GREGORIO WEBER

Emeritus Professor of Biochemistry and Biophysics
School of Chemical Sciences
University of Illinois
600 South Mathews
Urbana, Illinois 61801

DATE OF BIRTH

July 4, 1916; Buenos Aires, Argentina
U. S. Resident since l962, U. S. Citizen since 1971.

TRAINING

Undergraduate

University of Buenos Aires, School of Medicine, Doctor of Medicine, 1943.

Graduate

Cambridge University, Ph.D. Biochemistry, 1947.

SOCIETIES

American Chemical Society
American Society of Biological Chemists - Editor, The Journal of Biological Chemistry, 1968
Biochemical Society, London
American Biophysical Society, Member of Council 1970-1973.

AWARDS

British Council Scholar, 1944-1947
Beit Memorial Fellow, 1948-1951
American Academy of Arts and Sciences, Elected Fellow, 1968
1st National Lecturer of the Biophysical Society, 1969
Corresponding Member of the National Academy of Exact Sciences of Argentina, 1971
Nato Lecturer in Europe, 1975
National Academy of Sciences (U.S.), Elected Member, 1975
Guggenheim Foundation Fellow, 1970

Rumford Premium of American Academy of Arts and Sciences, 1979

ISCO Award for excellence in Biochemical Instrumentation, 1983

First recipient of Repligen Award for the Chemistry of Biological Processes of the American Chemical Society, 1986

ACADEMIC APPOINTMENTS

University of Illinois

Emeritus Professor of Biochemistry, 1986 to present
Professor in the Center for Advanced Studies, 1971 to 1986
Professor of Biochemistry, 1962 to 1986
Visiting Lecturer, Summer, 1959

University of Buenos Aires

Visiting Research Lecturer of CONICYT, 1981
Honorary Professor, from 1987

University of California at Los Angeles

Visiting Professor, 1977

Harvard University

Visiting Professor, 1970

University of Washington

Visiting Professor, Summer, 1964

Stanford University

Visiting Lecturer, 1961, 1979

Brandeis University

Visiting Professor, 1960

Sheffield University

Reader in Biophysics, 1960-1962
Senior Lecturer in Biochemistry 1956-60
Lecturer in Biochemistry 1953-1956

Institute of Physiology of the University of Buenos Aires

Teaching assistant in Physiology and Biochemistry, 1939-1942

RESEARCH ACCOMPLISHMENTS

A. Complex Formation of Fluorophores; Detection of Internal Complexes in the Coenzymes

1. Weber, G. The quenching of fluorescence in liquids by complex formation. Determination of the mean life of the complex. *Trans Faraday Soc. 44*, 185-189 (1948).

   First paper to demonstrate that fluorescence quenching can take place after formation of molecular complexes of finite duration rather than collisions.

2. Weber, G. Fluorescence of riboflavin and flavin-adenine dinucleotide. *Biochem. J. 47*, 114-121 (1950).

   First demonstration of an internal complex in FAD.

3. Weber, G. Intramolecular transfer of electronic energy in dihydrodiphos phopyridine nucleotide. *Nature, London 180*, 1409 (1957). First demonstration of an internal complex in NADH.

B. U.V. Fluorescence of the Aromatic Amino Acids and Proteins

1. Weber, G. and Teale, F.W.J. Ultraviolet fluorescence of aromatic amino acids. *Biochem. J. 65*, 476-482 (1957).

   Paper that first described the fluorescence of the aromatic amino acids.

2. Weber, G. and Teale, F.W.J. Electronic energy transfer in heme proteins. *Faraday Soc. Discussions 27*, 134-141 (1959).

   First paper to demonstrate the use of electronic energy transfer in the study of proteins by comparing the fluorescence of heme proteins before and after removal of the heme.

3. Weber, G. Fluorscence-polarization spectrum and electronic-energy transfer in tyrosine, tryptophan, and related compounds. *Biochem. J. 75*, 335-345 (1960).

