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Ekwuribe

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[54] **CONJUGATION-STABILIZED POLYPEPTIDE COMPOSITIONS, THERAPEUTIC DELIVERY AND DIAGNOSTIC FORMULATIONS COMPRISING SAME, AND METHOD OF MAKING AND USING THE SAME**

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[21] Appl. No.: **59,701**

[22] Filed: **May 10, 1993**

[51] **Int. Cl.**[5] **C07K 7/40**; C07K 7/36; C07K 17/08; C08H 1/00

[52] **U.S. Cl.** **530/303**; 530/307; 530/309; 530/322; 530/345; 530/402; 530/351; 530/409; 530/410; 530/411; 435/188; 424/85.1; 424/85.4; 424/94.3

[58] **Field of Search** 435/188; 514/3, 4, 12; 530/303, 307, 324, 309, 345, 322, 402, 326, 409, 410, 411, 325, 351; 424/85.1, 85.2, 85.4, 85.5, 85.6, 85.9, 94.3

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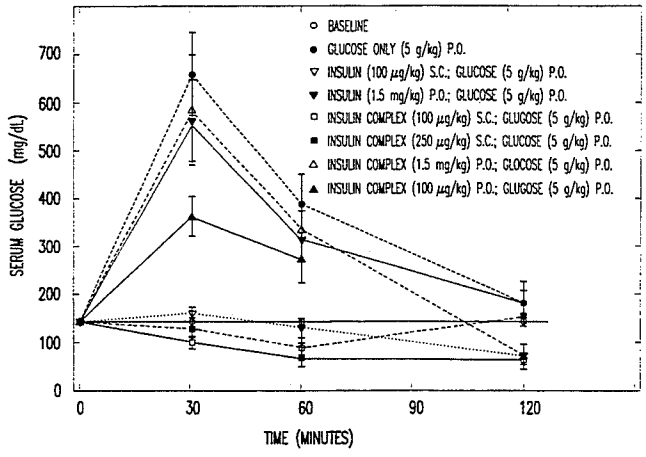
[57] **ABSTRACT**

A stabilized conjugated peptide complex comprising a peptide conjugatively coupled to a polymer including lipophilic and hydrophilic moieties, wherein the peptide may for example be selected from the group consisting of insulin, calcitonin, ACTH, glucagon, somatostatin, somatotropin, somatomedin, parathyroid hormone, erythropoietin, hypothalamic releasing factors, prolactin, thyroid stimulating hormones, endorphins, enkephalins, vasopressin, non-naturally occurring opioids, superoxide dismutase, interferon, asparaginase, arginase, arginine deaminease, adenosine deaminase, ribonuclease, trypsin, chymotrypsin, and papain. In a particular aspect, the invention comprises an insulin composition suitable for parenteral as well as non-parenteral administration, preferably oral or parenteral administration, comprising insulin covalently coupled with a polymer including (i) a linear polyalkylene glycol moiety and (ii) a lipophilic moiety, wherein the insulin, the linear polyalkylene glycol moiety and the lipophilic moiety are conformationally arranged in relation to one another such that the insulin in the composition has an enhanced in vivo resistance to enzymatic degradation, relative to insulin alone. One, two, or three polymer constituents may be covalently attached to the insulin molecule, with one polymer constituent being preferred. The conjugates of the invention are usefully employed in therapeutic as well as non-therapeutic, e.g., diagnostic, applications, and the peptide and polymer may be covalently coupled to one another, or alternatively may be associatively coupled to one another, e.g., by hydrogen bonding or other associative bonding relationship.

