1995

NASA/ASEE SUMMER FACULTY FELLOWSHIP

MARSHALL SPACE FLIGHT CENTER
THE UNIVERSITY OF ALABAMA IN HUNTSVILLE

PROTEIN CRYSTALLIZATION STUDIES

Prepared By:	James Evans Lyne, M.D., Ph.D.
Academic Rank:	Assistant Professor
Institution and Department:	The University of Tennessee Mechanical and Aerospace Engineering

NASA/MSFC:

Division:	Microgravity Science and Applications
Branch:	Biophysics and Advanced Materials
MSFC Colleague:	Daniel C. Carter, Ph.D.

1995 NASA/ASEE SUMMER FACULTY FELLOWSHIP PROGRAM

PROTEIN CRYSTALLIZATION STUDIES

JAMES EVANS LYNE

The Structural Biology laboratory at NASA Marshall Spaceflight Center uses x-ray crystallographic techniques to conduct research into the three-dimensional structure of a wide variety of proteins. A major effort in the laboratory involves an ongoing study of human serum albumin (the principal protein in human plasma) and its interaction with various endogenous substances and pharmaceutical agents. Another focus is on antigenic and functional proteins from several pathogenic organisms including the human immunodeficiency virus (HIV) and the widespread parasitic genus, *Schistosoma*. My efforts this summer have been twofold: first, to identify clinically significant drug interactions involving albumin binding displacement and to initiate studies of the three-dimensional structure of albumin complexed with these agents, and secondly, to establish collaborative efforts to extend the lab's work on human pathogens.

ALBUMIN STUDIES

Human serum albumin (HSA) is the most abundant of the plasma proteins with an average concentration in healthy individuals of 5 gm/dl. It is important in maintaining the colloid oncotic pressure and in binding an enormous variety of endogenous and exogenous ligands. Binding to HSA greatly increases the solubility of fatty acids and bilirubin in the plasma. Albumin also binds amino acids, steroids, and several metals and is implicated in the facilitated transfer of many ligands across organ-circulatory interfaces. By complexing with various pharmaceutical agents, albumin can strongly influence their therapeutic effect. In some cases, this may render agents inactive *in vivo* despite high efficacy in the absence of albumin *in vitro*.

Drugs displaced from albumin in clinically important interactions tend to have a small volume of distribution, a low therapeutic index, and undergo restrictive elimination.[10] Compounds which are present in high concentrations and are tightly protein bound such as non-steroidals, valproic acid, and sulfamethoxazole are particularly capable of displacing other agents and must be used with caution when they are given concomitantly.[14]

Efforts have begun to determine the three dimensional structure of albumin complexed with various compounds which are involved in clinically significant displacement interactions. Crystals of HSA bound to phenytoin, valproic acid, sulfamethoxazole, and tolbutamide have been grown from polyethylene glycol using the hanging drop and sitting drop techniques as previously described.[1] Ceftriaxone bound albumin will be crystallized in the near future. The crystals are to be used in x-ray diffraction studies to determine the three dimensional molecular structures of the albumin-ligand complexes. X-ray diffraction data collection has already begun on albumin bound to valproic acid, phenytoin, and sulfamethoxazole. Initial difference mapping indicates that valproic acid attaches in the IIIA binding site.

COLLABORATIVE STUDIES

Efforts have been made to establish collaborations which will expand the work of the Structural Biology Laboratory in the study of human pathogens. Schistosomiasis, a parasitic disease endemic in Africa, South America, and Asia, historically has been one of the most significant causes of morbidity and mortality in the world. The parasite, which is absorbed through the skin from contaminated water, is a blood fluke which, in its various forms, may cause liver and spleen enlargement, hepatic fibrosis, portal hypertension, diarrhea, and cystitis, as well as pulmonary and CNS symptoms. Although a highly efficacious curative agent is now available (praziquantal), disease control has proven very difficult because of high reinfection rates. Therefore, worldwide efforts have focused on the development of a vaccine, but none is yet available. Previous studies by the Structural Biology Lab resulted in the determination of the three-dimensional structure of the 26 kDa glutathione S-transferase from *Schistosoma japonicum*.[18] Glutathione transferase has been identified by other investigators as a possible vaccine candidate for both *S japonicum* and *S* *mansoni.*[19] However, schistosomes posses two glutathione transferases, and a successful vaccine would probably require an immune response to both. Therefore, it is desirable that the 28 kDa GST from *S japonicum* and both GSTs from *S mansoni* be studied crystallographically. The laboratory of Dr. Kathy Davern at the Walter and Eliza Hall Institute for Medical Research in Melbourne, Australia has a long history of successful studies of schistosomal GSTs. Dr. Davern has agreed to produce these proteins by recombinant techniques and supply them to NASA as part of a collaborative agreement reached this summer. Work has already begun on the protein production, and the GSTs are expected to be available for crystallization and structural studies within approximately six weeks.

REFERENCES

1) Carter, D. C., Ho, X.M., Munson, S.H., Twigg, P.D., Gernert, K.M., Broom, M.B. and Miller, T.Y., *Science*, 244, 1195-1198 (1989).

2) Carter, D.C. and He, X.M., *Science*, 249, 302-303 (1989).

3) He, X.M. and Carter, D.C., *Nature*, 358, 209-215 (1992).

4) Silverman, W., Anderson, D., Blanc, W., et al, *Pediatrics*, 18, 614-624, (1956).

5) Mandell, G.L. and Sande, M.A., Chap. 49 in *Goodman and Gilman's The Pharmacologic Basis of Theraputics*, sixth edition (eds. Gilman, A.G., Goodman, L.S., and Gilman, A.), 1106-1125 (Macmillian, New York, 1980).

6) Disturbances in Infants and Newborns, Chap. 189 in *The Merck Manual*, sixteenth edition (ed. Berkow, R.), 1972-2050 (Merck Research Laboratories, Rahway, N.J., 1992).

7) Fink, S., *Pediatrics*, 80, 873-875 (1987).

8) McElnay, J.C. and D'Arcy, P.F., *Drugs*, 25, 495-513, (1983).

9) Sellers, E.M., *Pharmacology*, 18, 225-227, (1979).

10) Rolan, P.E., *Br. J. Clin. Pharmac.*, 37, 125-128, (1994).

11) American Medical Association, *Drug Evaluations Annual 1994.*

12) Lewis, R.J., Trager, W.F., Chan, K.K., Breckenridge, A., Orme, M., Schary, W., *J. Clin. Invest.*, 53, 1607-1617 (1974).

13) O'Reilly, R.A., *New Engl J. Med.*, 302, 33-35 (1980).

14) Lindup, W,E., in *Progress in Drug Metabolism*, Vol. 10 (eds. Bridges, J.W., Chasseaud, L.F., and Gibson, G.G.), 141-185, (Taylor and Francis, London, 1987).

15) Koch-Weser, J. and Sellers, E.M., *New Engl. J. Med.*, 294, 311-316, 526-531 (1976).

16) Christensen, L.K., Hansen, J.M., and Kristensen, M., *Lancet ii*, 1298-1301 (1963).

17) Panegyres, P.K. and Rischbieth, R.H., *Postgraduate Med. J.*, 67, 98 (1991).

18) Lim, K., Ho, X., Keeling, K., Gilliland, G.L., Ji, X., Rucker, F., and Carter, D.C., *Protein Science*, 3, 2233-2244 (1994).

19) Grzych, J.M., Grezel, D., Xu, C.B., Neyrinck, J.L., Capron, M., Ouma, J.H., Butterworth, A.E., and Capron, A., *The Journal of Immunology*, 150, 527-535 (1993).