Induction of an antimicrobial biotin-binding egg white protein (avidin) in chick tissues in septic *Escherichia coli* infection

H. A. Elo, S. Räisänen and P. J. Tuohimaa[1]

Department of Biomedical Sciences, University of Tampere, Box 607, SF-33101 Tampere 10 (Finland), 1 June 1979

Summary. The induction of avidin in chick tissues was found in septic *Escherichia coli* infection. Avidin concentrations in the plasma roughly corresponded to those in the other tissues studied which suggests that avidin in chicks is a secretory protein.

Avidin is a biotin-binding protein found previously only in the oviducts or oviductal secretory products (egg white, egg jelly) of birds, lizards and frogs[2-4]. Avidin has not been found in mammals[2,5]. The production of this secretory protein in the oviduct of the laying hen is regulated by the ovarian function[2]. Although the function of avidin in chicken egg white is not clear, avidin is generally regarded as an antimicrobial substance[6,7]. Biotin is a growth factor for various yeasts, fungi and bacteria[8], and avidin forms a stable complex with biotin, thereby making it unavailable to the micro-organisms[2,9]. We have recently found that injured non-oviductal chicken tissues produce avidin[5,10]. If avidin production is induced in connection with bacterial or fungal infection, the phenomenon might have significance as a nonspecific defence mechanism against infection. *Escherichia coli* infections are usual in chicks[6,11], and therefore we studied whether septic *E. coli* infection induces avidin in chick tissues.

Materials and methods. *E. coli* was isolated from chick faeces and cultured for 18 h in brain-heart broth (Difco). Septic *E. coli* infection in 2-week-old White Leghorn chicks was caused by inoculating i.p. or i.v. given volumes of cultured cell suspension. Controls were injected i.p. with 0.5 ml of brain-heart broth and killed 24 h later. Tissues were taken at given times ranging from 12 to 32 h after the inoculation and stored at −20 °C until avidin was assayed with the [^{14}C]biotin-binding method and radioimmunoassay for chicken egg white avidin[12]. Another biotin-binding protein, distinct from egg white avidin, has recently been discovered in the chicken egg yolk and plasma[13]. This protein does not cross-react with antibody directed against avidin[14].

Results. The [^{14}C]biotin-binding method gave considerable avidin concentrations in the tissues of chicks inoculated with *E. coli*, while avidin was not found in the controls (table). There was no essential difference in avidin induction caused by i.v. or i.p. inoculation. Avidin concentrations were highest in the lung, and the concentrations in the plasma and intestine were approximately 2 times higher than those in the pectoral muscle. The induction in serious peritonitis was already high at 12–15 h after the inoculation. An induced biotin-binding protein in 5 inoculated chicks was recognized as avidin by the radioimmunoassay. Avidin values for the radioimmunoassay and [^{14}C]biotin-binding method (given in parentheses) were as follows: plasma 7.4 ± 0.9 (7.7 ± 1.4), intestine 11.2 ± 1.2 (13.4 ± 1.9), lung 33.3 ± 4.8 (19.4 ± 2.7) and pectoral muscle 5.6 ± 1.6 (6.0 ± 1.7) μg/g. The figures indicate that both methods gave the same avidin concentrations in the tissues studied, except for the lung, where the radioimmunoassay gave 70% higher concentration than the [^{14}C]biotin-binding method. Ultrasonicated *E. coli* cells (centrifugation at 2500 × g for 25 min, supernatant stored at −20 °C) did not indicate any [^{14}C]biotin-binding in the avidin assay.

Discussion. Avidin concentrations in the plasma of *E. coli*-infected chicks roughly corresponded to those in the other tissues studied which suggests that a secretory cell (cells) is (are) responsible for avidin production. This is a proof of some important function of avidin induction. The function of avidin in infected chick tissues is unknown. In vitro avidin inhibits the activity of biotinyl enzymes[7], which are involved in carboxylation and transcarboxylation reactions. Although a catalytic function has not been found for avidin, and is in fact improbable[7], this possibility cannot be excluded. One argument presented earlier against a general catalytic function was the restricted distribution of avidin[7], and this argument has now been refuted.

