JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

A Trail of Research Revisted

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The author describes how his interest in the nutritional value of soybeans led to a trail of research that had many ramifications. In an attempt to account for the poor nutritional value of raw soybeans and the beneficial effect of heat treatment, a protein displaying hemagglutinating activity and capable of inhibiting the growth of rats was isolated and characterized. This protein subsequently proved to be an example of a class of proteins that were later referred to as "lectins". Lectins were also isolated from kidney beans and shown to be even more toxic than the soybean lectin. Concurrent studies with the soybean trypsin inhibitors revealed that these proteins inhibited growth by stimulating the secretory activity of the pancreas and could in the long term cause acinar cell adenoma of the pancreas. Acute experiments with human subjects showed that the human pancreas also responded to the stimulatory effects of the so-called Bowman−Birk soybean inhibitor. The studies on soybean trypsin inhibitors were expanded to include a protease inhibitor present in blood, α-1-antitrypsin, a deficiency of which leads to emphysema in humans. The mechanism whereby this protein inhibits leucocyte elastase was investigated. On the basis of these results the intratracheal administration of a synthetic peptide inhibitor of elastase attached to albumin microspheres was found to prevent elastase-induced emphysema in hamsters.

KEYWORDS: Soybeans; lectins; trypsin inhibitors; pancreas; emphysema

I wish to thank the organizers of this symposium for allowing me the opportunity to indulge in a privilege which is sometimes graciously accorded those who have reached that stage in life when one is prone to reminisce upon one's past accomplishments, such as they might be, rather than any future aspirations. My story really begins over 50 years ago following my discharge from the U.S. Army in 1946. At that time I entered the Ph.D. program in Biochemistry and Nutrition at the University of Southern California. After devoting one year to intensive course work to get on track with respect to chemistry, which, after 4 years in the army as a mess officer, had become but a hazy memory, I was afforded the opportunity of returning to active duty. the carrot on the stick being that I would be free to complete the research I needed for my Ph.D. degree. To this end I was assigned to the Quartermaster Corps in Chicago, IL. One of the projects the army was interested in at that time was the increased utilization of soybeans as a substitute for meat protein in army rations. Thus began my involvement with soybeans.

From the early observation by Osborne and Mendel (*1*), it was well-known that soybeans very poorly supported the growth of rats unless subjected to heat treatment. It was generally believed at the time that the beneficial effect of heat treatment was due to the thermal inactivation of a trypsin inhibitor which had previously been isolated from raw soybeans by Kunitz (*2*). The main objective of my thesis research was to elucidate the mechanism whereby the trypsin inhibitor exerted its deleterious effect on animal growth.

On the basis of experiments in which the purified Kunitz inhibitor had been added to diets in which heated soybeans provided the sole source of protein, it became apparent that the trypsin inhibitor did not fully account for the poor growth seen with raw soybeans (*3*). Adding the trypsin inhibitor to heated soybeans failed to lower the growth rate of rats to the same extent as a diet containing raw soybeans. Thus began my search for the presence of some other component in soybean that might be responsible, at least in part, for its poor nutritive value in its raw form.

After receiving my Ph.D. degree and my release from the army in 1949, I joined the staff of the Biochemistry Department of the University of Minnesota. As is so often the case with fresh Ph.D.'s who assume a new academic position and feel the pressure to show some evidence of productivity as quickly as possible, I chose an area of research with which I was already most familiar, which, in my case, was to take up my reasearch where I had left off. I was still intrigued by the possibility that there was a factor in soybeans, in addition to the trypsin inhibitor, which was acting as a toxin or at least as a growth inhibitor. Fractions of an aqueous extract of raw soybeans were tested for toxicity by intraperitoneal injection into rats. Such an experiment revealed that the toxicity was not associated with the trypsin inhibitor but rather with those fractions which agglutinated the red blood cells of the rabbit (*4*). My reason for measuring hemagglutinating activity at this point was based strictly on a hunch because, in a very early report in 1908, Landsteiner and Raubitschek (*5*) had shown that the crude