4. Weber, G. Fluorescence-polarization spectrum and electronic-energy transfer in proteins. *Biochem. J. 75*, 345-352 (1960).

   3,4. First demonstration of electronic energy transfer among tyrosines, giving the critical distances of transfer from tyrosine to tryptophan and among tyrosine or tryptophan residues.

5. Lakowicz, J.R. and Weber, G. Quenching of protein fluorescence by oxygen. Detection of structural fluctuations in proteins in the nanosecond time scale. *Biochemistry 12*, 4171-4179 (1973).

   Describes the technique of using pressures of up to 100 atmospheres of oxygen to quench fluorophores in water. More important, it shows how this can be used to detect, for the first time and to the surprise of many, the existence of fast fluctuations in protein structure on the nanosecond time scale. The relevance of this work is shown in the increasing interest in experimental and theoretical work in protein dynamics.

C. Fluorescence Polarization and Rotational Diffusion

1. Weber, G. Polarization of the fluorescence of macromolecules. I. Theory and experimental method. *Biochem. J. 51,* 145-155 (1952).

   Contains the theory and the method of measurement of the polarization, gives the "polarization addition law", which is the basis for the computation of the polarization of the fluorescence for an arbitrary dipole distribution.

2. Weber, G. Polarization of the fluorescence of macromolecules. II. Polarization of the fluorescence of labeled protein molecules. *Biochem. J. 51,* 155-164 (1952).

   Introduces the Dansyl derivatives as suitable to determine the rotational diffusion of proteins of up to 10^5 molecular weight, the limit being given by the fluorescence lifetime of Dansyl derivatives.

3. Knopp, J.A. and Weber, G. Fluorescence polarization of pyrenebutyric bovine serum albumin and pyrenebutyric-human macroglobulin conjugates. *J. Biol. Chem. 244,* 6309-6315 (1969).

   Extends the method to molecular weights of $l0^6$ (relaxation times of up to 1 μs) by the introduction of a new fluorophore: the pyrene butyroyl residue with a lifetime of 100-150 ns.

4. Shinitzky, M., Dianoux, A.C., Gitler, C. and Weber, G. Microviscosity and order in the hydrocarbon region of micelles and membranes determined with fluorscent probes. I. Synthetic micelles. *Biochemistry 10,* 2106-2113 (1971).

   First paper to describe the use of the fluorescence of small molecules as probes for the viscosity of micelles. It owes much to Shinitzky.

5. Weber, G. and Mitchell, G.M. "Detection of anisotropic rotations by differential phase fluorometry." In *Excited States of Biological Membranes,* J.B. Birks (ed.), Wiley, London, pp. 72-76, (1976).

   This paper reports the first use of differential phase fluorometry to detect the anisotropic rotations of small molecules.

6. Weber, G. Theory of differential phase fluorometry: Detection of anisotropic molecular rotations. *J. Chem. Phys. 66*, 4081-4091 (1977).

   Birth of the theory and experimental proof that it works.

7. Mantulin, W.W. and Weber, G. Rotational anisotropy and solvent fluorophore bonds: An investigation by differential polarized phase fluorometry. *J. Chem. Phys. 66*, 4092-4099 (1977).

   A clear demonstration of phenomena previously reported by others that anomalously fast anisotropic rotations may be expected in molecules that do not form strong bonds with the solvent.

D. Study of Interactions of Proteins with Fluorescent Ligands

1. Weber, G. and Laurence, D.J.R. Fluorescent indicators of adsorption in aqueous solution and on the solid phase. *Biochem. J. 56*, xxxi (1954).

   Reports the findings that a number of aromatic secondary amines are strongly fluorescent in apolar solvents, but hardly in water, the most spectacular cases being the anilino-naphthalene sulfonates (ANS).

2. Weber, G. and Daniel, E. Cooperative binding by bovine serum albumin. II. The binding of 1-anilino-8-naphthalene sulfonate. Polarization of the ligand fluorscence and quenching of the protein fluorescence. *Biochemistry 5*, 1900-1907 (1966).

