S100B and Health: from the brain to plants via milk

S100B e Salute: dal cervello alle piante passando dal latte

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Abstract - The S100B protein was initially discovered in the brain during the 1960s. For several decades, research has been conducted on both humans and other vertebrates, with a focus on various organs and tissues. The characterisation of this phenomenon has also been undertaken in different biological fluids and cellular mechanisms. The contribution to medicine has been substantial, encompassing between 4,000 and 40,000 scientific contributions on PubMed, based on the level of query specificity. More recently, through in silico studies performed by bioinformatics methodologies, complemented by subsequent validation in vitro by immunodetection, the molecular configuration of S100B has been delineated in selected edible plants and fruits. It is a general law that nature employs the homologous gene information in species that are so distant in phylogeny. This observation, however, is opening up new scenarios for research and medicine. The hypothesis that S100B might play a role in nutrition was first postulated upon the observation that breastfeeding is a source of S100B, which is present in breast milk. The rationale behind nature's decision to incorporate this molecule into the infant's initial dietary intake remains a subject of scientific investigation. The main question is why this principle should be shared by all mammals and their offspring. Initially, S100B was theorised to be a neurotrophic factor; however, recent studies have revealed a novel perspective that identifies the target of S100B's physiological action in the microbiota. At least two pathways are currently under scrutiny: firstly, an endogenous pathway that is facilitated by enteric glial cells, and secondly, an exogenous pathway that is introduced by nutritional intake from birth, in the beginning through breastfeeding and subsequently lifelong, through the consumption of varied and balanced diets. S100B exploitation appears a promising tool for strengthening health and restoring imbalances, even under stressful conditions. The mechanisms would not be those involving probiotics, prebiotics, or nutrients, but rather a new approach based on a potential harmonization of the microbiota. The emphasis is on the biodiversity of the microflora, which is increasingly recognised as a key factor at a local level or exerting effects on other organs through gut-brain axis. Advancements in genetics and bioinformatics are unveiling novel technological horizons.

Riassunto – La proteina S100B fu inizialmente scoperta nel cervello durante gli anni '60. Per decenni è stata studiata nell'uomo e altri vertebrati, in diversi organi e tessuti, fino a caratterizzarla in vari fluidi biologici e meccanismi cellulari. Il contributo alla medicina è stato amplissimo e intenso, comprendendo su PubMed tra i 4000 e 40000 contributi scientifici, a seconda del livello di specificità posto nell'interrogare questo database di riferimento per la letteratura biomedica. Oggi, attraverso studi in silico basati su metodi di bioinformatica confermati poi in vitro con metodi immunologici, la struttura molecolare di S100B è stata dimostrata persino in alcune piante e frutti commestibili. Non deve stupire che la natura utilizzi la medesima informazione genica in specie e contesti così lontani nella filogenesi, ma, questa osservazione sta aprendo nuovi scenari per la ricerca e la medicina. Il sospetto che S100B potesse avere un ruolo nell'alimentazione emerse già osservando che l'allattamento al seno era una fonte di questa proteina, presente nel latte materno. Perché la natura avrebbe dovuto mettere energia per introdurre nel primo alimento del neonato questa molecola? Perché questo principio è universale per tutti i mammiferi e i loro cuccioli? Inizialmente si pensò fosse un fattore neurotrofico, ma oggi si sta aprendo una prospettiva rivoluzionaria, che identifica il bersaglio dell'azione fisiologica di S100B nel microbiota. Almeno due vie sono in studio, una endogena attraverso le cellule enterogliali ed una esogena attraverso l'alimentazione dalla nascita con l'allattamento e poi nel corso della vita con diete variate ed equilibrate. S100B appare un promettente strumento per rinforzare la salute e ripristinare squilibri, anche in condizioni di stress per l'organismo. I meccanismi non sarebbero quelli di probiotici, prebiotici, o nutrienti, bensì quelli di un potenziale armonizzatore del microbiota. Il focus è in quella biodiversità della microflora, che sempre più spiega effetti "in loco" o a distanza su altri organi attraverso complesse interazioni, come l'asse intestino-cervello, o fattori trofici ancora da investigare. I progressi della genetica, bioinformatica e scienze "omiche" stanno aprendo nuovi orizzonti conoscitivi e tecnologici, che investono anche questa piccola proteina S100B, nota da tempo, ma dalle rinnovate e promettenti prospettive.