33 Claims, 2 Drawing Sheets



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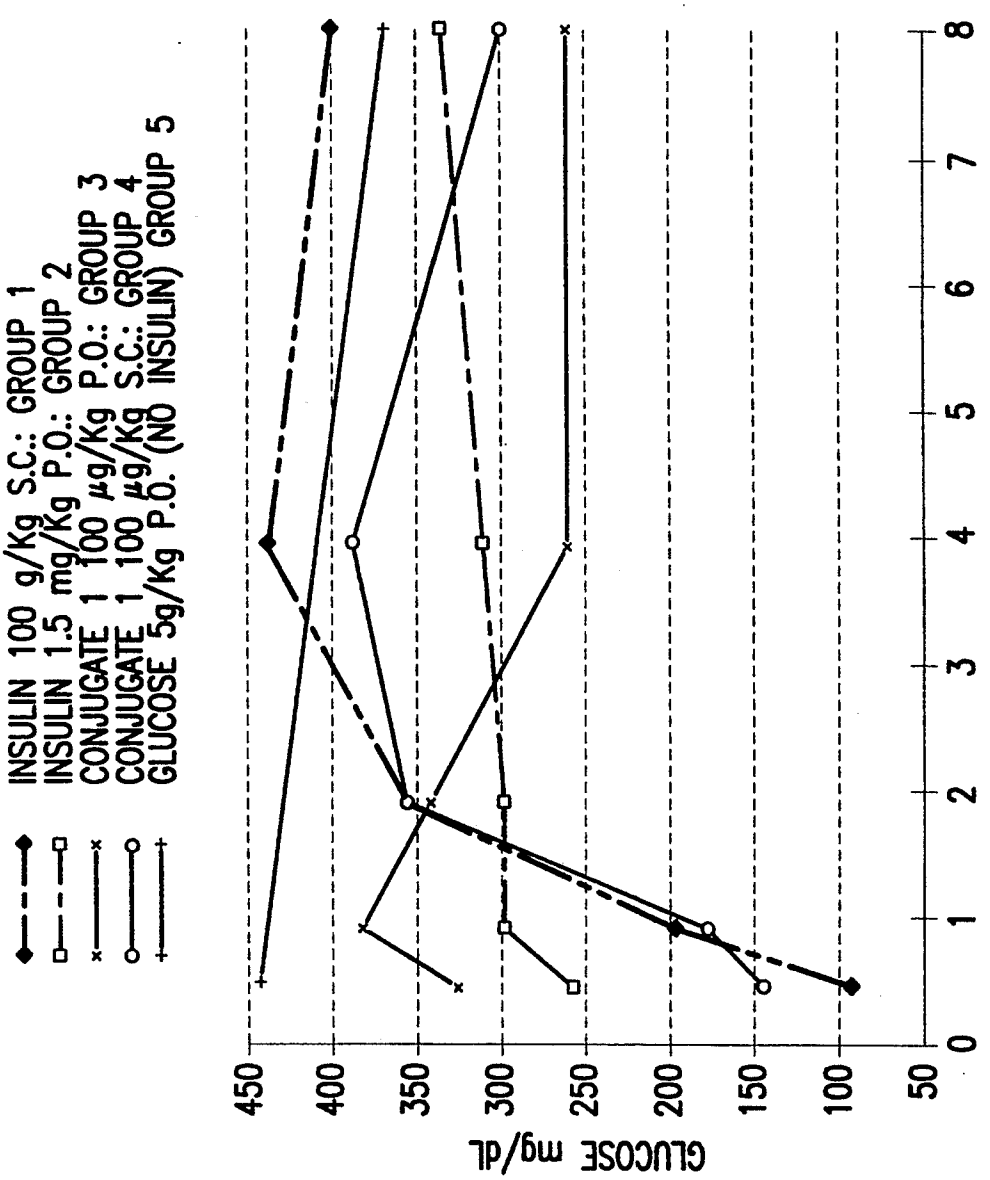