Avidin and its induction have properties which are characteristic of rapid nonspecific defence mechanisms against infections such as interferon[15], lysozyme[16] and iron-binding proteins[17]. Avidin inhibits in vitro the growth of microorganisms which require biotin as a growth factor[2,9]. Many chicken pathogens e.g. the fungus *Candida albicans* and bacteria like *Listeria monocytogenes, Staphylococcus aureus, Clostridium botulinum* and *Streptococcus faecalis*[11] belong to the biotin-requiring microorganisms[8]. It is interesting to note that some *Streptomyces* species produce an antibiotic MSD 235 which consists of 2 different components[18,19]: the low mol.wt component inhibits biotin synthesis in gram-negative bacteria, and the high mol.wt one (streptavidin) is, like avidin, a biotin-binding protein. Chicken egg white avidin is a basic glycoprotein with an unusually high isoelectric point at pH 10 (Green[7]). Lysozyme is also a basic protein[16]. Basic proteins have a tendency to bind to the cell membranes, and many basic natural polypeptides and the synthetic polylysines exhibit antimicrobial activity[20]. The basic polyamino acids[20] and proteins (e.g. lysozyme[21]) can also promote phagocytosis. The induction of avidin in chick tissues is rapid (table), and stimuli such as septic bacterial infection, tissue injury[5,10] and toxic doses of various

Avidin induction in some tissues of chicks caused by experimental septic *Escherichia coli* infection

Volume and route of inoculation	Dead animals*	Time after inoculation	Avidin concentration (μg/g of wet tissue)** Plasma	Intestine	Lung	Pectoral muscle
Controls	0/5		0.1 ± 0.0	0.0	0.0	0.0
0.1 ml i.v.	4/10	32 h	15.6 ± 4.4	5.9 ± 1.6	17.8 ± 3.7	7.1 ± 1.3
0.1 ml i.p.	1/10	32 h	9.1 ± 2.0	11.5 ± 4.0	11.7 ± 2.9	5.3 ± 0.9
0.25 ml i.p.	3/10	24 h	7.2 ± 1.4	11.7 ± 2.1	18.0 ± 1.2	7.6 ± 1.0
0.5 ml i.p.***	0/14	12–15 h	10.4 ± 1.6	13.0 ± 1.7	18.2 ± 1.2	3.2 ± 0.3

* Dead animals include those which either died or were killed because of bad condition before the time indicated. These animals were rejected. ** Avidin was assayed with the [^{14}C]biotin-binding method. The means ± SEM for 5–14 animals are given. *** Animals were in bad condition before being killed.

drugs[10,22] cause the induction. In the local muscular burning injury, avidin induction is restricted to the injured area[10]. The ability of induced avidin to retain biotin in the tissues as an avidin-biotin complex might explain an increase in total biotin concentration in the chicken plasma, and red blood cells found in malaria parasite *(Plasmodium lophurae)* infection[23]. Green[24] has presented evidence for a genetic relationship between avidins and lysozymes. He states that if both avidin and lysozyme perform an antibacterial function, it is possible that these proteins will be produced together in situations where the organism requires protection against bacteria. If avidin is assumed to have a defensive function against micro-organisms, it is easy to understand its occurrence in the egg white, which contains many bacteriostatic or bactericidal proteins[6], and induction in bacteria-infected or injured chicken tissues.

1 Acknowledgments. We thank Mrs O. Kurronen, Miss T.-M. Mattila and Mr C. Hartmann for technical assistance and Dr M.S. Kulomaa for the performance of radioimmunoassay. We are very much obliged to Dr T. Metsä-Ketelä for valuable criticism. The work was supported by grants from the Ford Foundation (No 760-0526) and the Scientific Foundation of the Tampere City.

