Abstract of Research Plan

NAME, ADDRESS, AND TELEPHONE NUMBER OF APPLICANT ORGANIZATION

Protein Solutions, Inc.
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YEAR FIRM FOUNDED	NO. OF EMPLOYEES (include all affiliates)
1988	5

TITLE OF APPLICATION

Assay of Carbohydrate Status Using Dipstick Biosensors

KEY PERSONNEL ENGAGED ON PROJECT

NAME	ORGANIZATION	ROLE ON PROJECT
C-Y Wang	Protein Solutions, Inc.	P.I./Chief Scientist
J.D. Andrade	Protein Solutions, Inc.	Research Scientist
D.C. Min	Protein Solutions, Inc. and the University of Utah	Graduate Student

ABSTRACT OF RESEARCH PLAN: State the application's broad, long-term objectives and specific aims, making reference to the health-relatedness of the project. Describe concisely the research design and methods for achieving these goals and discuss the potential of the research for technological innovation. Avoid summaries of past accomplishments and the use of the first person. This abstract is meant to serve as a succinct and accurate description of the proposed work when separated from the application. If the application is funded, this description, as is, will become public information. ***Therefore, do not include proprietary or confidential information.*** DO NOT EXCEED 200 WORDS.

Carbohydrate biosensors are needed to screen essential mono- and disaccharides, including glucose, fructose, galactose, and lactose in blood and urine. We propose to study the feasibility of a multi-channel instrumentless device. Such dipstick type sensor will benefit patients with diabetes who need careful diet control as well as new born infants with inborn errors of carbohydrate metabolism.

Protein Solutions, Inc. (PSI) has considerable experience in bioluminescence and has successfully developed direct reading quantitative biosensors for ATP-dependent processes. A front end enzyme, hexokinase, consumes ATP by converting glucose to glucose-6-phosphate was coupled with the direct reading ATP measuring device. Preliminary results demonstrated the quantitative assay of glucose in the range from 1-100 mg/dl using direct eye detection.

The scientific studies needed to assure the feasibility of direct reading, four channel carbohydrate sensor will be performed in the Phase I Project. A prototpye sensor will also be tested and evaluated in the end of Phase I project.

Provide key words (8 maximum) to identify the research or technology.

Biosensor/Carbohydrate/Bioluminescence/Firefly/Luciferase/ATP/Metabolism

Provide a brief summary of the potential commercial applications of the research.

PSI is working with special major corporate partners to provide resources for the Phase III manufacturing and marketing effort. PSI expects to manufacture and market this "four-in one multi channel carbohydrate sensor" for the home awareness and education market. PSI will license the technology to a larger firm in the clinical chemistry area and to a consumer products firm for the intelligent diaper product area.

Budget Justification

Using continuation pages if necessary, describe the specific functions of the personnel and consultants. Read the instructions and justify costs accordingly.

Mr. (Dr.) C-Y Wang will serve as Principal Investigator with 100% effort on the project in his 3/4 time position with Protein Solutions. He will be assisted by J. Andrade, Surface Scientist, who receives no salary; R. Scheer, Materials Scientist, who is employed half time by PSI and will spend 1/4 effort on the project; Mr. D. Min, Graduate Student, who will work 3/4 time on the project; and Mr. Roshen Koshy, a technician, who will work part time on the project.

Although no consultant costs are budgeted, Dr. Vladimir Hlady, Associate Professor of Bioengineering, and Dr. Russell Stewart, Assistant Professor of Bioengineering, both serve on Protein Solutions, Inc.'s Scientific Advisory Board. They are both affiliated with the University of Utah. Mr. Wang has worked in Dr. Stewart's laboratory for the past two years helping to develop the recombinant luciferase which is a key part of this technology. That material will continue to be produced in Dr. Stewart's laboratory, although PSI has licensed the technology and Mr. Wang has begun to transfer that technology and those capabilities to the PSI labs. The detailed luminescent studies will be done in Dr. V. Hlady's lab. Protein Solutions, Inc. is a member of the Center for Biopolymers at Interfaces at the University of Utah and thus has formal direct access to the laboratories of these faculty.

In addition, Dr. Larry Kricka, Dept. of Pathology at University of Pennsylvania, a recognized national and international expert on the application of bioluminescence for clinical analysis (Kricka et al. 1984), also serves on PSI's Scientific Advisory Board, and will provide advice and input as required.