10.1021/jf020185o CCC: $22.00
Published on Web 09/24/2002

extracts of many edible legumes, including soybeans, displayed hemagglutinating activity. Although it is true that Stillmark (*6*), who is generally credited with the discovery of lectins, had shown over 100 years ago that ricin, the toxic principle of the castor bean, displayed hemagglutinating activity, little attention had been paid during the ensuing years to the possible toxicity that might be associated with the hemagglutinins present in edible beans. In fact, Goddard and Mendel (*7*) regarded the hemagglutinins of most legumes to be nontoxic, and, because of this, they proposed that they might be of "considerable practical importance in the preparation of antisera".

Using hemagglutinating activity as a measure of biological activity, the hemagglutinin was subsequently purified by salt fractionation to the point of homogeneity as evidenced by moving boundary electrophoresis and sedimentation in the ultracentrifuge (*8*). (It should be appreciated that at this time, 1952, the protein chemist was not blessed with the simple and sensitive techniques for evaluating the purity of proteins that we have today.) During the course of purification, it was noted that the toxicity of the various fractions when injected into rats paralleled their ability to agglutinate rabbit erythrocytes.

As an aside, it should be mentioned that I took the liberty of naming this protein "soyin", a term which was intended to indicate its possible relationship to the other toxic hemagglutinins that were known at that time, such as ricin and abrin, names derived from the plants from which they originated (i.e. *Ricinus communis* and *Abrus precatorius*, respectively). It was subsequently brought to my attention that the name "soyin" had been previously used to denote the proteolytic activity present in a crude extract of the soybean, although the enzyme responsible for this activity had not been further characterized (*9*). Nevertheless, in deference to these authors, and, to avoid further confusion, the term "soyin" was no longer used in subsequent papers from our laboratory and has since been referred to as simply the soybean agglutinin (SBA). It may be of interest to note that, at the time we had succeeded in isolating the soybean agglutinin, 1952, the term "lectin" had yet to be proposed. It was two years later that Boyd and Shapleigh (*10*) proposed the term "lectin" (taken from the Latin *legere*, to pick out or choose) to describe a class of proteins that agglutinate cells and exhibit sugar binding specificty.

Up to this point we had succeeded in showing that there was a hemagglutinin in soybeans which was toxic when injected into animals. It remained to be proved, however, that this protein was in fact responsible, at least in part, for the poor nutritive value of raw soybeans when consumed in the diet. To prove this point, it became necessary to develop a method for the large-scale preparation of the soybean agglutinin so as to obtain sufficient quantities that could be incorporated into a diet fed to rats. We devised a labor-intensive procedure that enabled us to obtain 2 g of purified agglutinin per kilogram of raw soy flour and fed this preparation to rats on a diet containing heated soy flour at a level which approximated the hemagglutinating acivity of an equivalent level of raw soy flour (*11*). A significant reduction in the growth of rats compared to the unsupplemented diet was observed. This decrease, however, was only about half of the growth depression caused by the raw soybean. The remaining half of the growth depression of raw soybeans is apparently due to the trypsin inhibitor (*12*).

At this point my interests became more biochemically oriented. During the ensuing years (1953−1958) we determined some of the important physicochemical parameters of SBA including its molecular weight (*13*), its amino acid composition (*14*), the effect of chemical modification (*15*), and its requirement for metal ions for full activity (*16*). The quantitative evaluation of hemagglutinating activity was also refined by developing a photometric method for measuring hemagglutination (*17*). End-group analysis revealed for the first time that SBA was composed of subunits consisting of several polypeptide chains (*14*), a feature which later turned out to be a characteristic feature of the quaternary structure of most lectins. One of the surprising findings that emerged from these studies was the fact that this protein contained a significant amount of carbohydrate (6−10%). The fact that SBA was shown to be a glycoprotein may not be particularly surprising to the modern-day biochemist, but at that time the finding of a sugar moiety in a plant protein was accepted with reservation. It was thought that glycoproteins were strictly of animal origin and that the finding of a sugar with a plant protein was most likely due to non-covalent contamination. The conclusion that SBA was in fact a protein molecule to which a carbohydrate chain was covalently attached was elegantly confirmed by Sharon's group in Israel (*18*).