   Describes how polarization measurements may be used to determine the distribution of ligands among the protein molecules that bind them.

3. Anderson, S.R. and Weber, G. Fluorescence polarization of the complexes of 1-anilino-8-naphthalene sulfonate with bovine serum albumin. Evidence for preferential orientation of the ligand. *Biochemistry 8*, 371-377 (1969).

4. Kolb, D.A. and Weber, G. Quantitative demonstration of the reciprocity of ligand effects in the ternary complexes of chicken heart lactate dehydrogenease with NADH and oxalate. *Biochemistry 14*, 4471 (1975).

   Gives a rigorous quantitative demonstration of the reciprocity of effects among bound ligands. It is shown that in the presence of excess oxalate the free energy of binding of NADH decreases by 1.3 kcal and that of oxalate in the presence of NADH by 1.1 kcal. The figures are equal within experimental errors. Recent work from other laboratories has followed the ideas and technique developed in this study.

E. Techniques

1. Weber, G. Photoelectric method for the measurment of the polarization of the fluorescence of solutions. *J. Opt. Soc. Amer. 46,* 962-970 (1956).

   Details the one and only no-calibration, absolute method for the measurement of polarization of fluorescence of solutions. Recent extensions of the method includes: Jameson, D.M., Weber, G., Spencer, R.D. and Mitchell, G. (1978). Fluorescence polarization: Measurements with a photon-counting photometer, *Rev. Sci. Instrum. 49*(4), 510-514; Chryssomallis, G.S., Drickamer, H.G., and Weber, G. (1978): The measurement of fluorescence polarization at high pressure, *J. Appl. Phys. 49*(6), 3084-3087; A.A. Paladini and G. Weber (1981): Absolute measurement of polarization of fluorescence at high pressure, *Rev. Sci. Inst. 52*, 419-426.

2. Weber, G. and Teale, F.W.J. Determination of the absolute quantum yield of fluorescent solutions. *Trans. Faraday Soc. 53*, 646-655 (1957).

   Gives what is now the classical method of measurement of absolute quantum yield of fluorescence. Very few values of the many quoted in our original paper have been found to be in error.

3. Hastings, J.W. and Weber, G. Total quantum flux of isotropic sources. *J. Opt. Soc. Am. 53,* 1410-1415 (1963).

   An adaptation of method in paper 2 for measurement of quantum yield of chemi- and bioluminescence. As far as we know there have been no improvements or even suggestions for other methods.

4. Spencer, R.D. and Weber, G. Measurement of subnanosecond fluorescence lifetimes with a cross-correlation phase fluorometer. *Annals New York Acad. Sci. 158,* 361-376 (1969).

5. Spencer, R.D. and Weber, G. Influence of Brownian rotations and energy transfer upon the measurements of fluorescence lifetime. *J. Chem. Phys. 52,* 1654-1663 (1970).

6. Weber, G. Resolution of the fluorescence lifetimes in a heterogeneous system by phase and modulation measurements. *J. Phys. Chem. 85,* 949-953 (1981).

7. Jameson, D.M. and Weber, G. Resolution of the pH dependent heterogeneous fluorescence decay of tryptophan by phase and modulation measurements. *J. Phys. Chem. 85,* 953-958 (1981).

   Papers 4, 5, 6 and 7 are developments of phase fluorometry. Paper 5 replaces Jablonski's theory and equations, which are incorrect, by the correct ones. Paper 6 gives the theory of resolution of fluorescence decay due to complex independent emissions, a possibility previously limited to pulse fluorometry. Paper 7 applies the theory to a particular case where an independent check of the accuracy can be carried out.

8. Paladini, A.A. and Weber, G. Absolute measurements of fluorescence polarization at high pressures. *Rev. Sci. Instrum. 52,* 419-427 (1981).

   Describes the extension of the determinations of fluorescence polarization to solutions under pressures of 3 Kbar or less.

