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The Different Biologically Active Forms of Alpha-Fetoprotein as Functional Biomarkers in Pregnancy, Disease and Cancer: A Review and Update

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Abstract

Human alpha-fetoprotein (HAFP) has been known to manifest in multiple structural forms as previously reported in the biomedical literature. Such structural forms of HAFP have included isoforms, epitopes, carbohydrate and pH heterogenous forms, and structural variants. However, within the last decade, studies have reported variant forms of HAFP not necessarily of a structural nature as reported in the literature. These newer forms of HAFP are largely functional forms in contrast to structural variants. Such functional forms are a result of slightly denatured intermediates (molten globule forms), conformational variants, misfolded proteins, conformationally transformed HAFP molecules, non-secreted forms, and synthetic peptide segments/sequences derived from the HAFP polypeptide. These latter bioactive functional forms of HAFP have been discussed in the present report. Such recently described forms of the oncofetal protein are deemed of importance since they can be utilized as functional bioactive markers during pregnancy, perinatal, perineurium and juvenile stages, in addition to benign and/or malignant tumor growths in adults.

Keywords: alpha-fetoprotein pregnancy; tumor AFP; denatured forms; cell cycle; conformational variants; misfolded proteins; cancer; intermediate forms

Introduction

Alpha-fetoprotein (AFP) is a tumor-associated fetal protein (an oncofetal protein) found in mammals including primates such as man, chimps, monkeys, and Gorillas. Other mammals that synthesize AFP include dogs, cats, mice, rats, cattle, and horses (see PubMed, protein bank). Moreover, vertebrate classes such as sharks, bony fishes, amphibians, reptiles, and birds also display an AFP-like glycosylated protein (not AFP) similar to mammalian alpha-albumin (1). Furthermore, AFP in mammals belong to an albuminoid gene family consisting of five members; albumin, AFP, alpha-albumin, Vitamin D binding protein, and the ARG-protein [2, 3]. Human AFP (HAFP) consists of a single chain polypeptide containing 610 amino acids including an amino-terminal signal sequence. HAFP contains a 3-5% glycosylated carbohydrate grouping within the 69kD glycoprotein [4]. A 3-dimensional rendering of the AFP molecular

structure exhibits a 3-domain protein configured by intramolecular loops dictated by disulfide bridging; this results in helical V or U-shaped structures as displayed by Luft et al [5]. The data in Table-1 shows the previous forms of AFP which have been published to date. Then forms of AFP in Table 1 have been previously published in a report as genetic variants, isoforms, antigenic epitopes, enzymatic fragments, heterogenous forms, and short cell adhesion amino acid sequences [2]. However, other forms of AFP, not related to molecular structure have emerged in recent literature; such forms can be listed as: 1) denatured intermediate forms; 2) misfolded forms; 3) conformationally altered forms; 4) non-secreted (cytoplasmic) AFP; 5) conformationally-altered transformed AFP; 6) small ligand-bound tumor AFP; and 7) peptide segments as amino acid sequences derived from AFP (Table-2).

Table 1: Alpha-fetoprotein (AFP) Structure and Function: Relevance to Isoforms, Epitopes, and Micro heterogenic and Conformational Variants.

Alpha-fetoprotein Forms	Biological Roles, Activities
1). Genetic Variants* (mRNA Variants)	Fetal Liver: 2.2 kb, 1.7 kb to 50 to 65 kD Protein Neonate Liver: 1.5 kb to 48 kD Protein. Adult Liver: 1.37 kb to 37 kD Protein Hepatoma Liver: 1.35 kb to 37 kD Protein
2). Soluble Forms A. Free Forms B. Bound Forms	Candidate proteins bound to AFP could include IgG, IgM, actin, TGF- β , and protease inhibitors released by treatment with 0.4 molar KCL
3). Enzymatic Fragments	Apoptotic segments displayed 4 major AFP fragments of 38, 32, 23, and 26 kD (after hydrolis) from Domains 1 and 3 and from Raji lymphocyte cell cultures
4) Molecular heterogeneity A). Carbohydrate Forms B). pH isoforms	A) AFP contains carbohydrate moieties in one-to-three N-linked glycans sites which bind lectins (sugar heterogeneity). AFP can be also separated into 2 forms by pH isoelectric focusing chromatography.
5) Antigenic Variants (epitopes)	AFP contains 6 major antigenic determinant sites when used to raise antibodies. A major epitopic site could span 10,000 Daltons. Minor epitopic sites number up to 12 epitopes for an average antibody production.
6). Cellular adhesion sequences (3-6 amino acids).	Short AFP peptides segment of 3-6 Amino acids detected on AFP match to sequences of Laminin-S, fibronectin, Collagen I and V, Laminin A, and Lamin B1.