Resources

FACILITIES: Specify the facilities to be used for the conduct of the proposed research. Indicate their capacities, pertinent capabilities, relative proximity, and extent of availability to the project. Include laboratory, clinical, animal, computer, and office facilities at the applicant small business concern and any other performance site listed on the FACE PAGE. Identify support services such as secretarial, machine shop, electronics shop, and the extent to which they will be available to the project. Use continuation page(s) if necessary.

PSI's laboratory space is located in a suite in the University of Utah Research Park located at 391 Chipeta Way. This includes a fume hood, general chemical laboratory, and appropriate office space. Some of the work will be done in the labs of the Center for Biopolymers at Interfaces (CBI) at the University of Utah by PSI personnel using the CBI affiliation discussed above.

MAJOR EQUIPMENT: List the most important equipment items already available for this project, noting the location and pertinent capabilities of each.

Centrifuge, HPLC, Ovens, Computers, CCD Camera, Cameras

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EDUCATION:

1996 Ph.D. (pending), Bioengineering, University of Utah. Dissertation, " Firefly Luciferase: Activity, Stability and Sensor Application," under the direction of Prof. J.D. Andrade.

1985 B.S., Chemical Engineering, National Taiwan University, Taipei, Taiwan.

EXPERIENCE:

July, 1996-present Protein Solutions, Inc., **Research Scientist.** Direct research in recombinant protein expression and purification, design of bioluminescent based biosensor for applications in the health care industry.

1989-1996 University of Utah, Bioengineering Department, **Research assistant.** Responsible for basic studies of proteins at interfaces.

1990-1992 University of Utah, Bioengineering Department, **Teaching assistant.** responsible for classes: Systemic Biomechanics, Skeletal Biomechanics and Biocompatibility.

1987-1989 Taiwan Power Company. **Reaearch Chemist**. Major researcher for special project in oil/coal analysis.

HONORS:

University of Utah. University of Utah Graduate Fellow (1991-1992).

PUBLICATIONS:

1. C. Y. Wang and J. D. Andrade, "Denaturation of Firefly Luciferase", in Bioluminescence and Chemiluminescence: Current Status, P. Stanley and E. Kricka, eds, Wiley, 1991; p.427-432.
2 C. Y. Wang and J. D. Andrade, "Interfacial Behavior of Firefly Luciferase" in Bioluminescence and Chemiluminescence: Status Report, A. A. Szalay ed., Wiley, 1993; p.99-103.
3. C. Y. Wang and J. D. Andrade, "Purification and preservation of firefly luciferase," in Bioluminescence and Chemiluminescence: Fundamentals and Applied Aspects, A. K. Campbell, L. J. Kricka and P. E. Stanley eds., Wiley, 1995; p.423-426.
4. D. J. Min, C. Y. Wang and J. D. Andrade, "Air/warer monolayer studies of bioluminescent enzymes," in Bioluminescence and Chemiluminescence: Fundamentals and Applied Aspects, A. K. Campbell, L. J. Kricka and P. E. Stanley eds., Wiley, 1995; p.494-497
5. J. D. Andrade, C-Y Wang, V. Hlady, P. M. Triolo, and R. J. Scheer, "Method of Measuring Chemical concentration and/or light intensity based on spatial separation and resolution," provisional US patent application, 1995.
6. C. Y. Wang, Ph.D. Dissertation in preparation. "Firefly Luciferase: Activity, Stability and Sensor Application", 1996.
7. C. Y. Wang, S. Hitz, J. D. Andrade, and R. Stewart, "Biotinylation of Firefly Luciferase in vivo: a Recombinant Protein with a Specific Immobilization Site" submitted to Analytical Biochemistry (1996).

A. Specific Aims

The overall research objective is to determine the feasibility of a multi-channel, direct reading, carbohydrate sensor for point of care application. Specific ATP dependent enzymes convert the monosaccharides or disaccharides to other metabolites thereby depleting ATP. The depleted ATP is quantitatively measured by firefly luciferase bioluminescence. The end point of measurement is photons produced from the ATP in each reaction channel. A novel technology is used to present the bioluminescent signal in a quantitative, thermometer-like, spatially distributed format. A clear inflection in light intensity reflects the concentration of specific carbohydrate.