F. Energy Transfer

Besides B2, B3, B4, D2, and D3 which deal with applications, the following two papers were real firsts in this field:

1. Weber, G. Concentration depolarization of the fluorescence of solutions. *Trans. Faraday Soc. 50,* 557 (1954).

   Gives the general formulation of depolarization by successive transfers that has been universally adopted afterwards.

2. Weber, G. and Shinitzky, M. Failure of energy transfer between identical aromatic molecules on excitation at the long wave edge of the absorption spectrum. *Proc. Natl. Acad. Sci. USA 65,* 823-830 (1970).

   Described the "red edge effect" in energy transfer among identical molecules, amply confirmed by more recent observations.

G. Protein-Ligand Interactions

1. Weber, G. Ligand binding and internal equilibria in proteins. *Biochemistry 11,* 862 (1972).

2. Weber, G. Addition of chemical and osmotic free energies through negative interaction of protein bound ligands. *Proc. Natl. Acad. Sci. USA 69,* 3000-3003 (1972).

3. Kolb, D.A. and Weber, G. Cooperativity of binding of anilino-naphthalene-sulfonate to serum albumin induced by a second ligand. *Biochemistry 14,* 4476-4481 (1975).

4. Weber, G. Energetics of ligand binding to proteins. *Adv. Prot. Chem. 29,* 1-83 (1975).

5. Weber, G. Energetic advantage of ion countertransport in chemiosmotic conversion. In *Frontiers of Biological Energetics,* Academic Press, Vol. 1, pp. 12-18 (1978).

   Papers 1 to 5 describe a general approach to the thermodynamics of multiple ligand binding to proteins, through the simple concept of the "standard free energy couplings" between pairs of bound ligands. This approach is extended to include the covalent reactions in which the protein takes part and can give a rational explanation of the inter conversion of chemical and osmotic energies in metabolism and of the phosphorylation of ADP by ionic gradients. Paper 5 treats explicitly the case of the Na^+ K^+ ATPase.

6. Xu, G-J and Weber, G. Dynamics and time-averaged chemical potential of proteins: Importance in oligomer association. *Proc. Nat. Acad. Sci USA 79,* 5268-5271 (1982).

   The anomalous dissociation of yeast enolase into monomers is explained on the assumption that the chemical potential of the dimer or monomer in equilibrium is not a constant but depends upon the extent of reaction. This is a novel and quite unorthodox concept but may be of great importance in the description of the properties of oligomeric proteins. Careful experimentation will determine whether it has a wider application as we anticipate in this paper.

7. Weber, G. Asymmetric ligand binding by haemoglobin. *Nature 300,* 603-607 (1982).

   The relation between asymmetric ligand binding and asymmetric titration curve is developed. The asymmetry observed in the case of haemoglobin is shown to be possible only if the α–α and β–β subunit interactions change by different amounts on oxygenation.

8. Weber, G. Order of free energy couplings between ligand binding and protein subunit association in hemoglobin. *Proc. Natl. Acad. Sci. USA 81,* 7098-7102 (1984).

9. Macgregor, R.B. and Weber, G. Estimation of the polarity of the protein interior by optical spectroscopy. *Nature 319,* 70-73 (1986).

10. Weber, G. Free energy couplings between ligand binding and subunit associaton in hemoglobin are of first order. *Biochemistry 26,* 331-332 (1987).

H. <u>Pressure Effects Upon the Complexes of Small Molecules, Proteins and Protein-Ligand Complexes</u>

1. Weber, G., Tanaka, F., Okamoto, B.Y. and Drickamer, H.G. The effect of pressure on the molecular complex of isoalloxazine and adenine. *Proc. Natl. Acad. Sci. USA 71,* 1264-1266 (1974).

   Paper 1 demonstrates that a typical hydrophobic (stacking) complex is stabilized by pressure.