FIG. 1

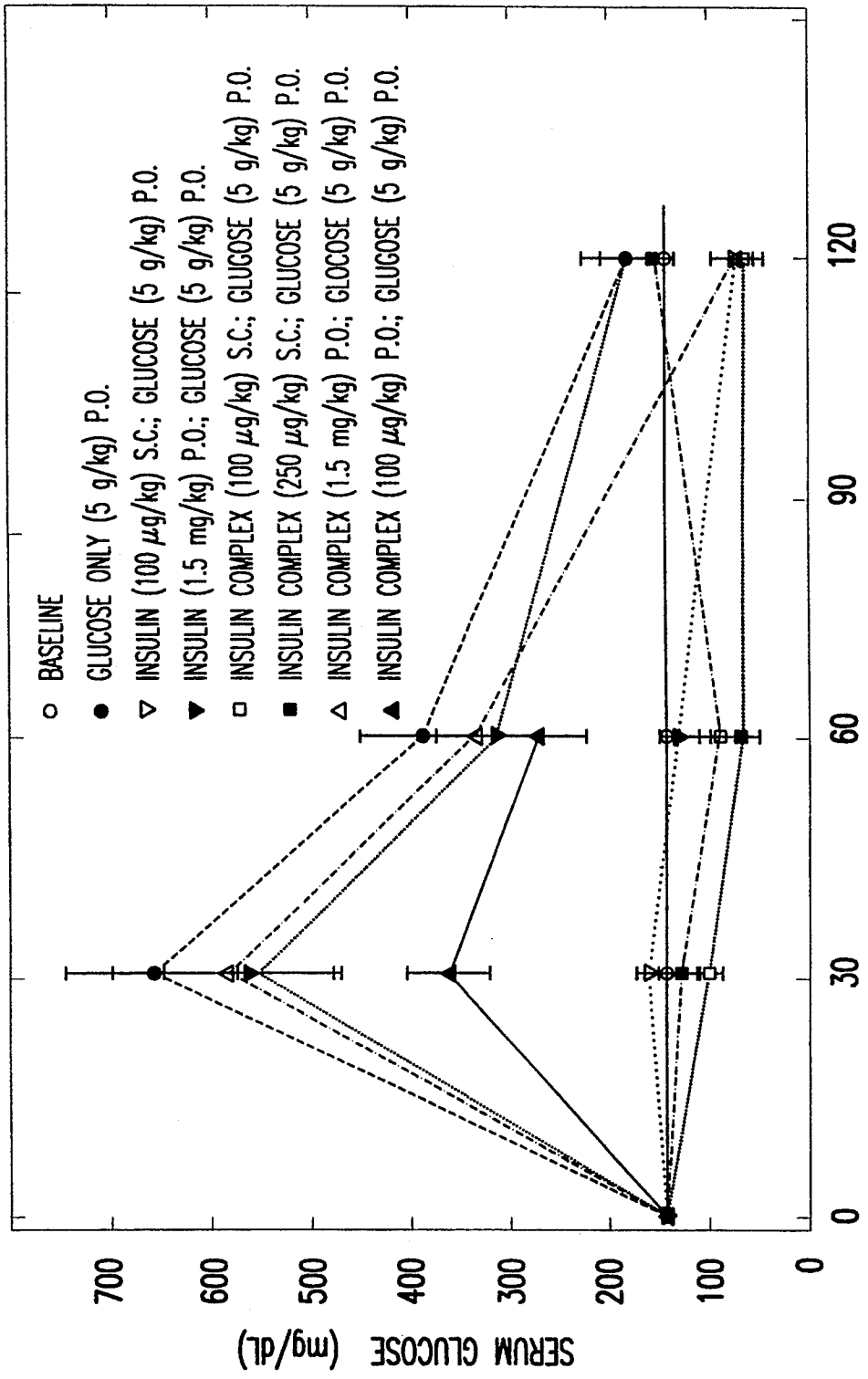

FIG.2

CONJUGATION-STABILIZED POLYPEPTIDE COMPOSITIONS, THERAPEUTIC DELIVERY AND DIAGNOSTIC FORMULATIONS COMPRISING SAME, AND METHOD OF MAKING AND USING THE SAME

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to conjugation-stabilized (poly)peptide and protein compositions and formulations, and to methods of making and using same.

2. Description of the Related Art

The use of polypeptides and proteins for the systemic treatment of certain diseases is now well accepted in medical practice. The role that the peptides play in replacement therapy is so important that many research activities are being directed towards the synthesis of large quantities by recombinant DNA technology. Many of these polypeptides are endogenous molecules which are very potent and specific in eliciting their biological actions.

A major factor limiting the usefulness of these substances for their intended application is that they are easily metabolized by plasma proteases when given parenterally. The oral route of administration of these substances is even more problematic because in addition to proteolysis in the stomach, the high acidity of the stomach destroys them before they reach their intended target tissue. Polypeptides and protein fragments, produced by the action of gastric and pancreatic enzymes, are cleaved by exo and endopeptidases in the intestinal brush border membrane to yield di- and tripeptides, and even if proteolysis by pancreatic enzymes is avoided, polypeptides are subject to degradation by brush border peptidases. Any of the given peptides that survive passage through the stomach are further subjected to metabolism in the intestinal mucosa where a penetration barrier prevents entry into the cells.

