977) Science (Washington, D.C.) 195, 789-790.

5. Casey, C. E., Walravens, P. H., and Hambidge, K. M. (1981) Pediatrics 68, 394-396.

6. Dreyfuss, G., Adams, S. A. and Choi, Y. D.. (1984) Mol. Cell. Biol. 4, 415-423.

7. Giulian, G. G., Moss, R. L., and Greaser, M. (1983) Anal. Biochem. 129, 277-287.

8. Lonnerdal, B., Stanislowski, A. G. and Hurley, L. S. (1980) J. Inorg. Biochem. 12, 71-78.

9. Hurley, L. S. and Lonnerdal, B. (1982) Nutr. Rev. 40, 65-71.

10. Evans, G. W. and Johnson, P. W. (1980) Pediatr. Res. 14, 876-880.

11. Sandstrom, B., Cederblad, A. and Lonnerdal, B. (1983) Am. J. Dis. Child. 137, 726-729.