In this Phase I project, the following objectives will be accomplished:

1. Analyte and enzyme selection and characterization
2. Enzyme immobilization and kinetics
3. Dehydration and stability
4. Calibration and reference
5. Device design
6. Device testing and evaluation

B. Significance

Diseases of carbohydrate metabolism are caused by specific enzyme deficiencies, which block important metabolic pathways and lead to metabolic derangement. The diseases can be classified according to the sugar involved:

Glucose; Defects in disturbed glucose metabolism results in hyperglycemia, osmotic diuresis, thirst, and weight loss (Milla et al. 1978). Accelerated ketone body production results in ketoacidosis.

The hyperglycemia is caused by consistent overproduction of glucose in the liver coupled with inefficient disposal of glucose in peripheral tissues. A striking feature of diabetes is the shift in fuel usage from carbohydrates to fats. Glucose is also excreted in the urine when the blood glucose level exceeds the reabsorptive capacity of the renal tubules. Although dipsticks for glucose urine analysis are available, sensors providing a more complete carbohydrate profile are not available.

Fructose; Fructose is an important source of dietary carbohydrates. Excess doses of fructose are toxic and cause hyperuricemia, hyperlactatemia, and ultra-structural alterations in liver and intestinal cells. Prolonged fructose ingestion in infants leads to poor feeding, vomiting, hepatomegaly, jaundice, hemorrhage, a proximal renal tubular syndrome, and finally, hepatic failure and death (Fernandes et al., 1995).

Fructosuria results from an inherited deficiency of fructokinase, resulting in diminished conversion of fructose to fructose-1-phosphate. Infants and adult patients with hereditary fructose intolerance are perfectly healthy and asymptomatic as long as they do not ingest any food containing fructose and sucrose. Thus during breast feeding, no metabolic derangement occurs. The newborn infant who is not breast-fed but receives a cow's milk formula with fructose or sucrose substituting for lactose is in grave danger.

Galactose; The main dietary source of galactose is the disaccharide lactose, the principal carbohydrate of mammalian milk. The name galactosemia has been given to a toxicity syndrome associated with the administration of galactose to patients with an inherited disorder of galactose utilization. This syndrome is caused by a deficiency of

either galactokinase, galactose-1-phosphate uridyl transferase or uridine diphosphate galactose-4-epimerase. The deficiency of those enzymes, coupled with galactose ingestion results in failure to thrive, vomiting, liver disease, cataracts, and mental retardation (Segal, 1989).

Lactose: Primary adult-type hypolactasia is the most common form of genetically determined disaccharide deficiency. Subjects with this hypolactasia have no feeding problems during infancy because the enzyme is present at birth.

Lactose intolerance due to lactase deficiency can cause gastrointestinal problems, including diarrhea, irritable bowel, or recurrent abdominal pain, particularly in children. Congenital lactase deficiency is a very rare disease. On a lactose-free diet, children show good growth and psychomotor development.

The diagnosis of these syndromes may be made by the identification of glucose, fructose, galactose, and lactose in the urine. Except for glucose, the diagnostic test of each sugar is usually expensive and is often done by thin layer chromatography or gas-liquid chromatography (Kraffczyk, 1972; Fernandes et al., 1995). Multi-channel carbohydrate biosensors, which are sensitive, fast, cheap, and instrument free, are of significant interest in the detection of sugar in urine.

Relevant Experience

The PI has studied firefly bioluminescence and its sensor applications for six years. His dissertation, "Firefly Luciferase: Activity, Stability and Sensor Applications", and his publications covered the properties of firefly luciferase and its application to bioluminescent analytical devices. Under the guidance of Dr. Russell Stewart, the PI has expressed recombinant luciferase in E. coli, obtaining highly pure luciferase in large quantities (Wang et al., 1996).

