Electronic Characteristics of Substrates for Ceruloplasmin

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In a frequently cited report Levine and Peisach noted that the rates of oxidation of a number of aryl polyamines and polyphenols by ceruloplasmin can be directly related to the sum of Hammett sigma values for the substituents in the aromatic substrates, and concluded that an increased ring electron density in the substrate is associated with increased activity with ceruloplasmin.[1] This conclusion was recently criticized by Curzon and Speyer,[2] who pointed out that Hammett sigma values, as calculated by Levine and Peisach, relate only to the effect of each electron-supplying group at the other electron-supplying group, and do not give a measure of net π-electron density in the aromatic ring.

More reliable information concerning the π-electron distribution in aromatic molecules can be obtained by wave mechanical techniques. Such calculations have now been carried out by the Hückel molecular orbital method,[3] and Table 1 gives the ring π-electron densities obtained for a number of representative compounds. Statistical estimation of the correlation between ring π-electron density and maximum activity with ceruloplasmin, as determined by Levine and Peisach (Table 1),[1,4] gave a correlation coefficient (0.16) which was not different from zero at the 5 % level of significance. It may be concluded that the kinetic data reported by Levine and Peisach do not provide any evidence that maximum activities are quantitatively related to ring π-electron densities of the substrates. There does not

Table 1. Molecular orbital data for aromatic compounds which have been tested as substrates for ceruloplasmin,[5–7] and maximum activities (V, given in μl O_2 consumed per hour per μmole enzyme) as determined by Levine and Peisach.[1,4] Ring π-electron densities (RED) were calculated as the sum of the π-electron densities at the six ring carbon atoms of each compound. Energy levels of the highest occupied molecular orbital (EHOMO) are expressed in units of the resonance integral β for neighbouring carbon atoms ($\beta < 0$).

Compound	RED	EHOMO	$V \times 10^{-3}$
Substrates			
p-Phenylenediamine	6.203	0.439	424
m-Phenylenediamine	6.216	0.597	20
o-Phenylenediamine	6.207	0.460	254
N-Methyl-*p*-phenylenediamine	6.215	0.360	178
N,*N*-Dimethyl-*p*-phenylenediamine	6.230	0.264	265
N,*N*-Dimethyl-*m*-phenylenediamine	6.245	0.371	60
Triaminobenzene	6.294	0.338	135
p-Aminophenol	6.157	0.593	169
N,*N*,*N*′,*N*′-Tetramethyl-*p*-phenylenediamine	6.257	0.182	
Würster's Red	5.793	0.435	
Epinephrine	6.145	0.379	
Norepinephrine	6.165	0.510	
Serotonine	6.218	0.569	
Non-substrates			
Quinol	6.107	0.884	(145)
Catechol	6.107	0.927	(8)
Phenol	6.056	1.065	
p-Cresol	6.087	0.967	
Aniline	6.112	0.681	
3,4-Dihydroxyphenylalanine	6.132	0.840	

even seem to be any qualitative relationship of this kind. Maximum activities for *N,N*-dimethyl-*p*-phenylenediamine and the corresponding radical cation Würster's Red have been shown to be of closely similar magnitude,[5] in spite of the fact that the ring π-electron density of Würster's Red is lower than that of the unsubstituted benzene ring, and thus lower than that of all of the non-substrates listed in Table 1.

The fact that Würster's Red, despite its low π-electron density, functions as a substrate for ceruloplasmin calls for an explanation. Examination of Table 1, which also gives energies of the highest occupied molecular orbital (EHOMO) of the respective compounds, shows that all of the substrates for ceruloplasmin, including Würster's Red, are characterized by exceptionally high EHOMO levels ($0.1-0.6\ \beta$), whereas non-substrates such as quinol, aniline, and 3,4-dihydroxyphenylalanine have more normal EHOMO values ($0.6-1.0\ \beta$). This observation suggests that a low ionization potential of the substrate is essential for the enzymatic reaction, and gives some support to the view that substrate binding takes place through the formation of a charge-transfer complex.

Further experiments are in progress in order to investigate whether the above characteristic property of the substrates reflects a quantitative relationship between EHOMO values and reaction rate parameters such as V and K_m. The experiments of Levine and Peisach were carried out before the influence of iron ions on the enzymatic reaction velocity was recognized,[6] and their kinetic data are not sufficiently reliable for such an analysis.

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Group-specific Adsorption of Glycoproteins

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Some of the plant proteins called phytohemagglutinins or lectins are known to form complexes with carbohydrates and glycoproteins.[1-4] Although such protein-carbohydrate interactions are usually revealed by studying mixtures in solution, these proteins can also interact with insoluble granular carbohydrates and as consequence be more or less strongly adsorbed.[5-7] The proteins can then be displaced from the adsorbent by an eluent containing competing soluble carbohydrate in appropriate concentration. Complementary adsorbents for carbohydrates can be formed by chemical fixation of such proteins to inert permeable supports and the adsorption and desorption of carbohydrates should be governed by the same principle.

Concanavalin A was chosen for the production of a specific carbohydrate adsorbent * since it is easily available in large quantities and shows affinity for certain carbohydrates.[8,9] Agarose was found to be a satisfactory matrix material for the fixation of concanavalin A.

Detailed studies of the adsorption properties of concanavalin A-agarose will be reported elsewhere but we present here the results of some orientation work done with human serum. Serum, containing a considerable number of glycoproteins of varying carbohydrate content was chosen as a model system to explore the feasibility of sorting out glycoproteins from a complex mixture. Some serum glycoproteins are reported to interact with concanavalin A.[10,33,11]

The adsorbent was prepared by coupling concanavalin A purified by the Sephadex adsorption method [5] to beads of cyanogen-bromide activated 2 % agarose gel.[12,13] The agarose beads were the commercial product Sepharose 2 B from Pharmacia Fine Chemicals, Uppsala, Sweden. Con-

* Patent pending.