*Indicates mRNA data from both human and rodent AFP.

*For each of the subject topics 1-6 listed in Table-1, please refer to the #2 reference included in the previously published paper "Alpha-fetoprotein Structure and Function: Relevance to Isoforms, Epitopes, and Conformational Variants", Exp. Biol. Med, Volume 226, Issue-5, pages 377-408, 2001.

Table 2: Alpha-fetoprotein (AFP) Functional Forms and Variants.

Alpha-fetoprotein forms	Biological Roles, Activities	References Cited
1). Denatured Intermediate Forms	A secondary structure form lacking a rigid tertiary structure is referred to as a "molten globule (MGF) of the protein; the MGF is considered a slightly denatured protein state	6, 7, 8, 9
2). Conformational Variants	Forms of AFP that have undergone a conformational change in their tertiary structure exposing hidden occult sites.	2, 7, 8, 10, 11, 12
3). Misfolded Protein Forms	An unfolded form of AFP due to faulty chaperoning by HSP 70, HSP 90, and Bip molecules during periods of heat shock, oxidative stress; ischemia, and anoxia.	15-19 20-23, 25, 26
4). Transformed alpha-fetoprotein (Tr-AFP)	A form of intact AFP which has undergone a conformational unfolding to expose a growth inhibitory peptide, segment that suppresses fetal growth during pregnancy.	10, 29, 30, 31, 33, 36-38
5). Non-secreted alpha-fetoprotein (CyAFP) cytoplasmic form	A form of AFP derived from a 37 kD AFP retained in the cell as a non-secreted form. It is thought to dimerize with truncated steroid receptors and transcription factors.	39, 40-44, 45-47, 50, 51, 53
6). Liganded tumor AFP (T-AFP) which influences tumor immunity	A form of tumor-secreted AFP bound to a low molecular weight ligand such as a fatty acid component	55-57, 58-61
7). Synthetic AFP peptide segments: A). GIPA B). GIPB C). GIPC D). GIP-34 E) LDSYQCT	Peptide fragmented/segments isolated from AFP includes a portion from AFP 14-20, namely LDSYQCT involving sugar transport. Also, a segment termed Growth Inhibitory peptide (GIP) consisting of 3 internal parts, GIP-A, B, C which regulates growth and inhibits cancer growth via cell cycle phase arrest and redox control).	4, 13, 14, 51 68-70 72-74 75-81

The objectives of the present review and commentary are several-fold in nature. First, this treatise is meant

to alert and familiarize the readership concerning the several different functional forms in which AFP may

be present. In contrast to functional forms, several structural variants have previously been reviewed by the author in an earlier publication [2], summarized in Table 1. Secondly, more recent publications have revealed new functional forms of AFP not previously reported because of their more recent appearance in the literature. Thirdly, these recently published forms of AFP are under discussion in the present report concerning their non-structural, liganded, and conformational changes which can provide new insights into their proposed functional significance.

Denatured Intermediate Forms of AFP

The first functional biomarker type of AFP to be discussed is a slightly denatured intermediate form of a protein, known as a “molten globule form” (MGF) first described for AFP by Uversky [6-8]. The MGF of a protein was originally described for proteins in the presence of a lowered pH state under isoelectric conditions. The characteristics of such protein forms can be addressed as follows. First, proteins in the MGF are present in a highly flexible form; second, they reside largely as non-secreted forms in the cytoplasm; third, they can undergo rapidly-induced translocation between the cell cytoplasm and the intercellular spaces; fourth, they can easily traverse bilipid membranes; fifth, they engage in chaperone binding, recognition, and transport; and lastly, they undergo rapid modification and degradations in liposomes and by ubiquitin systems [7-9]. Overall, MGFs are particularly susceptible to exposure to high ligand concentrations which cause the proteins (i.e., AFP) to undergo conformation changes which transition to the MGF; [6] these changes can result in exposure of hidden and occult sites of molecular crevices produced by the initial tertiary folding of the protein within the cell (See Below).