Under a contract between Protein Solutions, Inc. and the University of Utah's Center for Biopolymers at Interfaces (CBI), the PI has been working on the trehalose and/or sucrose stabilization of firefly luciferase for the past two years (Wang and Andrade, 1995). Firefly luciferase, an enzyme that catalyzes bioluminescent reaction (DeLuca and McElroy, 1978), has been entrapped in agarose gels and is stable for up to 6 months (longer storage time have not yet been done). These gels maintain their optical clarity in the dehydrated state, and can be rehydrated after extended periods with high levels of activity. With the awarding of grant from NSF (STTR Phase I grant, NSF DMI-9413561), Mr. Wang, and Dr.'s Scheer and Andrade have extended this gel preservation technique to apyrase and have developed a device for measurement of ATP concentration based on the spatial position of light output. Apyrase, an enzyme purified from potato with distinct activity for dephosphorylation of ATP and ADP, (Molnar and Lorand, 1961) is utilized to consume ATP in such a way as to provide a spatial signal.

The spatial position of the emitted light is proportional to the analyte (ATP) concentration is the unique approach to ATP detection. The human eye can then be used to assess spatial position rather than absolute light intensity. The spatial positioning is achieved by carefully controlling the ATP concentration using an ATP "filter", a second ATP consuming enzyme, apyrase. Apyrase has a turnover rate approximately 100 times

faster than luciferase and serves to regulate ATP concentration, thereby modulating the spatial position of the bioluminescence.

Measurements of enzyme and substrate activity after prolonged gel storage were performed for different gel preparation and storage conditions. These measurements gave us the information necessary to decide which enzymes or substrates could be successfully stored and which preservation method was preferred.

After learning that both luciferase and apyrase could be stored for prolonged periods in a dehydrated state, refinement of the luciferase and apyrase concentration levels for appropriate readout were performed. Appropriate concentration levels were determined through a systematic examination of light output relative to the concentrations of luciferase, apyrase, and ATP. Each of the gel components, including the luciferase and apyrase enzymes, and the analyte components, including the luciferin substrate and coenzyme A, were reviewed. The optimum concentration for each component was determined as: luciferase in the range of 0.1 mg/ml, luciferin in the range of 0.5 mM, and coenzyme A in the range of 0.5 mM. The apyrase concentration is dependent on the range of ATP to be measured.

To measure an ATP concentration, a sample containing ATP is distributed to the luciferase/apyrase gel at which time the ATP is consumed by the two competing enzymes. Because the turnover rate of apyrase is two orders of magnitude higher than that of luciferase, the apyrase quickly moderates the ATP concentration. The amount of ATP reacting with the luciferase to produce light depends on both the initial ATP concentration and the concentration of apyrase.

To demonstrate and test the dependence of light output on both of these concentrations, a 2-D (two dimensional) gradient of ATP concentration ($5x10^{-4}$ to $5x10^{-7}$ mole/l ATP) vs. apyrase concentration (2 units/ml to 0 units/ml) was produced. (See Figure 1.) This is a positive surface profile directly related to light intensity. The image was captured with a CCD camera. With high initial concentrations of ATP, a large concentration of apyrase is required to modulate the light intensity. With low initial concentrations of ATP, only small concentrations of apyrase are required to modulate the light intensity. The goal of such modulation is to generate a specific light cutoff point indicating a specific concentration of ATP in the analyte solution.

A typical ATP sensor will use a single apyrase gradient. If the ATP concentration is low, the inflection point of the spatial distributed bioluminescence is to the far left in Figure 1, whereas if the ATP concentration is high, the inflection is to the far right (see also Figure 2). This unique spatial or position detection technology is used to provide a generic ATP sensor. The ability to inexpensively, easily sense ATP is enabling technology which makes this proposal possible.

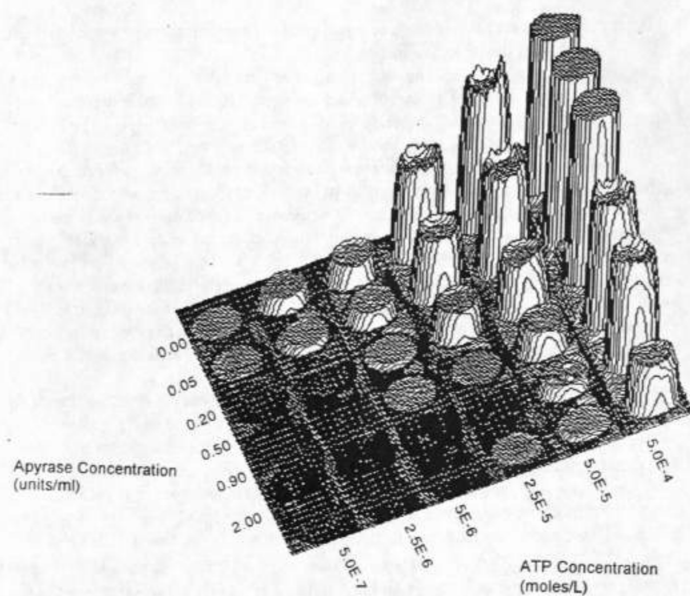


Figure 1. Typical CCD 3-D profile of light intensity for a six by six gradient array. Note the change in the bioluminescence along the apyrase gradient for different ATP concentration (see also Figure 2).