2. Li, F.M., Hooke, J.W., III, Drickamer, H.G. and Weber, G. Effects of pressure upon the fluorescence of the riboflavin binding protein and its flavin mononucleotide complex. *Biochemistry 15,* 3205-3211 (1976).

3. Li, T.M., Hooke, J.W., III, Drickamer, H.G. and Weber, G. Plurality of pressure-denatured forms in lysozyme and chymotrypsinogen. *Biochemistry 15,* 5517-5580 (1976).

4. Visser, A.J.W.G., Li, T. M., Drickamer, H.G. and Weber, G. Volume changes in the formation of internal complexes of flavinyltryptophan peptides. *Biochemistry 16,* 4883-4886 (1977).

5. Torgerson, P.M., Drickamer, H.G. and Weber, G. Inclusion complexes of poly-β-cyclodextrines. A model for pressure effects upon ligand-protein complexes. *Biochemistry 18,* 3079-83 (1979).

   Papers 4 and 5 show that the mechanical constraints owing to covalent bonds are important in determining the volume changes upon formation of intramolecular complexes. It is believed that such mechanical constraints are paramount in determining the effects of high pressure upon the monomeric globular proteins.

6. Torgerson, P.M., Drickamer, H.G. and Weber, G. Effect of hydrostatic pressure upon ethidium bromide association with tRNA. *Biochemistry 19,* 3957-60 (1980).

   Papers 1 to 6 form a comprehensive study of pressure effects upon molecular complexes and proteins in solution, carried out in collaboration with Professor H.G. Drickamer at the School of Chemical Sciences, University of Illinois at Urbana. The second paper seems of particular interest in that it demonstrates that "pressure denaturation" of proteins is a complex phenomenon, in which different parts of the protein change conformation over distinctly different pressure ranges.

7. Paladini, A.A. and Weber, G. Pressure-induced reversible dissociation of enolase. *Biochemistry 20,* 2587-2593 (1981).

   Paper 7 applies the concepts developed in papers 1-6 to proteins. It is predicted that the dissociation of oligomeric proteins under pressure must be a general phenomenon of which this paper gives a first demonstration.

8. Weber, G. and Drickamer, H.G. The effect of high pressure upon proteins and other biomolecules. *Quart. Rev. Biophys. 16,* 89-112 (1983).

9. King, L. and Weber, G. Conformational drift of lactate dehydrogenase. *Biophys. J. 49,* 70-72 (1986).

10. Weber, G. Phenomenological description of the association of protein subunits subjected to conformational drift. Effects of dilution and of hydrostatic pressure. *Biochemistry 25,* 3626-3631 (1986).

11. King, L. and Weber, G. Conformational drift of dissociated lactate dehydrogenase. *Biochemistry 25,* 3632-3636 (1986).

12. King, L. and Weber, G. Conformational drift and cryoinactivation of lactate dehydrogenase. *Biochemistry 25,* 3637-3640 (1986).

    Papers 9 to 13 describe the pressure dissociaton of dimers and tetramers and establish the generality of the conformational drift of separated protein subunits.

13. Silva, J.L., Miles, E.W. and Weber, G. Pressure dissociaton and conformational drift of the β subunit of tryptophan synthase. *Biochemistry 25,* 578-5786 (1986).

14. Ruan, K. and Weber, G. (1988) Dissociation of hexokinase. *Biochemistry 27*, 3295_3301.

15. Silva, J.L. and Weber, G. (1988) Pressure-induced dissociation of Brome Mosaic virus. *J. Mol. Biol. 199*, 149-159.

16. Ruan, K. and Weber, G. (1989) Hysteresis and conformatinal drift of pressure-dissociated glyceraldehydephosphate dehydrogenase. *Biochemistry 28*, 2144-2153.

Papers 12 and 16 propose a new explanation for the inactivation of oligomeric proteins in the cold: A cycle of incipient dissociation, conformational drift of the isolated monomers and reassociation into inactive tetramers that can rearrange themselves into the active form upon warning. Paper 15 describes the reversible dissociation of a virus capsid by pressure.