Molecular Complexes between Methoxyamphetamines and Riboflavin Derivatives

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Molecular complexes in the ratio 1:1 were formed in aqueous solution between various methoxy-substituted phenylisopropylamine (amphetamine) hydrochloride or oxalate salts and riboflavin derivatives (flavin mononucleotide and flavin adenine dinucleotide). The association constants for these complexes were determined by the fluorescence quenching method as well as by the absorption method. The nature of the complex formation may be due to charge-transfer, electron-transfer, electrostatic and hydrophobic forces. The steric effect also plays an important role in the complex formation. The absorption wavelength and association constant of the complexes are correlated with the published biological activities of the methoxyamphetamines.

In our previous paper [1], we reported the formation of π-molecular complexes between variously substituted methoxyamphetamines and 1,4-dinitrobenzene. The equilibrium constants for these processes were determined by nuclear magnetic resonance chemical shift measurements in carbon tetrachloride solution. For most complexes the measured association constants are linearly related to the values for the threshold dose for the hallucinogenic activity of the methoxyamphetamines. It was found, however, that three of the amphetamine derivatives, 3,4-dimethoxyamphetamine, 2,3,4-trimethoxyamphetamine and 3,4,5-trimethoxyamphetamine have abnormally high association constants with the 1,4-dinitrobenzene, and therefore did not correlate well with their observed biological activity. It was concluded from these results that these particular amphetamines exhibit this anomaly because they encounter less steric hindrance in forming the molecular complexes with the small 1,4-dinitrobenzene acceptor than they would with larger kinds of receptor sites, even though the smaller molecule may possess electronic features characteristic of a biological system. In order to effect a better simulation of biological acceptor-donor interaction than possible with 1,4-dinitrobenzene, it was of interest to examine the formation of complexes of this kind in aqueous solution and with acceptors of larger molecules having greater steric complexity. Flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) are good electron acceptors. They readily form stable molecular complexes with phenols [2, 3], indoles [4], aromatic hydrocarbons [5], purines [6, 7] and chlorpromazine [8]. Recently, Budini and Marinangeli [9] have reported that the free energies of complex formation of flavin derivatives and various psychoactive compounds of diverse molecular structure are linear functions of the electron donating abilities of the drug molecules.

In this paper, the formation of π-molecular complexes between the psychoactive methoxyamphetamines and both FMN and FAD are described. The extent of the complex interactions has been measured in aqueous solution at room temperature by both fluorescence quenching and absorption spectrometry. Both the complex stability, as measured by the association constants, as well as the position, λ_{max}, of charge-transfer bands of the complexes were found to give good linear correlation with the threshold dose levels for all the methoxyamphetamines studied.

Materials and Methods

Materials

The FMN, FAD and *p*-methoxyphenylethylamine hydrochloride were obtained from Calbiochem and were used without further purification. Methoxy-substituted amphetamine hydrochloride or oxalate salts were supplied by Fox Chemical Co. and were

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chromatographically pure. The 3,4-methylenedioxyamphetamine, mescaline hydrochloride and amphetamine sulfate were obtained from Aldrich Chemical Co. The 3,4-methylenedioxyamphetamine was converted to the hydrochloride salt by reaction with concentrated hydrochloric acid. The hydrochloride salt was recrystallized from ethanol-water 1:1 twice: m.p. 181 – 182 °C.

Solid 2,4,6-trimethoxyamphetamine-FMN complex

The complex was prepared by dissolving 0.001 M each of 2,4,6-trimethoxyamphetamine hydrochloride and FMN monosodium dihydrate in 5 ml of water and cooling the solution at 0 °C for several hours. The solid was filtered and recrystallized from water; its m.p. was 161 °C decomposition. NMR results and elemental analysis indicated that the solid was a 1:1 complex.

The analysis for $C_{29}H_{40}N_5O_{12}P \cdot NaCl \cdot 2\,H_2O$ was as follows:

Calcd: C 44.93 H 5.68 O 28.92 N 9.04 P 4.00,
Found: C 44.76 H 5.87 H 28.42 N 9.13 P 3.50.

Complexes between other methoxyamphetamines and FMN have higher solubilities in water than FMN and could not be isolated in pure form.

