

**PROTEIN-DYE
INTERACTIONS:
DEVELOPMENTS AND
APPLICATIONS**

PROTEIN-DYE INTERACTIONS:

Developments and Applications

Edited by

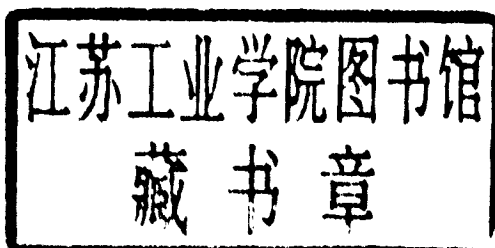
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Preface

This volume contains the papers and reports presented at the First International Conference on Dye-Protein Interaction, held 24–28 July 1988 at the University of Compiègne, France. This was the first international meeting dealing entirely with dye-protein interaction. The major focus of the conference was on the better understanding of the mechanism of interaction of proteins with different triazine dyes and the synthesis of novel structural dyes having good biomimetic activities. The potentials and limits of their use in biotechnology, mainly for purification, were stressed. Current contributions in developing dye-based affinity methods were highlighted in such areas as affinity partition, affinity precipitation and new support matrices for efficient affinity chromatography, etc.

The interrelation between metal chelates and dyes in terms of their interactions with proteins was underlined. It is our belief that this proceedings volume will be a stimulus for broad and creative applications of dye affinity concepts in many fields of biomedical research and biotechnology.

In addition, a discussion session emphasised the necessity for understanding the toxicological aspects of these dyes, their fragments and their metabolites. This helped to trigger plans for future work, and this topic will be one of the priorities in a future meeting on dye-protein interactions.

The help of the International Scientific Committee, which included Drs C. R. Lowe (UK), G. Kopperschläger (GDR), E. Stellwagen (USA), D. Thomas (France), G. Birkenmeier (GDR), S. Rajgopal-Narayan (USA), J. P. Dandeu (France), D. Muller (France) and E. Dellacherie (France), in organising this meeting is gratefully acknowledged.

We are grateful to the Université de Technologie de Compiègne (UTC), France, for the support and the infrastructural facilities provided for the meeting. Financial support from INSERM (French National Institute for Medical Research), including that for travel grants to the speakers, is gratefully acknowledged. The following organisations, Groupe Français de Bio-Chromatographie (GFBC), Université Paris VII, and the following industries, Pharmacia, IBF, Sanofi, Bertin, Merck, Prolabo and J. T. Baker, provided financial contributions.

Finally, we are indebted to the authors for their important contributions to this volume, to Miss Nathalie Honoré for her secretarial help in preparing the volume, and to Elsevier Science Publishers Ltd for its timely publication.

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INTRODUCTION

A SHORT HISTORICAL REVIEW ON THE INTERACTION BETWEEN DYE-STUFFS AND BIOLOGICAL MATTER

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In the aftermath of the 1968 student revolution, there was a certain contempt for established knowledge and traditions. The pendulum is now swinging in the opposite direction and even young scientists realize the importance of knowing and appreciating the roots of science, that is, the achievements of their own forerunners. Not only is it intellectually stimulating to obtain a widened perspective regarding the present position of science in society, but the history of science may tell us about discoveries in the distant past which can, sometimes, be transformed into modern forms for solving present day problems. With these thoughts in mind, I would like to give a very brief historical account of the prerequisites for dye-stuff based chromatography. I shall consider three relevant branches: the chemistry of dyes, dye affinity for biological matter and group selective adsorption as applied to biomolecules.

Dye-Stuffs Chemistry

Man's first contact with dyes and pigments is literary true and concerns their affinity for his skin. It is archeologically supported (1). Stone age man, like primitive people up to the present, painted their skin. Painting of corpses was included in ancient funeral rites. The oriental women of the Bronze Age attempted to charm their men by dyeing their hair with henna, the color principle of Lawsonia inermis and that fashion is to some extent still in vogue among females. To enlighten the dull and monotonous everyday trivialities our ancestors dressed themselves in textiles, dyed with extracts of madder (alizarin) and Isatis tinctoria (indigo) (2). According to

Bible the Hebrews under patriarchal times were using kermes - a red anthraquinone derivative - and the Phoenicians traded in the imperial purple from Tyros (dibromoindigo).