Conformational Variants

Proteins are synthesized in the endoplasmic reticulum (ER) and therein undergo molecular changes into tertiary-folded proteins prior to their exocytotic transport through the Golgi network via exit into the vascular circulation. The protein chaperones engaged in the protein folding process include the heat-shock proteins such as; 1) HSP-90; 2) HSP-60, and; 3) BiP (immunoglobulin binding protein), while the co-chaperones encompass: A) calnexin, B) calreticulin, C) protein disulphide isomerase, and D) peptidyl prolyl isomerase [2]. As a result, the protein acquires

a tertiary fold which streamlines the shape of the protein for travel through the circulatory system. The folding process also assures that the hydrophilic amino acids remain on the outer protein surfaces in order to maintain and increase the solubility of the protein in serum; by the same token, the hydrophobic amino acids are tucked into the inner molecular surfaces of the folded protein [7, 8, 9]. Thus, a conformational change in a tertiary-folded protein can reveal and expose multiple hydrophobic and amphipathic amino acids segments that were previously concealed in inner sites prior to the conformational change. One such AFP example is discussed below (see the transformed AFP in pregnancy Section) [10, 11]. Micro-environmental changes among cells and tissues can induce stress in the various biological systems of the mammalian body's intra-and extracellular mileaux. Such stress conditions causing conformational changes in proteins can include many different physical forces as follows. These stresses could include: oxidative, osmotic, ionic, temperature, pH changes, and high concentrations of fatty acids, steroids, and growth factors [11, 12]. These micro-environmental stresses are known to occur in fetal, newborn, juvenile, and even in adult stages during the overall ontogeny of mammals. A well-known published example of an exposed (occult) peptide segment site on AFP is the “growth inhibitory peptide” (GIP) sequences residing on the AFP third domain [4, 13, 14] (see below Section VIII). The conformational change that exposes the GIP site that temporarily converts the normally growth promoting full-length AFP molecule into a growth inhibitor protein referred to as “transformed AFP” (see below). This transformed version has been studied and described under clinical pregnancy settings [36-38].

Misfolded Proteins, Fetal Defects, and AFP

Evidence has accumulated to the effect that defective and/or altered protein folding in the ER may form a possible basis (via mutations and/or modifications) for many human sicknesses and birth defects [21, 14]. The misfolding of proteins have led to debilitating diseases and disorders such as Cystic Fibrosis, Marfan's syndrome, Tay-Sachs disease, Sick cell anemia, Scrapie's disease, Alzheimer's, and other disorders [22]. Misfolding of proteins has been attributed to many factors such as the individual amino acid sequences, chaperonins, clathrin-coated membrane, events, and enzymes that catalyze the

folding [15-19]. As discussed above, the chaperonins (HSP70, HSP60) serve to mask hydrophobic unstructured regions of the newly synthesized proteins in order to prevent aggregation of the partially-folded chains [20-22]. Improper folding, assembly, and localization of nascent proteins can cause proteins to accumulate abnormally in extracellular regions in and around cells. It has been proposed that congenital disorders that exhibit altered or decreased concentrations of HAFP could contribute to chromosomal trisomies such as observed in Down syndrome and trisomy-13 [23,24]. These trisomy disorders are characterized by low AFP concentrations in both maternal/fetal serum and amniotic fluid. Such defects could be attributed to low AFP hepatic synthesis or in conformational alterations of the secreted AFP protein that could elude serological detection [23-26]. It remains plausible that such fetal defects might be associated with misfolding and/or mis-assembly of the fetal protein following either hepatic synthesis in or during trans passage from the fetal to the maternal circulation via the placental membranes [27, 28, 29]. Present data seem to favor the conformational alterations of AFP resulting from either impaired fetal kidney processing (pyelectasis) or altered transmembrane passage into the maternal bloodstream [11, 12, 30].

Transformed AFP (TrAFP)