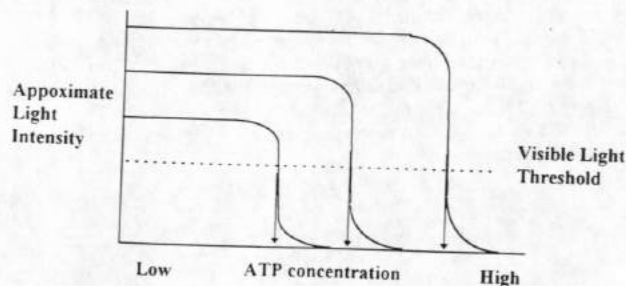
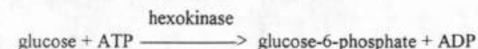


Figure 2. ATP concentration detected via the spatial distribution of ATP bioluminescence using apyrase gradient.

—Hexokinase consumes ATP in its conversion of glucose to glucose-6-phosphate,.



Thus putting hexokinase on the "front end" of our ATP sensor, together with a known initial ATP concentration, results in ATP depletion due to the phosphorylation reaction. The depletion of ATP is a direct measurement of glucose concentration. The result of this preliminary study showed that a front end enzyme can be coupled with luciferase/apyrase system to measure carbohydrate concentration.

The measurement of ATP concentration itself is of limited commercial interest. However, hundreds, perhaps even thousands, of enzymes exist which are involved with ATP consumption or ATP production, most of which are specific to another chemical substrate, for example, glucose, fructose, etc (Lundin, 1982). These "front end" enzymes permit the development of individual sensors or sensor channels for each of those substrates.

We can also develop sensor for enzyme activity. In this case both the substrate and ATP are included within the sensor. A decrease in ATP concentration is a measure of kinase activity. So both substrate sensor and enzyme activity sensor can be developed using our quantitative ATP detection.

D. Experimental Design and Methods

1. Analyte end enzyme selection and characterization

The pre-requisite for front end enzyme is substrate specificity. Monosaccharides such as glucose, fructose, and galactose are phosphorylated by specific kinases in the presence of ATP. Although we have already selected the four carbohydrate analytes, we will continue to review the literature, and consult with experts in the areas of diabetes and disorders of carbohydrate metabolism to be sure that these are indeed the four most useful carbohydrate analytes for general diagnostic purposes. As our technique and technology is so versatile, it is likely that in Phase II, and in subsequent generations of sensors, other carbohydrate analytes would be included. The following enzymes are selected as front end enzymes for each channel to react with a specific monosaccharide (Table 1):

(a) Glucokinase (enzyme 2.7.1.2): A glucose specific enzyme which consumes ATP in converting glucose to glucose-6-phosphate. Diabetes mellitus is due to a deficiency of this enzyme.

(b) Fructokinase (enzyme 2.7.1.4): This liver enzyme catalyzes the phosphorylation of fructose to form fructose-1-phosphate.

(c) Galactokinase (enzyme 2.7.1.6): This galactose specific enzyme converts galactose to galactose-1-phosphate.

Lactose is a disaccharide which can be hydrolyzed by β-galactosidase (enzyme 3.2.1.23) to glucose and galactose, and followed by reacted with glucokinase (enzyme 2.7.1.2). The enzymes will be purchased from commercial sources. Because enzyme turnover rate is direct related to the completeness of the phosphorylation reaction, the kinetics of enzymes in solution will be studied. The monosaccharide solutions used will be prepared based on the normal concentration in urine (Table 1). The amount of enzyme

used will be adjusted according to its turnover rate. Issues of the specificity of the kinase, the availability of that kinase, and the stability, robustness, and microenvironment requirements of that kinase will also be addressed.