Key words: S100B, Microbiota, Diet, Breastfeeding, Gut-Brain Axis, Elisa, NGS, Bioinformatics, Epidemiology

Key messages:

- S100B levels in gut lumen are associated to eubiosis, exerting a role in microbiota regulation with potential applications in therapy, prevention and health promotion.
- Nutritional, Pharmaceutical or Phytotherapy-based approaches may open new perspectives for developing novel strategies to apply S100B in diet under regular or stressing conditions.

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A protein discovered in the brain and studied in different tissues

S100B is a small (10 kDa) protein, capable of forming dimers and tetramers, and binding calcium (1). Originally discovered in brain extracts in the 1960s ***(Fig. 1)***, it was later characterised in other tissues. One of its defining characteristics is its solubility in a 100% saturated solution of ammonium sulphate (2), hence the name. It is expressed by several cells, including glial astrocytes in the central nervous system, and also in various organs and biological fluids. This protein is well conserved phylogenetically, being found in various animal species in forms that are homologous to those found in humans, indicating a relevant biological role. Moreover, similar molecules belonging to the S100 protein family include EF-hand domains and helix-loop-helix (HLH) structures. The genes that encode these proteins are located in the q21 region of the long arm of chromosome 1, except for the S100B protein, which is located in a different region of the human genome, on chromosome 21q22. Transcription of this gene into mRNA and translation into amino acids has been observed in various organs and tissues, including the kidney, adrenal gland, cartilage, immune system, eye, adipose tissue, skin melanocytes, Leydig cells in male gametes and the mammary gland, as well as in trophoblasts and stromal cells in the chorial villi of the placenta (3).

From the earliest studies on the brain, growing evidences support a fundamental role for S100B in the functioning of the organism ***(Fig. 2)***. From the initial focus aimed at elucidating its physiological and trophic function, the following decades