Absorption spectrophotometric method

The absorption spectrum of the amphetamine-FMN complexes overlapped the absorption spectrum of FMN. The interaction between the amphetamines and FMN was studied with a Cary 14 spectrophotometer in a 1 cm quartz cell equipped with a thermostable sample-cell jacket. The measurements of absorption maximum of the complexes were made at a fixed acceptor concentration (3.2×10^{-4} M) with different amounts of excess donor in water solution at room temperature (25 ± 0.5 °C). The concentration range of the donor was 8×10^{-3} to 1×10^{-1} M. The acceptor was in both the sample and the reference cells. The association constants, K, were determined by the modified Benesi-Hildebrand's equation [8, 10]:

When

$$[D]_0 \gg [A]_0, 1/\Delta A = 1/K(\varepsilon_{DA} - \varepsilon_A)[A]_0[D]_0 + 1/(\varepsilon_{DA} - \varepsilon_A)[A]_0 \quad (1)$$

where $[A]_0$ and $[D]_0$ are the initial concentrations of acceptor and donor in moles/liter; ε_{DA} is the molar absorptivity of the complex; ε_A is the molar absorptivity of the acceptor; ΔA is the absorbance difference of the acceptor with and without the donor compound at the longest wavelength of absorption maximum of the complex. A plot of $1/\Delta A$ against $1/[D]_0$ should be linear, and yield K as the intercept/slope ratio and ε_{DA} as $\varepsilon_A + 1/\text{intercept}$ $[A]_0$.

Fluorescence quenching method

Fluorescence spectra of FMN or FAD with and without the donor compound were obtained with a Perkin-Elmer fluorescence spectrophotometer model MPF-2A equipped with a constant temperature jacket. The concentration of the flavin derivatives was 5.6×10^{-6} M and the concentration range of the donor was 6×10^{-4} to 1×10^{-2} M. The excitation wavelength was set at 460 nm. Quenching of flavin fluorescence was followed at 520 nm. The measurements were performed in water and in 0.07 M potassium phosphate buffer solutions at pH 7.

The following equation was used to determine the association constant [6]:

$$I_0/I - 1 = [D]_0 K \quad (2)$$

where I_0 is the fluorescence intensity of flavin, and I is the fluorescence of flavin in the presence of different concentrations of donor compounds. The symbol $[D]_0$ is the initial concentration of the donor compound (methoxyamphetamine).

Results and Discussion

Molecular complex formation of flavin mononucleotide (FMN) with the various methoxyamphetamines in solution alters the characteristic absorption spectrum of the FMN molecule. An example of this effect on the FMN spectrum is shown in Fig. 1. In water solution pure FMN (curve a) shows

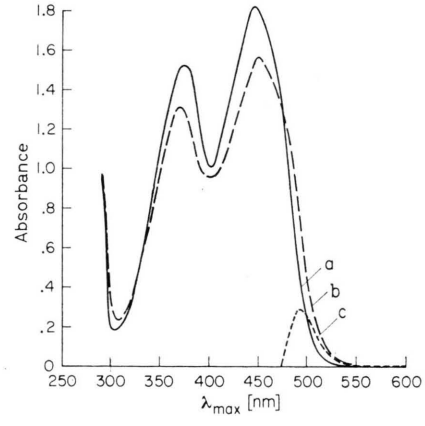

Fig. 1. Absorption spectra of FMN and a complex in water solution: a (———) FMN (1.6×10^{-4} M) against water; b. (— — —) FMN (1.6×10^{-4} M) plus 2,4,6-trimethoxyamphetamine hydrochloride (6.18×10^{-2} M) against water; c. (· · · ·) difference spectrum of FMN-2,4,6-trimethoxyamphetamine, b against a.

two absorption maxima in the near UV region, one at 375 nm, and another at 447 nm. The absorption spectrum of the equilibrium mixture of FMN and 2,4,6-trimethoxyamphetamine is shown as curve b. Complex formation with FMN results in a decrease in the absorption maxima. Since the principal peak of the molecular complex spectrum is masked by the free FMN spectrum, the absorption maximum at longest wavelength of the molecular complex can be obtained by measuring the difference spectrum, which is shown as curve c in Fig. 1. It is known that the lower energy of the absorbing electrons of the complex compared to FMN broadens and shifts the long wavelength FMN band toward the red. Similar changes in the spectra of flavin derivatives when involved in molecular complex formation have been reported previously for other donor molecules [7, 11]. It is observed that at constant concentration of donor and acceptor the intensity of this region increases as the temperature decreases since more complex is formed at lower temperature [10]. There is no methoxyamphetamine absorption in this region. The wavelength of the maximum absorption at longest wavelength of the complex can be measured to an accuracy of ± 0.5 nm.

The wavelengths of the absorption maxima at longest wavelength, the molar absorptivities, and association constants of the molecular complexes obtained from analysis of the spectra of these complex mixtures of FMN and the various methoxyamphetamines are summarized in Table I. Usually when charge-transfer complexes are formed, a new band appears at the longer wavelength and the intensity of the original absorption band decreases. The intensity loss of the original absorption band depends upon the amount of complex formation. Oxidized riboflavin is a weak acceptor and usually does not exhibit a charge-transfer band which could be regarded as evidence for the formation of classical charge-transfer complexes with the aromatic hydrocarbons [5]. For molecular complexes the absorption in the region of 490 nm is probably due to the charge-transfer complex formation. According to the theory of the charge-transfer complex [12, 13], the wavelength of the charge-transfer band for a series of complexes with a common acceptor species increases approximately linearly with the ionization potential of the donor, which is theoretically equal to the energy of the highest filled molecular orbital (E_H). The energy of the highest filled molecular

Table I. The absorption maxima and association constants of complexes between methoxyamphetamine hydrochloride salts and FMN at 25 ± 0.5 °C compared with the energy of highest filled molecular orbital and hallucinogenic activity of various methoxyamphetamines.