2 R. Hertz, Physiol. Rev. *26,* 479 (1946).
3 P.D. Jones and M.H. Briggs, Life Sci. *11,* 621 (1962).
4 V. Botte, S.J. Segal and S.S. Koide, Gen. comp. Endocr. *23,* 357 (1974).
5 H.A. Elo, M.S. Kulomaa and P.J. Tuohimaa, Comp. Biochem. Physiol. *62B,* 237 (1979).
6 R.G. Board and R. Fuller, Biol. Rev. *49,* 15 (1974).
7 N.M. Green, Adv. Protein Chem. *29,* 85 (1975).
8 B.W. Langer, Jr, and P. György, in: The Vitamins, 2nd edn, vol.2, p.352, Ed. W.H. Sebrell, Jr, and R.S. Harris. Academic Press, New York 1968.
9 R.E. Eakin, E.E. Snell and R.J. Williams, J. biol. Chem. *140,* 535 (1941).
10 H.A. Elo, M.S. Kulomaa and P.J. Tuohimaa, Acta endocr. *90,* 743 (1979).
11 M.S. Hofstad, B.W. Calnek, C.F. Helmboldt, W.M. Reid and H.W. Yoder, Jr, (ed.). Diseases of Poultry, 6th edn. The Iowa State University Press, Ames 1972.
12 M.S. Kulomaa, H.A. Elo and P.J. Tuohimaa, Biochem. J. *175,* 685 (1978).
13 R.D. Mandella, H.W. Meslar and H.B. White III, Biochem. J. *175,* 629 (1978).
14 H.W. Meslar, S.A. Camper and H.B. White III, J. biol. Chem. *253,* 6979 (1978).
15 D.H. Metz, Adv. Drug Res. *10,* 101 (1975).
16 E.F. Osserman, R.E. Canfield and S. Beychok (ed.). Lysozyme. Academic Press, New York 1974.
17 E.D. Weinberg, Microbiol. Rev. *42,* 45 (1978).
18 E.O. Stapley, J.M. Mata, I.M. Miller, T.C. Demny and H.B. Woodruff, Antimicrob. Ag. Chemother. *1963,* 20.
19 L. Chaiet, T.W. Miller, F. Tausig and F.J. Wolf, Antimicrob. Ag. Chemother. *1963,* 28.
20 E. Katchalski, M. Sela, H.I. Silman and A. Berger, in: The Proteins, 2nd edn, vol.2, p.405. Ed. H. Neurath. Academic Press, New York 1964.
21 M. Klockars and P. Roberts, Acta haemat. *55,* 289 (1976).
22 P.K. Heinonen, T. Metsä-Ketelä and P. Tuohimaa, J. Reprod. Fert. *52,* 159 (1978).
23 W. Trager, J. exp. Med. *77,* 557 (1943).
24 N.M. Green, Nature *217,* 254 (1968).

Inhibition of growth and lactic acid synthesis in *Lactobacillus casei* by maltol and its reversal by glutamic acid

N. Banerjee, R. Bhatnagar[1] and L. Viswanathan

National Sugar Institute, Kanpur (India), 11 June 1979

Summary. Maltol inhibited growth and lactic acid synthesis by *Lactobacillus casei.* The inhibition was partially overcome by the addition of casein hydrolysate and yeast extract. Some amino acid mixtures were also effective, among which glutamic acid was able to reverse the inhibition completely.

Introduction. Maltol is a product of the Maillard Reaction[2], a reaction occurring between carbohydrates and proteins or amino acids at elevated temperatures. The Browning Reaction (as it is also called) is found to occur commonly in milk products, bread, etc. In some systems, like microbiological growth media where carbohydrates and amino acids are usually added, the reaction takes place during heat sterilization. Similarly, sugar cane molasses, which is widely used as a raw material for industrial fermentation, also contains a large number of these brown products. Microbiological media containing these substances may not be suitable for a specified purpose as the micro-organisms may be affected by these substances, and this might change the normal course of their activity. It has been shown by

Table 1. Inhibition of growth and acid accumulation in *L. casei* culture by maltol and its reversal

Parameters studied	Concentration of maltol (mg/ml)	Percent inhibition over control* in parameters			
		Additions to growth medium			
		Nil	Tryptone 1 mg/ml	Yeast extract 1 mg/ml	Casein hydrolysate 1 mg/ml
Growth as turbidity	0.5	12	24	9.8	15
	1.0	25	36.3	11.1	16
	2.0	30	42.3	11.1	18
Total acids	0.5	16.6	16.6	0	0
	1.0	33	33.3	0	0
	2.0	40	36.6	4	0
Lactic acid	0.5	22.7	19.5	0	2.4
	1.0	34	23	0	0
	2.0	38.2	39	0	4.8

* Control is grown on medium alone.