Tumor Res., 8, 7-10, 1973

Some biochemical properties of human α-1 fetoprotein

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This morning, Professor Gitlin has reviewed his classical work on the ontogeny and normal biology of alpha-fetoprotein (AFP)[1]. I have been requested by our distinguished Chairmen to review some of the biochemical and immunochemical aspects of alpha-fetoprotein which define this carcinoembryonic protein. I think it will become apparent that the newer analytic tools have shed much light on the basic structure of this protein, but that there are still many questions to be resolved.

Until 1970, AFP was operationally defined immunologically and electrophoretically as a unique antigen. It was defined as an antigen recognized by heterologous antisera in fetal and hepatoma sera, but not in normal sera. Ever since Abelev's original and classical discovery in 1963[2], many workers have confirmed the immunologic identity of all AFP's identified in fetal or hepatoma sera within a given species, including man. The antigen is synthesized by fetal liver and gut cells and secreted into serum and secretions. The protein was also defined electrophoretically, having a characteristic post-albumin or rapid $alpha_1$ mobility on electrophoresis in alkaline pH. The protein was known to have a molecular weight of approximately 70,000 and no carbohydrate was detectable histochemically. AFP was shown to be a single homogeneous protein by electrophoresis in a variety of supporting media by gel filtration and by ultra centrifugation. Since 1970, AFP has been purified and further characterized by a number of laboratories and I would like to review and compare some of this data.

Professor Gitlin was the first to obtain an approximate molecular weight of about 70,000 by gel filtration[1]. However, other workers have reported molecular weights varying from 61,000 to 76,000[3~7]. It is important to remember that the apparent molecular weight of a protein can differ depending on the method used, because of the effect of molecular shape, degree of hydrophobicity, and many other factors. We have determined an apparent molecular weight of 72,000, by the molecular sieving effect of SDS polyacrylamide gel electrophoresis[6]. This method has a higher degree of resolution than previous methods and seems more preferable for analytical comparisons. Despite the variability, it is clear that AFP is a slightly larger molecule than human serum albumin.

Structurally, the protein appears to be a single-stranded polypeptide chain. We have found no evidence of sub-unit structure or dissociable polypeptides by denaturation with SDS, urea, or β mercaptoethanol. We have found no evidence of polymeric forms or aggregation, as reported by others, either by SDS gel electrophoresis or gel filtration chromatography.

There is general agreement that the molecular size of fetoprotein derived from fetal or hepatoma sources is indistinguishable. Two laboratories have analyzed the amino acid content of AFP derived from fetal and hepatoma sources and found no significant difference[3,5]. Ruoslahti *et al.* have confirmed the identity of fetal and hepatoma AFP, by tryptic digestion and peptide mapping[5]. However, there seems to be significant differences in the amino acid composition that have been reported. In addition, the sedimentation coefficient has been reported to be between 4.5 and 5.0 Svedberg units by three different laboratories[1,3,7]. I believe a more precise estimation of these physical and chemical parameters is still needed for analytic comparisons with other fetoproteins and other studies.

AFP was initially thought to be free of carbohydrate due to the lack of PAS stainability, and lack of effect of neuraminidase on the electrophoretic mobility of fetoprotein. However, Nishi and Ruoslahti chemically measured 3 and 4.3% of the weight of their purified AFP preparations to be carbohydrate[3,5]. The exact amount of carbohydrate will be difficult to determine in view of the possible contamination with glucose derivatives from the Sephadex columns employed in the purification. Sialic acid was detected in trace amounts by Ruoslahti.

In 1969, in the course of our isolation and characterization of human fetoprotein, we attempted to define the isoelectric point (pI) of AFP by isoelectric focusing. We obtained a pI of 4.78 in a number of experiments, focusing in narrow range ampholytes, using polyacrylamide as a supporting medium. Other workers found a pI ranging from 4.75 to 5.08[5,7]. The pI measured after elution of a liquid solution probably is less accurate than analytic gel isofocusing due to unavoidable mixing during column elution. Acrylamide gels can be sliced, eluted with water and the pH of each slice measured directly. AFP was detected in the gel slice eluants by means of electroimmunodiffusion. However, resolution even by this method is limited by the size of the gel slices. When we placed a similar gel into an antibody impregnated agarose gel, after focusing to equilibrium, and performed crossed antibody electrophoresis, *two* immuno-precipitin peaks were unexpectedly defined.

The 2 fetoprotein peaks were found to be separable by preparative isoelectric focusing in a liquid medium stabilized by a sucrose density

gradient. The major peak was isoelectric at 4.8 and a second, smaller peak was eluted at approximately pH 5.2. The two major peaks were refocused separately, demonstrating that the heterogeneity was not due to a reversible, pH dependent, conformational change as has been reported for human albumin. The isolated two major molecular forms of AFP were also subjected to extended agarose electrophoresis, and crossed antibody electroimmunodiffusion. The two major peaks remained stable showing no interconversion[8].