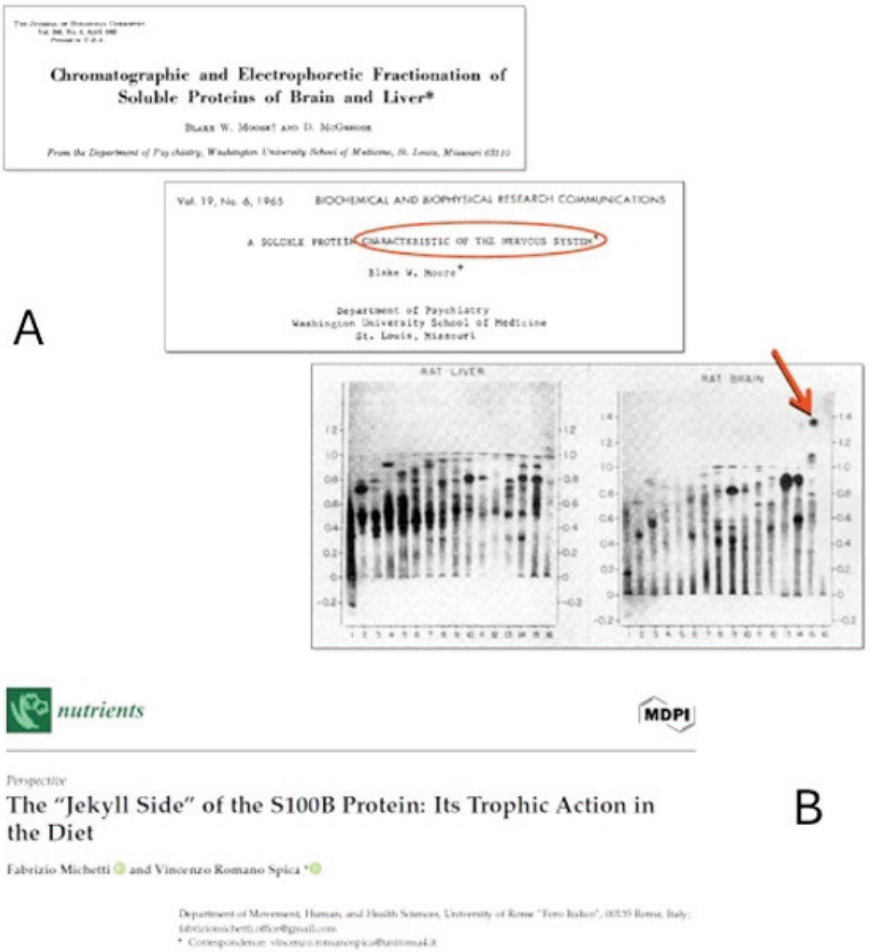

Fig. 1 - Early work (A) reporting the discovery and characterisation of a brain protein originally named S100. The name was derived from the observation of its solubility in a 100% solution of ammonium sulphate at neutral pH. Subsequently, other molecules of the same family were identified, distinguished by letters and/or numbers, for a complex of more than 20 members. However, S100 -also referred to as S100B: beta- possesses a number of autonomous characteristics, such as being located on chromosome 21q22, as opposed to the 1q21 locus where the genes encoding the other members of this protein family are located. It has the ability to interact and bind with other molecules and can form homodimers, heterodimers and tetramers. Its presence in the brain, and in particular its expression in the glia, led to it being assayed in blood even in hospital practice as a valid prognostic marker released in head trauma. However, its expression has also been demonstrated in various other tissues and biological fluids. Recently (B), a trophic and physiological action has been reconsidered and new perspectives have also been considered through the intake of the protein through particular foods present in the diet, re-evaluating the 'Dr. Jekyll' side as opposed to the 'Mr Hyde' side related to the study of protein modifications in pathological phenomena. Recent findings have opened up new perspectives for a physiological role of S100B in protecting and promoting health through modulation of the microbiota.

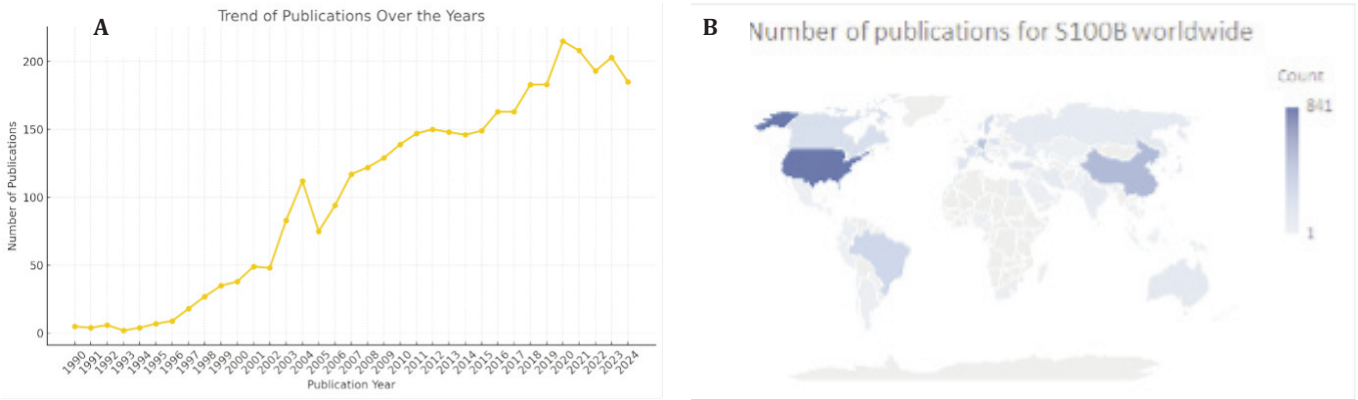

Fig. 2 - **A)** Analysis of yearly publications trend. The web science database includes 3559 records on S100B protein and about 39,000 for «S100». The line indicates the publication trend from 1990 until 2024, showing the number of papers which are published each year. This trend starts increasing in 1990 and till then 2024 its impact is increasing. It shows an upward trend which dominantly indicates the increment in the research trends concerning the S100B protein in different scientific areas.
B) Analysis of countries. A country map was generated to find the leading countries in the research fields of S100B protein. Among the records which were analysed, the publications originated from 89 countries. The top 10 countries in terms of publication count include USA, China, Germany, Italy, Brazil, England, Sweden, Canada, Japan, France. The USA emerged as the foremost contributor. The second and third position carried by China and Germany.

witnessed a shift away from the 'normal' condition to studying, instead, its response in various 'pathological' conditions (4, 5).