As discussed above, HAFP has been shown to display multiple molecular forms, complexes, and variants. Recently, such AFP interacting forms were reported to demonstrate binding interactions with: A) cell surface receptors; B) intracytoplasmic binding agents; and C) inter-molecular complexing proteins such as IgG [29]. Moreover, the native serum circulating AFP has long been employed in the clinic as a “gold-standard” biomarker [32]. Such disease biomarkers include hepatocellular carcinomas and germ cell malignancies in addition to pregnancy markers for neural tube defects, anencephaly, Down Syndrome, and chromosomal abnormalities [30-32]. A slightly denatured intermediate form of AFP has recently been reported in the biomedical literature as a pregnancy variant (10). This non-structurally altered functional form of AFP termed “transformed AFP” (TrAFP) has been identified and measured in maternal and fetal serum from patients in clinical pregnancy studies [10]. TrAFP has been confirmed to be a transient (temporary) unfolded version of the

compacted tertiary structured full-length AFP polypeptide. The TrAFP form is produced as a result of a conformational change in the native folded AFP molecule following exposure to various developmental environmental stresses in the fetal milieu. Such stresses have been discussed in the above section (see conformational variants) and are known to occur in the fetal and maternal serum as well as at the fetal/maternal placental interface [11, 12]. The conformational change in the full-length AFP molecule exposes a peptide sequence of 34 amino acids that is known to serve as a growth inhibitory segment [4, 13, 14]. This inhibitory activity stands in dire contrast to the known function of intact HAFP as an overall growth enhancing agent. Since its publication in the 2007 to 2009 era, TrAFP has been utilized as a clinical biomarker for predicting third trimester adverse outcomes and perinatal deterioration as reported in previously published studies (36-38). Examples of such predictive adverse perinatal conditions included: a) intrauterine growth retardation; b) fetal growth restriction; c) threatened pre-term labor; d) fetal hemodynamic redistribution, e) fetal distress, and f) fetal chronic hypoxic stress [36-38].

Cytoplasmic Alpha-fetoprotein (CyAFP)

A non-secreted form of AFP, termed cytoplasmic (CyAFP), has been described in another previously published report [39]. The CyAFP represents a cytoplasmic-bound form of AFP that remains in the cell and displays cross-talk signaling intracellular functions. The CyAFP appears to be a truncated AFP molecular form of 37 kD produced as a mRNA translated protein in both adult normal liver and in Hepatomas. Thus, the synthesis of CyAFP without secretion has been shown to occur in both malignant and non-malignant cells encompassing hepatomas, hepatic ascites fluid, immature rodent uterine cells, MCF-7 human cultured breast cancer cells, and biopsied cytosols from human breast cancer patients (40-44). In regard to functions, the CyAFP partakes in two physiological activities, namely; 1) CyAFP may function to serve as a means for gating and regulating the nuclear membrane pores transpassage preventing the inappropriate entry of nuclear receptors and transcription factors into the nucleus; and 2) CyAFP may function as a protein-to-protein interaction factor by dimerizing with nuclear receptors and transcription factor in order to regulate and mitigate the flow and passage of such factors into the

nucleoplasm [39]. Such functions of AFP could directly or indirectly affect signal transduction, interaction, and transpassage of nuclear receptors and factors into the nucleus. Thus, CyAFP appears to have the capability to form molecular hetero-complexes by dimerization with cytoplasmic stored steroid receptors and transcription factors [45-47]. AFP has been shown to display a dimerization region in the third domain of the AFP molecule which serves as a receptor binding motif for co-localization and interacting of AFP with transcription-associated factors [45, 46]. Such proteins modulate intercellular signaling and cross-talk interaction leading to regulation of transcription factors for initiation of growth in cancer cells. This observation is in keeping with AFP as a known factor in growth enhancement during fetal differentiation and growth progression [48, 49]. CyAFP also has a critical role in ontogenetic growth and metastasis of adult liver cancer. The presence of a leucine zipper-based heptad dimerization motif on the carboxy terminal third domain of AFP has been described in a prior publication using computer modeling [45]. Such “in silico” modeling described the presence of a pattern of hydrophobic 8-amino acid repeats which resembled the dimerization heptads known to exist in both nuclear hormone receptors Group-I and nuclear thyroid receptor Group-II superfamilies [45, 46]. The Group I receptors encompass the steroid receptors for estrogen, androgen, glucocorticoid, progesterone, and Vitamin-D. The Group-II family include the nuclear receptors for retinoic acid receptor (RAR), RXR thyroid receptor, T3R thyroid receptor, and the c-erbA receptors [50]. Thus, the above data suggests that CyAFP has a role in intracellular signaling and regulation. Previous reports demonstrated that CyAFP concentrations correlated with levels of a metastasis-related protein termed CXCR4, a chemokine receptor found in hepatoma tissues [51]. Not only did CyAFP serve as a modulator of CXCR4, it also influenced AKT/mTOR signaling in the cell cycle, in various growth activities, and with apoptosis involvement. These same investigations reported that CyAFP interaction with CXCR4 expression interacted with PTEN to activate AKT phosphorylation of additional CXCR4 receptors [50, 52]. Thus, CyAFP harbors a function to activate CXCR4 migration and metastasis of hepatoma cells. As a further example, CyAFP can promote the malignant transformation of liver cells to malignant cells via the hepatitis-B virus (HBV)-X protein (HBX)