Carbohydrates	Concentration in urine (mg/dl)	"Front End" Enzymes
Glucose	5.2	Glucokinase (enzyme 2.7.1.2)
Fructose	2.1	Fructokinase (enzyme 2.7.1.4)
Galactose	10.0	Galactokinase (enzyme 2.7.1.6)
Lactose	9.5	β-galactosidase (enzyme 3.2.1.23) and Glucokinase (enzyme 2.7.1.2)

Table 1 Carbohydrates, concentration in urine, and typical enzymes selected (Tietz, 1986).

Recombinant luciferase, which is an integral part of our ATP sensing technology, will continue to be expressed and purified in Dr. Stewart's laboratory. The properties and purity of recombinant luciferase has been well characterized by the PI (Wang et al. 1996).

2. Enzyme immobilization and kinetics

Our present ATP sensing technology is based on the incorporation of firefly luciferase and an ATP consumase (apyrase) in an agarose gel. Sucrose or trehalose is used as a preservative and stabilizer for the enzymes and the gel matrix (Carpenter and Crowe, 1988). The new issue here is to entrap the carbohydrate specific enzyme along with sucrose or trehalose in agarose gel by the same technique. To prevent enzyme denaturation, a low-gelling temperature agarose is used as matrix and the entrapping process is performed under 30°C.

The activity of entrapped enzyme is estimated by adding monosaccharide and excess ATP. The residual ATP is then guided through the wicking fiber to the second unit, the direct reading ATP biosensor. Luciferase and apyrase will react with residual ATP and provide the analysis required. The front end enzyme concentration will be adjusted according to the results of objective 1. A CCD camera will record the light profile, from which the front end enzyme activity can be determined. The reaction time for front end enzyme and the length of wicking fiber will be adjusted for optimum resolution.

Based on the characterization of the kinases in Task One, we will consider enzyme size, kinetics, and stability in terms of the optimum immobilization strategy for each enzyme. Initially, this will involve encapsulation in a low melting agarose gel. As our experience with that technology is at present limited to luciferase and hexokinase, and not to kinases in general, we fully expect that the immobilization strategy will have to be optimized for each of the selected kinases.

3. Dehydration and stability

The next objective is the preparation of dry reagent and assessment of the storage stability of the enzymes. The gel incorporated with enzymes is fully dehydrated in air, maintaining transparency, and later rehydrated with the analyte sample of interest

(monosaccharide, ATP and $MgSO_4$). The residual ATP is guided to the ATP measuring unit. The protectant for front end enzyme activity, the optimum front end enzyme concentration in agarose gel and the short to medium term stability of dry reagent will be evaluated in this stage. We expect that the dehydrated gel can be easily rehydrated with maximal enzyme activity. Indeed, this has been shown for hexokinase.

4. Calibration and reference

A reliable sensor requires several calibration and reference channels. We will include a reference channel for the volume of liquid absorbed and delivered into the sensor. This is a standard channel on all modern semi-quantitative dipstick sensors. That channel, or a second reference channel, will include a background ATP analysis. The ATP channel will also suffice as a general channel for determining whether or not there may be inhibitors of luciferase in that particular urine sample. Our experience to date shows that this is unlikely, but nevertheless, a specific luciferase inhibition test must be included.

We will also include reference channels to test for inhibition of the specific kinases involved, and for unusual cross reactivity problems. We expect that there will be somewhere in the range of 2-4 such reference channels. There will be preliminary work on these reference/calibration channels during Phase I, and more extensive and substantive work in Phase II as we move towards a prototype sensor.

The solutions with urea at different pH will be loaded to the front end of each channel. Inhibition effects will be evaluated. If necessary, the urine sample can be diluted or neutralized before test.

The channels for glucose, fructose, galactose, lactose and standard ATP will be calibrated. The relationship between photon readout and different monosaccharide concentrations will be determined. A CCD camera will be used to help analyze the data. Luciferase/apyrase ratio will be adjusted to sharpen the inflection point for ease in reading (see Figure 2). Calibration markers for each carbohydrate concentration from 0 to 100 mg/dl will be precisely marked. In the Phase II Project, an uniform marker and range for all carbohydrates will be developed by adjustment of enzyme concentrations.