Alterations in serum or tissue levels have been reported in a variety of diseases, ranging from neurological disorders to obesity and diabetes, and including certain cancers, such as melanoma. S100B has even been identified as a promising prognostic marker in cases of severe acute respiratory syndrome (SARS-CoV-2) infection (6). It is only very recently that scientific research has redirected the focus of this investigation towards the trophic role that was at the origin of its discovery. This has resulted in the highlighting of the dual nature of the protein, with one aspect being beneficial to health and the other being associated with pathological phenomena, somewhat akin to the duality of the characters Dr. Jekyll and Mr. Hyde (7). In the field of medicine, it is well-established that a given molecule can exert both positive and negative effects. A salient example of this phenomenon is the utilisation in pharmacology of plant poisons as therapeutic agents, such as atropine or colchicine. Additionally, also proto-oncogenes and suppressor genes, have been identified as fundamental DNA sequences essential for life, regulating cell cycle. This notion has been substantiated by seminal research contributions, notably the 1989 Nobel Prizes in Physiology and Medicine, bestowed upon Harold Varmus and Michael Bishop for their discoveries concerning the origin of 'cancer' oncovirus genes from 'normal' cellular genes. For S100B, several scientists have reached the conclusion that low (nanomolar) concentrations could be considered physiological by exerting a trophic effect ('Jekyll side'), whereas high (micromolar) concentrations could exert a toxic/proinflammatory role ('Hyde side'). It is evident that modified levels may not be causative of the disease; rather, they are the consequence of alterations induced by pathogenic agents or mechanisms. In both hypotheses, however, various intracellular biochemical pathways have been identified, while extracellular S100B is believed to interact with surrounding cell types mainly through the receptor for advanced glycation end products (RAGE), a ubiquitous transmembrane receptor similar to immunoglobulins that binds to a wide range of extracellular ligands and intracellular effectors (7, 8). At present, the 'Hyde side' of S100B is the primary focus of the scientific community, particularly in relation to its use as a biomarker for various diseases. In contrast, the physiological role of this protein, i.e. its main biological and trophic function (the 'Jekyll side'), remains to be fully unravelled.

From humans to animals even in plants

The discovery that the molecular structure of S100B is present in plants (9) brought about a revolution in

knowledge about this protein. While it was already known that the genetic sequence of this small protein was highly conserved across phylogenies, it had never been systematically studied in plants before. However, it was already known that S100B was present in animals and in particular in mammalians. The human amino acid sequence is, highly similar to those of cattle, donkeys and sheep, as well as cats, leopards, camels, elephants, cetaceans, guinea pigs and rats. Surprisingly, nature has also made homologous genes available to produce S100B in species that are more distantly related to humans, such as earthworms, planarians and silkworms. Immunoreactivity for S100B has also been observed in cockroaches (10).

Finally, researchers came to discover S100B in plants while conducting a clinical-pharmaceutical study on the action of S100B inhibitors in neurodegenerative diseases and inflammatory bowel diseases, particularly Crohn's disease (5, 11). To exclude S100B contamination in the mice's feed during *in vivo* experiments, it was monitored by immunodetection. This led to the hypothesis that S100B molecular domains could also be found in plants. *In silico* bioinformatics pipelines were developed and applied to investigate the available genomes of the entire *Viridiplantae* clade, which comprises over 500,000 species. Supercomputers revealed that the theoretical molecular structure of S100B was present in a small percentage of plants (less than 0.02%), including edible species. This was later confirmed immunologically by showing the presence of antigens compatible with the molecular architecture of S100B *in vitro*, both in animals and plants ***(Fig. 3)***. Further studies in botany, genetics, and plant physiology are needed to understand the significance of proteins containing the S100B domain being expressed in different plant tissues, including leaves and fruits. Some of these edible species are rare, but others are very common worldwide, such as apples and spinach.

From breastfeeding to diet in adults

S100B has been found to be present in biological fluids, including milk secreted by the mammary apocrine gland (12). This characteristic is common to all mammals, meaning the S100B protein is present in the initial food consumed by every human and other baby mammal.