system [53]. Finally, CyAFP is associated with a down-regulation of GADD153 via binding to the RAR receptor and interference with its nuclear trans passage resulting in unregulated GADD153 expression [53-54]. In summation, the above studies document that CyAFP interacts with genes and gene products that affect signal transduction pathways between cytoplasmic factors and the nucleus.

Tumor-associated Alpha-fetoprotein (TAFP)

Ligand-free AFP has often been associated with naturally-occurring (normal) AFP and has been designated as NAFP. Usually, NAFP is derived from cord serum obtained by blood drawn from the umbilical cord in pregnancy. In comparison, AFP derived and purified from hepatoma tumor tissue has been termed “TAFP”. Recent and past studies have demonstrated that AFP is heavily involved in immune activities that could play a role in hepatoma immunotherapy [55, 56, 57]. Such AFP inaction with immune-associated cells include; T-cell, dendritic cells, CD4+ helper cells, CD8+, natural killer cells, and CD1- bearing monocytes [58-61]. Recent studies have shown that TAFP functions in a manner different than NAFP in reports forwarded by Butterfield and her associates [62]. The studies by Butterfield et al demonstrated that tumor-derived AFP (TAFP) could impair the differentiation and stimulating activity of T-cells and the immune responses of human dendritic cells (62, 63). Further studies showed that blood-derived monocytes from healthy donors, cultured in the presence of hepatoma-derived AFP (TAFP), but not NAFP, significantly inhibited T-cell proliferative responses [64]. Moreover, tumor-derived AFP could directly drive human natural killer-cell activation and subsequent cytotoxic cell death [63]. In addition, hepatoma-derived AFP uptake was found to reduce CD1 molecules on the surface of monocyte derived dendritic cells [65]. As a possible negative feedback response, tumor-derived AFP was further found capable of suppressing fatty acid metabolism and oxidative phosphorylation in dendritic cells [66]. In all the above situations, the tumor suppressive and activation processes were solely dependent on the presence of TAFP, but not NAFP. It was subsequently determined that TAFP had co-purified (chaperoned) with a low-molecular weight (LMW) ligand or entity transported as a factor bound to AFP [65]. This LMW ligand bound to AFP was found to influence and contribute to the impairment of dendritic cell

differentiation and function and was a driving force for killer cell activities and tumor cell death [64]. The LMW ligands has yet to be fully identified, but a fatty acid-like substance or lysophospholipid factor are possible candidates [66, 67, 26].

A Transformed Exposed Sequence on AFP; The Growth Inhibitory Peptide (GIP) Segment

The “Growth Inhibitory Peptide” (GIP), which is exposed by a conformational change in the full-length AFP molecular, has been isolated and synthesized as a 34-mer peptide and can be manufactured as such. Thus, the peptide- GIP has been synthesized from full-length AFP and purified as a stand -alone peptide which has been characterized, bioassayed, and studied physiologically in both in vitro and in vivo studies [4, 13, 14]. Hence, the 34-mer GIP has been studied independently of the full-length AFP molecule and has been further studied as three independent fragments, namely, a 12 amino terminal segment (GIP-A), a 14 amino acid mid-piece, (GIP-B) and an 8-amino acid carboxy-terminal fragment (GIP-C) (13) (see Table-2). The biological activities of the 34-mer GIP have been studied in multiple vertebrate animal classes ranging from sea forms, amphibians, mammals, and primates including man. Overall, the biological activities of GIP have been described in brineshrimp, frogs, chick embryos, mice/rats and man, all of which have one feature in common, i.e., the property of growth regulation (14). However, the GIP segment, exposed on the full-length AFP, functions just the opposite of full-length AFP, that is, growth inhibition. In summary, AFP can both up-regulate and down-regulate growth by means of the full-length AFP and the exposed GIP fragment, depending on the immediate environmental conditions surrounding the AFP molecule (see section on Conformational Variants). The summary of functions engaged by the 34-mer GIP are multiple in number. The GIP segment is involved in the following developmental activities: 1) Immature rodent uterine growth [69]; 2) tail growth in amphibian metamorphosis [70]; 3) shedding of egg envelopes in brine shrimp [14]; 4) protection from insulin and estrogen toxicity in pregnancy [71]; 5) inhibition of blood vessel angiogenesis in tumors [14]; and 6) prevention of hyperestrinisin in pregnant mice [71]. In studies of human cancer growth, GIP was capable of: A) inhibiting estrogen-dependent and independent breast cancer growth [72, 73]; B)