Although most of my earlier studies had been devoted to SBA, my lingering interest in nutrition prompted me to study the possible nutritional significance of the lectins known to be present in edible legumes other than soybeans. It was well-known, for example, that raw kidney beans (*Phaseolus vulgaris*) are very poorly tolerated by rats, and animals on a diet containing these beans die within a week or so. This prompted us to develop a scheme for the large-scale purification of the kidney bean lectin, which proved to be a very potent inhibitor of the growth of rats (*19*) and chicks (*20*). A detailed study of its physicochemical properties revealed it also was a glycoprotein (*21*, *22*), comprising several subunits (*23*) and possessing a requirement for metal ions (*24*). The lectin from *P. vulgaris* was also found to be lethal to the larvae of the cowpea weevil (*25*), thus providing the first direct evidence that lectins probably play an important role in protecting plants from insect predators.

Finally, coming full circle back to my original interest in the nutritional effects of lectins, it is now well established that the toxicity of the kidney bean lectin is due to the fact the lectin binds to the epithelial cells lining the small intestine, causing disruption of the villi of the brush border (*26*). We extended these studies by demonstrating that one of the consequences of the damage inflicted by the lectin is an impairment of the absorption of nutrients across the intestinal wall (*27*).

An important aspect of my continued interest in the antinutritional properties of soybeans involved studies on the trypsin inhibitors they contain. The main physiological effect of the soybean trypsin inhibitor (SBTI) in rats is to stimulate the pancrease to increase its output of digestive enzymes (trypsin, chymotrypsin, and elastase) by negative feedback control (*28*). This effect is manifested by an endogenous loss of protein and a concomitant depression of growth (*29*). Histopathological changes of the pancreas also occur, which have been characterized as hypertrophy and hyperplasia (*30*). One of the most significant outcomes of these studies was the observation that the long-term feeding of diets containing SBTI to rats ultimately led to pancreatic nodular hyperplasia and acinar cell adenoma (*31*). Although these results are difficult to relate as to their significance in humans, we did demonstrate that the direct introduction of SBTI into the small intestinal tract of human subjects caused the pancreas to significantly increase its output of digestive enzymes (*32*). One can only speculate as to what this finding might signify if soybeans, in which the SBTI had not been inactivated, were to contribute a significant portion of the human diet over a long period of time.

As a spin-off from studies with SBTI, we decided to take advantage of our experience with protease inhibitors in a study of α-1-antitrypsin (AAT), a blood protein that serves to protect lung tissue from being degraded by leucocyte elastase. A genetic deficiency of this protein or its inactivation by cigarette smoke leads to the development of emphysema in humans. We developed methods for the isolation of AAT (*33*) and leucocyte elastase (*34*) and studied the mechanism of their interaction (*35*). A synthetic peptide inhibitor of AAT was covalently bound to albumin microspheres as a carrier (*36*), and the intratracheal administration of this complex was found to prevent elastase-induced emphysema in hamsters (*37*).

I would like to conclude this trip down memory lane by acknowledging the contributions made by my students and colleagues, without whom much of the research I have described would not have been possible. In particular, I wish to express my sincere thanks to all of the participants who have made this symposium a reality.

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Received for review February 11, 2002. Revised manuscript received May 3, 2002. Accepted May 6, 2002. Portions of this paper are reprinted with permission from *Carbohydr. Res.* 1991, *213*, 1−5. Copyright 1991 Elsevier Science.
JF020185O