It is widely believed that S100B may be one of the beneficial factors that determine the advantages of breastfeeding, and it may also play a natural role in the development of newborns through neurotrophic action (13, 14). However, recent evidence

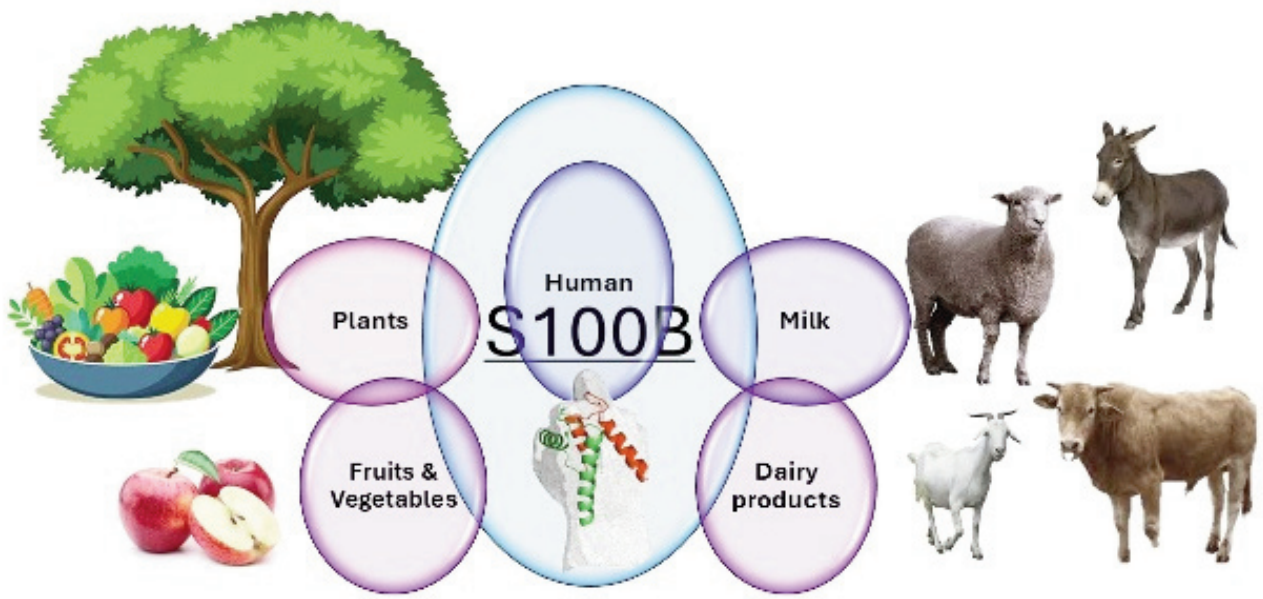

Fig. 2 - The S100B protein was originally discovered in the brain, but has recently been shown to be present in the diet as well, through various foods, including milk and some dairy products, certain fruits and vegetables. The action initially provided through breastfeeding could therefore be maintained even after weaning in various types of diets, including the Mediterranean diet, which is rich in vegetables, fruit and dairy products.

suggests that S100B also plays a role within the intestinal lumen by interacting with the microbiota. This occurs indirectly, without the protein crossing the intestinal barrier and being absorbed into the circulation. Various experimental, *in silico* and *in vivo* observations suggest that S100B influences the microbiota, which may then act on other systems or apparatuses. For example, it may modulate the functional axes of the microbiota-organs, including the gut-brain axis (15, 16). The gut microbiota is now considered a true organ, despite being composed of genetically distinct non-eukaryotic cells, but rather a prokaryotic community of bacteria and other microorganisms, including fungi, protozoa, and viruses. The intestinal bacterial microflora is extremely complex, comprising 600–1,000 different species and more than 10^{13}–10^{14} prokaryotic cells in total, contributing about 1–2 kg to the weight of each individual. Using an *ad hoc* developed algorithm, the researchers generated the 'microbiota proteome': all the proteins that can be expressed based on knowledge of the genomes present in a given microflora (15). This approach confirmed the potential interaction between S100B and various bacterial ligands and revealed how their distribution changed between healthy subjects and those with Crohn's disease. Furthermore, *in vivo* experiments on mice revealed that biodiversity indices, particularly the Shannon index, followed S100B levels in the intestinal lumen (16). Higher S100B values in the colon were found to correlate with an increase in the Shannon index, which may serve as an indicator of eubiosis. The whole of these results suggests that the presence of S100B in mother's milk may support infant development and perform direct neurotrophic actions on the nervous system, as well as indirect actions through harmonising the microbiota and mediating via the gut-brain axis. Building on this speculation, the hypothesis that breast-feeding, through S100B, plays a crucial role in initiating the correct formation of the microbiota and defining the imprinting of the microflora for an established biodiversity that will accompany the individual throughout life is worth exploring. Therefore, vaginal delivery, involving exposure to the first lactobacilli and microflora through the birth canal and subsequent interaction with environmental microflora, would constitute the basis on which breastfeeding and nutritional factors, including S100B, can contribute to establishing a healthy intestinal ecosystem in newborn babies from the very beginning of life.