blocking growth of tamoxifen-resistant breast cancer cells [74]; C) suppression of cell-to-cell contact inhibition in breast cancer cells [69]; D) inhibition of cancer growth in 38 of 60 different cultured cell lines including breast, prostate, ovarian, central nervous system cancers, melanoma, kidney, lung, and colon [74]; E) growth suppression in multiple breast cancer cell lines including MCF-7, T47D, Bt-547, and sarcoma mammary isografts in the mouse 6WI-1 using in vivo hollow fiber cancer assays, and F) inhibition of platelet aggregation [13, 14]. The remarkable aspect of all in vivo assays was the total lack of any GIP-induced harmful and/or toxic side effects. This is because GIP is not a cytotoxic agent, but rather a cytostatic agent causing only growth inhibition [13]. Finally, as a cell surface membrane disrupter, GIP has been demonstrated to inhibit and suppress cell spreading, migration, cell-to-cell contact, cell-to-extracellular matrix interaction and cancer metastasis in animal models [14]. The mechanism of action of the growth inhibition and cell membrane disruption of GIP-34 has been discovered and is well understood. Overall, the growth suppression of GIP-34 involves interference with the cell growth cycle, multiple cells signaling transduction cascades, and protein-to-protein cross-talk interactions [68]. Blockage of the cell growth cycle results in: 1) cell cycle “G1-to-S phase” arrest; 2) prevention of p27 and p21 cyclin inhibition via ubiquitin degradation; 3) protection of p53 from inactivation of phosphorylation; and 4) blockage of K⁺ ion channels and transient receptor potential channels by estrogen and epidermal growth factors [68, 69]. Additionally, acting as a chemotherapeutic adjunct agent, GIP is capable of affecting tamoxifen resistance, uterine hyperplasia, blood clotting, Herceptin antibody resistance, radio-resistance in cells, cardiac arrhythmias, and doxorubicin bystander cell toxicity [4]. Finally, GIP-34 could further serve as a cancer preventative and therapeutic agent by: acting as a decoy ligand for the CXCR4 chemokines receptor for cancer metastasis; mimicry of disintegrins by inhibiting cancer cell growth, migration, and angiogenesis, [69]. 3) blocking circulating tumor cells from initiating the metastatic process [73, 74]; 4) disabling cell-to-stromal cell communication [77]; 5) inhibiting cytoskeletal factor activities required for cell migration [76]; and 6) acting as a anti-microbial peptide for cell entry, drug delivery, and pore /channel formation [80-84].

Conclusion

It is obvious from the above discussion that AFP can assume non-structural changes in its protein referred to as conformational changes. The change in shape of a secreted tertiary-folded protein can expose hidden occult sites or epitopes which were previously concealed. One such hidden peptide site (GIP) has presently been demonstrated to possess multiple biological activities of its own as an isolated peptide, opposite to that of the full-length AFP protein. It is the latter conformational transformations of AFP discussed in the present report that distinguished it from those of previous structural AFP publications. In nature, the tertiary-folded AFP can readily be observed and measured in the bloodstream; however, after the protein leaves the vascular system and travels into the surrounding tissues and intercellular fluid compartments, it is subject to conformational change. As discussed above, proteins can assume a “molten globular form” when engaged in transpassage across bilipid cell membranes (6-8). The blood pH of 7.2 is frequently different in the intracellular fluids and can also differ in the cytoplasm of cells. Furthermore, lowering the environmental cellular pH to 3.1 has been shown to convert AFP into a molten globule form which allows for easier transmembrane passage into the cytoplasm of cells (6, 7).

It has been the purpose of the present report to introduce the reader to the various bioactive versions of which the AFP molecule may exist. These forms are not based solely on molecular structure, but on induced conformational changes and variations due to the micro-environment in which the AFP molecule finds itself. The multiple forms of AFP presented in the present report provides evidence that such forms of AFP do indeed exist and can be detected and measured; such forms could possibly be employed as functional biomarkers in future studies.

Declarations

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