Man must adjust his life according to the sources available to him in his own environment and for the American Indians it became natural to collect certain logs in order to extract brilliantly colored compounds. So, from the Indians we inherited the use of brazilin and hematoxylin to dye wool fabrics, later to be abandoned for that purpose but instead introduced as tools in histology. One of the pioneers in the field of plant dyes was the French lipid chemist Michel Eugène Chevreul, the director of the Gobelins, a dye factory founded in 1662 (3). In the early nineteenth century he also studied the chemistry of quercitrin and morin. Chevreul died at the age of 103. His life span overlapped the time that foreshadows the advent of the era of synthetic dyes.

The first synthetic dyes appeared during the eighteenth century. The invention or discovery of picric acid is fading back into obscurity but it was probably the first one in the series of millions of dyes to come. The isolation of aniline is a milestone in the history of dyes as is the 18 year old William Henry Perkin's synthesis of Aniline purple, 'Perkin's mauve' or mauveine of 1856 (4). As is so often the case, important and original discoveries are not easily traced back to their origin. O. Unverdorben seems to be the first dye investigator to have prepared aniline (5) initially as a dry-distillation product of indigo in 1826 under the name of 'crystalline', but later to be renamed after an-nil, the arabic word for indigo. Aniline was rediscovered at least twice during the following 15 years but the immense importance of the substance as the building block of a new era of organic chemistry first became apparent after Perkin's debut. Isolation of mauveine from a dirty product is an excellent example of a serendipitous discovery: Perkin tried to synthesize quinine from aniline! Like Michael Faraday, once a young

assistant to Humphry Davy, Perkin, as A.W. Hofmann's pupil, eventually surpassed in knowledge and in skill his teacher - as it should rightly be and, unfortunately, so seldom seems to be the case nowadays. Perkin senior was also a clever businessman. He took patents, founded a factory that became known also for its excellent marketing and information service to the customers - unheard activities at that time. About seventy five years after Unverdorben's isolation of aniline from indigo, following Kekulé's, Bel's and van't Hoff's epoch making contributions to structural organic chemistry, the synthesis of indigo from aniline became feasible. The circle was closed. Whenever possible, we should follow the latter's lead: from simplicity to complexity.

The dye industry is concerned with the development of coloring materials for textiles and printing, but pioneers of histology and bacteriology also became dye consumers.

Biological Staining

F.V. Raspail, working in Paris in the early part of last century, may perhaps be considered as the founding father of histology and histochemistry (6). But he had many forerunners. Furthest away in time was Anthony Leevenhoek, the 'father of the microscope'. He tried with modest success to use saffron to enhance the contrasts of his microbial objects.

After Perkin's synthesis of mauve the time was ripe for synthetic dyes to enter the scene of biology and medicine. Bencke was the first in the line to use aniline dyes (7) and E. Klebs the first, in 1868, to detect an enzyme (peroxidase) by staining (8). Mischer's isolation of nuclear chromatin in 1873 with the aid of methyl green must be mentioned and, of course, Koch's and Ehrlich's seminal contributions during the 1880's (9). With the aid of methylene blue Robert Koch was able to discover the tubercle and colera bacteria.

Paul Ehrlich's early work on vital dyes turned him into chemotherapy, a science which he himself founded in the latter part of the last century and which kept him occupied for the rest of his life. Also, in this field, the problems related to the specific affinities of dyes for biological matter are encountered. Starting out from N.O. Witt's theory of relationships between the color of dyes and chemical constitution (10), Ehrlich postulated the presence in the biologically active substances of toxophoric and haptophoric groups with affinity for biological counterparts - the receptors (11).

Ehrlich used trypanosomes, the cause of African sleeping sickness, as targets for his intended 'magic bullets'. Hundreds of dyes were tested and their action was recorded although only a minor portion of the results was published. Starting with the well-known methylene blue and benzopurpurin he later came across substances of considerable potency which became widely known as trypan red, trypan blue and trypanflavine.