5. Device design

The preliminary device design is given in Figure 3. The actual design will be developed in Phase I and optimized and further developed as a prototype for testing and evaluation in Phase II. Basically, each of the sensing channels, i.e., specific carbohydrates, will be developed as an individual, single channel sensor, but with the appropriate reference and calibration channels. Each of these sensors will then be packaged as a multi-layer sensor with appropriate spaces to permit capillarity feed of the urine to be analyzed. As indicated in Figure 3, the sensor will be designed to draw a constant volume of urine into each of the four analyte channels, deliver the urine progressively through a pre-processing layer, a constant concentration ATP layer, a kinase region, and then the ATP-depleted solution delivered to the gradient sensor region which does the quantitative ATP analysis via firefly luciferase bioluminescence.

Phase I activity will focus on the three monosaccharide channels. Only very preliminary work will be done on the more complex disaccharide (lactose) channel during Phase I (see time plan Table 2).

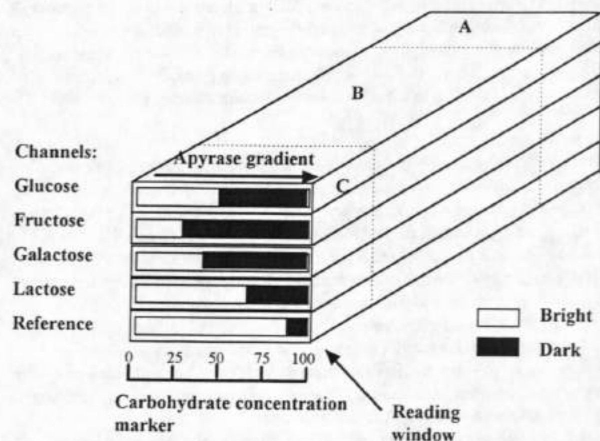


Figure 3. The four channel carbohydrate biosensor design. Zone A is the dipping end and the front end enzyme (kinase) reaction zone. The carbohydrates in the sample are phosphorylated in this zone. Zone B provides capillarity transport of the ATP analyte solution from zone A to zone C. Zone C is photon producing region. Luciferase catalyzes the ATP dependent bioluminescence. The apyrase gradient provides the sharp spatial transition of visibile light in the reading window. Carbohydrate concentration is reciprocal to the length of light band.

6. Device testing and evaluation

Most of the Phase I testing will utilize pooled human urine to which is added the various sugars of interest in known concentration. Carbohydrate concentration in urine will be assayed by standard methods using reference and clinical laboratory

Task	Phase I	Phase II
1. Analyte and enzyme selection and characterization		
2. Enzyme immobilization and kinetics		
3. Dehydration and stability		
4. Calibration and reference		
5. Device design		
6. Device testing and evaluation		

Table 2. Time plan (each frame represents 2 months)

7. Phase II work plan

The phase II activities will likely focus on optimization of the multi-enzyme immobilization process, more complete evaluation and optimization of multi-enzyme kinetics, and more complete evaluation of the stability of the dry reagent components. We will also evaluate sealed packing methods to extend the probable shelf life from 3 to 6 months to about 2 years.

Calibration and reference channel for the three specific monosaccharides will be developed and implemented. About 6 months into Phase II, we will apply our glucose and galactose analysis skills to the development of the more complex lactose channel. The prototype device available at the end of Phase I will be subjected to extensive testing early in Phase II, followed by improved design and optimization to a final prototype device in mid-Phase II. Testing and evaluation of the final prototype device will be a focus of the final 9 months of Phase II.

8. Phase III

PSI is working with special major corporate partners to provide resources for the Phase III manufacturing and marketing effort. PSI expects to manufacture and market the sensors for the home awareness and education market. PSI will license the technology to a larger firm in the clinical chemistry area and to a consumer products firm for the intelligent diaper product area. Negotiation with three major firms are now in process.

E. Human Subjects-None

F. Vertebrate animals-None

G. Consultants-None
(but see Budget Justification for brief discussion of unpaid advisors)

H. Contractual Arrangements-None

I. Literature Cited

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Wang, C-Y, S. Hitz, J. D. Andrade, and R. Stewart, "Biotinylation of Firefly Luciferase in vivo: a Recombinant Protein with a Specific Immobilization Site" submitted to Analytical Biochemistry (1996).