In spite of these obstacles, there is substantial evidence in the literature to suggest that nutritional and pharmaceutical proteins are absorbed through the intestinal mucosa. On the other hand, nutritional and drug (poly)peptides are absorbed by specific peptide transporters in the intestinal mucosa cells. These findings indicate that properly formulated (poly)peptides and proteins may be administered by the oral route, with retention of sufficient biological activity for their intended use. If, however, it were possible to modify these peptides so that their physiological activities were maintained totally, or at least to a significant degree, and at the same time stabilize them against proteolytic enzymes and enhance their penetration capability through the intestinal mucosa, then it would be possible to utilize them properly for their intended purpose. The product so obtained would offer advantages in that more efficient absorption would result, with the concomitant ability to use lower doses to elicit the optimum therapeutic effect.

The problems associated with oral or parenteral administration of proteins are well known in the pharmaceutical industry, and various strategies are being used in attempts to solve them. These strategies include incorporation of penetration enhancers, such as the salicylates, lipid-bile salt-mixed micelles, glycerides, and acylcarnitines, but these frequently are found to cause serious local toxicity problems, such as local irritation and toxicity, complete abrasion of the epithelial layer and inflammation of tissue. These problems arise because enhancers are usually coadministered with the peptide product and leakages from the dosage form often occur. Other strategies to improve oral delivery include mixing the peptides with protease inhibitors, such as aprotinin, soybean trypsin inhibitor, and amastatin, in an attempt to limit degradation of the administered therapeutic agent. Unfortunately these protease inhibitors are not selective, and endogenous proteins are also inhibited. This effect is undesirable.

Enhanced penetration of peptides across mucosal membranes has also been pursued by modifying the physicochemical properties of candidate drugs. Results indicate that simply raising lipophilicity is not sufficient to increase paracellular transport. Indeed it has been suggested that cleaving the peptide-water hydrogen bonds is the main energy barrier to overcome in obtaining peptide diffusion across membranes (Conradi, R. A., Hilgers, A. R., Ho, N. F. H., and Burton, P. S., "The influence of peptide structure on transport across Caco-2 cells", *Pharm. Res.*, 8, 1453–1460, (1991)). Protein stabilization has been described by several authors. Abuchowski and Davis ("Soluble polymers-Enzyme adducts", In: *Enzymes as Drugs*, Eds. Holcenberg and Roberts, J. Wiley and Sons, New York, N.Y., (1981)) disclosed various methods of derivatization of enzymes to provide water soluble, non-immunogenic, in vivo stabilized products.

A great deal of work dealing with protein stabilization has been published. Abuchowski and Davis disclose various ways of conjugating enzymes with polymeric materials (Ibid). More specifically, these polymers are dextrans, polyvinyl pyrrolidones, glycopeptides, polyethylene glycol and polyamino acids. The resulting conjugated polypeptides are reported to retain their biological activities and solubility in water for parenteral applications. The same authors, in U.S. Pat. No. 4,179,337, disclose that polyethylene glycol rendered proteins soluble and non-immunogenic when coupled to such proteins. These polymeric materials, however, did not contain fragments suited for intestinal mucosa binding, nor did they contain any moieties that would facilitate or enhance membrane penetration. While these conjugates were water-soluble, they were not intended for oral administration.

Meisner et al., U.S. Pat. No. 4,585,754, teaches that proteins may be stabilized by conjugating them with chondroitin sulfates. Products of this combination are usually polyanionic, very hydrophilic, and lack cell penetration capability. They are usually not intended for oral administration.

Mill et al., U.S. Pat. No. 4,003,792, teaches that certain acidic polysaccharides, such as pectin, algesic acid, hyaluronic acid and carrageenan, can be coupled to proteins to produce both soluble and insoluble products. Such polysaccharides are polyanionic, derived from food plants. They lack cell penetration capability and are usually not intended for oral administration.

In Pharmacological Research Communication 14, 11–120 (1982), Boccu et al. disclosed that polyethylene glycol could be linked to a protein such as superoxide dismutase ("SOD"). The resulting conjugated product showed increased stability against denaturation and enzymatic digestion. The polymers did not contain moieties that are necessary for membrane interaction and thus suffer from the same problems as noted above in that they are not suitable for oral administration.

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