Already during the phlogiston period, adsorption of dyes and other substances was explained according to 'physical theory' or 'chemical theory'. Chevreul, who supported the former, coined the expression 'capillary affinity' for the force that binds dyes to tissues and Ostwald considered dyeing to be effected by 'mechanical affinity'. In this context it is of interest to mention the controversy between Svante Arrhenius and Paul Ehrlich. Arrhenius postulated that a chemical equilibrium was attained between a toxin and its antitoxin whereas Ehrlich considered the combination to be complete which in modern terminology must mean the involvement of covalent binding. Modern dyes may be fixed to a fiber by covalent attachment (reactive dyes) or by 'physical' adsorption, which in present terms can be formulated as attachment by non-covalent bonds. Dye based chromatography, in fact, utilizes both kinds of attachment: chemical fixation of the dye to a solid support and adsorption of soluble ligates (analytes).

In a review on the interactions between dye-stuffs and biological matter it is justified to mention the 'photodynamic action' discovered in 1900 by O. Raab (12): Biomolecules may be damaged by light in the presence of dyes. This phenomenon, an apparent risk factor in the adsorption process, can perhaps be used to advantage, for example in thin layer chromatography, provided that it can be effectively controlled.

Adsorption of Biomolecules

One of the roots of enzyme separation technology is to be found in the development of adsorption methods and, as said, adsorption is closely connected to the applied chemistry of dyes and colored matter. Chromatography as introduced by Tswet (13), and its forerunner, capillary adsorption, were known (14) but largely overlooked in the first three decades of our century. Willstätter, one of the leading figures in organic chemistry, made extensive use of batchwise adsorption to purify enzymes. He was in fact so successful that he obtained highly active enzyme preparations which he thought were devoid of proteins (15). He was not favorably inclined towards Tsvet's chromatography, and, his authority and opinion were serious obstacles to the career of the lonely working Tswet - a fact that delayed the full appreciation and acceptance of chromatography, a powerful tool for chemistry and biology.

In a survey, Lars Sundberg and I traced back the first attempts to use specific adsorption techniques for purification of enzymes to Starkenstein (16) and perhaps G. Hedin. These early experiments were made just after the turn of the century. Several biochemists followed in their footsteps and among the pioneers of bioaffinity chromatography, Campbell, Leuscher and Lerman (17) and Arsenis and McCormick (18), must be mentioned. By using dinitrophenyl- and isoalloxazine derivatives of cellulose as adsorbents they initiated the use of colored ligands in chromatography. The birth of modern chro-

matography is usually connected with Cuatrecasas et al. (19). Two requirements for its success were at hand in 1967: 1) a suitable carrier and 2) an efficient and sufficiently reliable coupling procedure (20).

It is not my task to review early work on the specific topic of the present symposium. Many of the pioneers are present here today and it is up to them and us, their followers, to show the advantages of using dye ligands in preference to alternatives. Let me just make some comments. Ehrlich formulated his affinity theory but its value for predicting action from structure was rather limited. Empirically, through trial and error, he had to screen thousands of related and unrelated substances to find therapeutica as active as desired. We have not advanced much further in predicting adsorption selectivity from the nature of the dye ligands. Dyes are of complex molecular structure and their interaction with biomacromolecules are not easy to understand even with help of modern valence theory.

From the history of chemotherapeutics we may learn to go from simplicity to complexity, a scientific 'loadstar' worth being pointed out repeatedly. Domagk and his associates introduced prontosil, the first sulfonamide drug, in the early 30s. A few years later Trefouel and coworkers in France, Nitti and Bouvet and others showed that antibiotic activity was retained in the simple degradation products of the azo dyes. Personally, I believe it very well worthwhile to study simple π -electron-rich molecules as ligands for group fractionation of biomolecules. Eighteen years ago Nermin Fornstedt and I used the dye-constituent sulfanilic acid coupled to agarose for group fractionation (21) (incidentally, epoxy-coupling was used for the first time to couple affinity ligands to polymer matrices). The subsite contribution to the affinities of such ligands is more easily accessible to rational chemical interpretation. However, we have to be prepared to accept arguments in favour of dye-ligand based chromatography. Synthesis of biomimetic dyes (22), introduc-

tion of affinity partitioning (23-24) and precipitation (25) with soluble dye-coupled polymers, systematic chromatographic screening procedures (26) and other techniques to come may, hopefully, provide us with extremely valuable tools. By studying complex adsorption behaviour we may also have a fair chance to discover unknown chemical interactions. Diving deep into dye-ligand-based chromatography may eventually yield unexpected profits.

Acknowledgements

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