Similarly, observations from plant studies showed that dietary intake of S100B could continue in the adult diet. Certain types of fruit and vegetables, such as apples, spinach and brassica vegetables, are rich in S100B and are traditionally part of the diet provided in early childhood. Other sources of S100B include certain dairy products such as yoghurt, ricotta and some cheeses. These foods, along with others, are common in various diets around the world and may contribute to harmonising the microbiota even in adulthood (17). Recent epidemiological studies and preliminary data show that it is possible to estimate and compare the amount of S100B in various diets. From this perspective, S100B becomes an innovative nutraceutical factor and a revolutionary additional indicator that can be used to design healthy, customised diets. Eubiosis is an increasingly relevant topic in medicine that is no longer limited to managing constipation or intestinal inflammatory diseases such as Crohn's/IBD. It also extends to preventing chronic degenerative diseases through the action of metabolic pathways (18).

In conclusion, S100B could play a role in directing the colonisation and formation of the microbiota in a sterile gut, such as that of an infant. The infant's meconium is surprisingly devoid of the microbes that will inhabit the adult intestinal lumen and will account for around a fifth of the weight of the faecal material. Once the microbiota has been established in the first months of life, however, S100B may continue to play its role in two ways: (i) an exogenous route involving a balanced diet containing certain vegetables and/or dairy products; and (ii) an endogenous route involving the production of the protein by glial cells in the enteric nervous system (ENS), which wraps around the intestinal tract in an impressive geometric network. In this context, the ENS would not just only manage peristalsis, but may control the 'human-microbiota' bidirectionally, regulating the biodiversity of the microflora (19, 20). From this perspective, S100B opens up new avenues for research and innovative applications in medicine and health.

Innovation and applications in different sectors

As a nutritional factor and harmoniser of the microbiota, S100B could therefore find fascinating applications in various fields. Experiments are already underway to develop targeted solutions and identify nutritional, pharmacological, or phytotherapeutic intervention strategies that could benefit

from these recent findings. The availability of plant sources of S100B could lead to new ways of balancing diets and implementing phytotherapy-based interventions. Additionally, the ability to more accurately estimate the amount of S100B in food will enable this new parameter to be incorporated into the preparation and customisation of eubiosis-aimed dietary programs. S100B-based products could be extremely useful in scenarios where a balanced diet cannot be guaranteed, such as in sports, extreme situations or travels where the balance of the microbiota needs to be protected from stress or debilitating conditions. Although the mechanisms of action have not yet been fully elucidated, there is evidence to support its effects and its promising applications in different fields and scenarios. For instance, preliminary experimental data suggests that an harmonising action on microflora could be transferred also to other fields in order to govern fermentation processes in several processes for the production of foods, pharmaceuticals or other specific technological areas. Modulating microbiota equilibrium may play a relevant role in managing microflora in other ecological niches, such as oral, vaginal, skin or even environmental microflora.

Conclusions

Not only is the S100B protein present in astrocytes of the brain and other tissues, but its molecular structure is also found in certain foods, including milk and other dairy products, as well as several edible plant species. There is evidence to suggest that the gut microbiota has a harmonising function, with cascading implications for eubiosis and potential trophic-protective actions on various organs. From birth, newborns take up this molecule through breast-feeding and subsequently through a balanced diet. The dosage of S100B in the diet or through targeted supplementation could provide new insights into scientific and technological research, disease treatment or prevention, and health promotion from childhood to elderly.

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