Donor compound	Absorption method			Emission method			Hallucinogenig activity [Mescaline units] [15]
	λ_{max} [nm]	ε_{DA} [l/mol,cm]	K † [l/mol]	K ‡ [l/mol]	K † [l/mol]	E_H [atomic units] [14]	
Amphetamine sulfate	487.0	5149	8.8	1	1.1	−0.5885	0
Isopropylamine HCl	—	—	—	—	0.07	?	0
2-Methoxyamphetamine *	488.5	5949	10.8	36	47	−0.5371	?
3-Methoxyampletamine *	487.5	5342	21.2	43	54	−0.5316	?
4-Methoxyamphetamine	488.1	5511	26.4	52	64	−0.5262	5
4-Methoxyphenylethylamine HCl	488	5922	19.5 (12.7) §	—	—	?	?
2,3-Dimethoxyamphetamine	487.5	5240	15.2	37	43	−0.5346	?
2,4-Dimethoxyamphetamine	490.5	6147	46	89	103	−0.5194	5
2,5-Dimethoxyamphetamine *	493.9	5735	41	81	110	−0.5012	8
2,6-Dimethoxyamphetamine	489.5	5760	58.6	82.5	104	−0.5334	?
3,4-Methylenedioxyamphetamine	491.1	6086	20.4	60	70.2	?	3
3,4-Dimethoxyamphetamine	491.1	5688	46.6	81	92	−0.5238	<1
3,5-Dimethoxyamphetamine	488.6	5627	66.9	95.9	109	−0.5240	?
2,3,4-Trimethoxyamphetamine *	488.9	5417	15	38	43	−0.5274	<2
2,3,5-Trimethoxyamphetamine *	489.7	5303	33.6	59	81	−0.5026	4
2,3,6-Trimethoxyamphetamine *	492.7	5387	30.1	47.5	79	−0.5112	13(<10)**
2,4,5-Trimethoxyamphetamine	496.8	5266	119.6	154	185	−0.5001	17
2,4,6-Trimethoxyamphetamine	492.1	6118	127.1	177	192	−0.5217	10 (12)**
3,4,5-Trimethoxyamphetamine	490.0	5728	40.7	60	87	−0.5218	2.2
Mescaline hydrochloride	489.6	5790	32	69.1	77	−0.5226	1

* Oxalate salt. † In water. ‡ Phosphate buffer, pH 7. § In 50% ethanol. ** Reported by Kalbhen [16].

orbital of methoxyamphetamines has been reported by Kang and Green [14]. A correlation between the energy of the highest filled molecular orbital of methoxyamphetamines and the longest wavelength of the maximum absorption of FMN-methoxyamphetamine complexes has been found and indicates that the new maximum absorption band of the complex is a charge-transfer band or at least in part due to the charge-transfer. There is no new absorption band in the region of 490 nm for isopropylamine hydrochloride and riboflavin mixture.

Kang and Green [14] found some correlation between the energy of the highest filled molecular orbital and the hallucinogenic activity of methoxyamphetamines measured by Shulgin *et al.* [15]. In comparing the activity of the methoxyamphetamines and the wavelength of the absorption band of the complexes in the region of 490 nm, a good correlation is observed. The activities for 2,4,6-trimethoxyamphetamine and 2,3,6-trimethoxyamphetamine reported by Kalbhen [16] were used for the correlation. The best fit calculation by the least-squares method gives the equation:

$$\text{Activity} = 1.59\,\lambda - 776.28$$

with the statistical significance $r = 0.832$, $p < 0.001$. This result indicates that the electron donating ability of the methoxyamphetamine is related to the biological activity of the methoxyamphetamines.

It has been proposed [17] that steric requirements are also an important factor for psychotropic activity. The association constants for donor-acceptor molecular complexes are not only related to the electronic properties of the two components but also related to the steric structure of the donor and acceptor molecules [18]. The association constants (stability) of the complexes of methoxyamphetamines and an electron acceptor may relate directly to the biological activity of the amphetamine derivatives if the electron acceptor has electronic and steric properties similar to those of the physiological receptor site.