This microheterogeneity of AFP, independently observed by Purvis, was confirmed by means of analytic isoelectric focusing in polyacrylamide, preparative isoelectric focusing in sucrose density gradients, ion-exchange chromatography, as well as by extended agarose electrophoresis and crossed antibody electroimmunodiffusion[8,9]. This charge heterogeneity is probably the reason a number of laboratories have been unable to isolate fetoprotein by means of ion-exchange chromatography. Virtually all fetal and hepatoma sera tested contained the two peaks. The quantitative amounts appeared stable in each patient but varied between different patients. When fetoprotein was treated with active neuraminidase, and reanalyzed by extended agarose electrophoresis and crossed antibody electroimmunodiffusion, a technique with far more resolving power than immunoelectrophoresis, a definite alteration in eletrophoretic mobility was demonstrated. A single, homogeneous peak with a slower mobility was demonstrated. This observation of 2 molecular forms alterable by sialidase appears quite consistent with the finding by Ruoslahti, who independently calculated the presence in AFP of two moles of sialic acid per mole of protein. The *mono*-sialic and *di*-sialic form of fetoprotein which circulate in the serum may have different metabolic fates, functions or different half lives. The presence of sialic acid in other serum glycoproteins has been shown to affect cell uptake and metabolic degradation. The two forms appear to be qualitatively and quantitatively identical by immunoprecipitation. Antisera made against isolated *di*-sialic AFP quantitatively precipitates both the *mono* and *di*-sialic forms, as well as the native protein. However, it is not known whether sialic acid affects the ability of the protein to compete with labeled fetoprotein in classical radioimmunoassays. Fetoprotein has been purified by a number of different methods in different laboratories, and is being widely used in radioimmunoassays. It is clear that standardization of reagents will be necessary for this clinically valuable cancer diagnostic immunoassay.

Although synthesis of proteins can be regulated at many levels and by many factors, the mechanism of reappearance of AFP has been assumed to be due to de-repression of a fetal gene. In order to test this hypothesis,

the gene product must be chemically identified with a *unique* primary structure, or a regulatory gene product identified. The small amount of AFP immunoreactivity detected by radioimmunoassay in normal sera, not yet completely identified as the *identical* gene product, suggests only a partial repression of AFP synthesis with maturation. The biologic function or effect of this material in normal adults is unknown. It seems clear that more knowledge about the structure, metabolism, biologic function, and mechanism of "de-repression" of AFP synthesis in malignancy may shed new light on the problem of human cancer.

REFERENCES

1. GITLIN, D. and BOESMAN, M.: Serum alpha-fetoprotein, albumin and globulin in the human conceptus. *J. Clin. Invest.*, **45**, 1826 (1966).
2. ABELEV, G. I., PEROVA, S. D., KHRAMKOVA, N. I., POSTNIKOVA, Z. A. and IRLIN, I. S.: Production of embryonal alpha-globulin by the transplantable mouse hepatomas. *Transplant. Bull.* **1**, 174 (1963).
3. NISHI, S.: Isolation and characterization of a human fetal α-globulin from the sera of fetuses and a hepatoma patient. *Cancer Res.*, **30**, 2507 (1970).
4. ALPERT, E., DRYSDALE, J. W., SCHUR, P. H. and ISSELBACHER, K. J.: Human alpha 1-fetoprotein: Purification and physical properties. *Fed. Proc.*, **30**, 246 (1971).
5. RUOSLAHTI, E. and SEPPÄLÄ, M.: Studies of carcino-fetal proteins: Physical and chemical properties of human α-fetoprotein. *Int. J. Cancer*, **7**, 218 (1971).
6. ADINOLFI, A., ADINOLFI, M. and COHEN, S.: Isolation and characterization of human foetal α-globulin (α_1F) from foetal and hepatoma sera. *Biochim. Biophys. Acta*, **251**, 197 (1971).
7. MASOPUST, J., TOMASOVA, H. and KOTAL, L.: Some physicochemical characteristics of human α_1-fetoprotein. *In*: Protides of Biological Fluids (ed. H. Peeters) Pergamon Press, 37 (1971).
8. ALPERT, E., DRYSDALE, J. W., ISSELBACHER, K. J. and SCHUR, P. H.: Human α-fetoprotein: Isolation, characterization, and demonstration of microheterogeneity. *J. Biol. Chem.*, **247**, 3792 (1972).
9. ALPERT, E., DRYSDALE, J. W. and ISSELBACHER, K. J.: Isoelectric focusing of human α-fetoprotein: An aid in purification and characterization of microheterogeneity. *Ann. N. Y. Acad. Sci.*, **209**, 387 (1973).