The association constants for the complexes of methoxyamphetamines and FMN were determined by the absorption method as well as by the fluorescence quenching method. It was found that the values of the association constants determined by fluorescence quenching are greater than those determined by the absorption method. Several mechanisms which appear to operate in the quenching of fluorescence of aromatic molecules in solution are as follows: a. electronic energy transfer, b. charge-transfer interactions, c. chemical reactions, d. collision, e. heavy atom or paramagnetic molecules due to intersystem crossing enhanced by spin orbit coupling. The charge-transfer and electron-transfer mechanisms of fluorescence quenching have been extensively investigated by Weller and co-workers [19, 20]. The quenching efficiency of both mechanisms depends on the oxidation and reduction potentials (ionization potential and electron affinity) of donor and acceptor. The absorption and fluorescence spectra of the FMN and methoxyamphetamine mixture are stable in the experimental condition. Chemical reactions seem unlikely. In the presence of very high concentrations of donor compounds, the plot of $(I_0/I) - 1$ against $[D]_0$ shows some deviation from linearity, but the quenching efficiency is higher at the lower temperatures. Therefore, quenching by collision is involved but is not an important factor. Oxygen, which provided the only paramagnetic molecules in the system, is not a good quencher in aqueous solution. In our experiment no new emission band was observed in the complex formation. The higher values of association constants from fluorescence quenching may be due to the fact that association constants determined by fluorescence quenching may result from a combination of charge-transfer, electron-transfer, and some other quenching effect such as collision quenching, whereas the association constant determined by the absorption method is only a measurement of the absorption of the new species, presumably the charge-transfer complex. A very good correlation between the association constant determined by these two methods can be expressed as:

$$K(\text{fluorescence quenching}) = 1.372\,K(\text{absorption}) + 29.83.$$

The experimental error for the fluorescence quenching and absorption methods is believed to be about 5% and 10%, respectively.

The complex formation might be charge-transfer in nature, but other binding forces may also be involved. The association constants determined by the emission method in buffer solution are lower than those determined in pure water solution, but the relative values are about the same as shown in Table I. Phosphate salts in buffer solution will increase the ionic strength of the solvent and will

decrease the formation of charge-transfer complexes and decrease the stability of the complex. It can be seen in Table I that changing solvent from water to 50% ethanol decreases the association constant for 4-methoxyphenylethylamine. Hydrophobic forces between the aromatic compounds may play a role in aqueous solution.

In general, the more methoxy groups on the amphetamine ring, the higher the association constant of the complex. This relationship indicates that electrostatic forces may be important in the complex formation. The steric hindrance effect also plays an important role in the complex formation. With two methoxy groups on the amphetamine ring adjacent to each other, especially on the 2 and 3 positions, the stability of the complexes decreases drastically.

The relationship between the hallucinogenic activities of the methoxyamphetamines and the association constants of the complexes between FMN methoxyamphetamines in water, as determined by the fluorescence quenching method, is shown as a correlation equation:

$$\text{Activity} = 0.082\,K - 2.34$$

The correlation is significant ($r = 0.847$, $P < 0.001$). Since FMN is not the physiological receptor site and has a different steric structure from the real receptor site, we should not expect a perfect correlation between activity and the stability of the complex. The deviation of some of the compounds such as 3,4-dimethoxyamphetamine and 4-methoxyamphetamine from the correlation line illustrates the apparent differences in steric requirements between the drug-FMN and drug-receptor complexes.

Table II shows the stability constants and other thermodynamic parameters for the complexes between FAD and some methoxyamphetamines. The stabilities of the amphetamine-FAD complexes are generally lower than those of the amphetamine-FMN, since FAD is a weaker acceptor. The thermodynamic parameters of the methoxyamphetamine-FAD complexes are similar to those of tryptophan-FMN [7]. The negative values of ΔH means that the association constants are higher at the lower temperatures (or the complexes are more stable at the lower temperature).

In conclusion, the complex formation between methoxyamphetamines and FMN or FAD is charge-transfer and electron-transfer in nature, although other binding forces such as hydrophobic, electrostatic or van der Waals forces may be involved in the complex formation. The steric hindrance effect also plays an important role in the complex formation. We have found correlations between the stabilities, or absorption maxima in the longest wavelength for the complexes and the biological activities of methoxyamphetamines. Although we cannot predict the structure of the physiological receptor site at the present time, flavin derivatives appear to simulate the electronic and steric structure of the real receptor site to a degree. Complex formation with the receptor site appears to be a major factor contributing to the physiological activity of methoxyamphetamines.

Table II. Thermodynamic parameters for the formation of FAD-methoxyamphexamine complexes in phosphate buffer solution at pH 7 determined by fluorescence.

Donor compound	K [l/mol]	ΔF°_{298} [kcal/mol]	ΔH°_{298} [kcal/mol]	ΔS°_{298} [cal/mol·°C]
4-Methoxyamphetamine	42.3	−2.227	−1.48	2.53
2,4-Dimethoxy-amphetamine	75.5	−2.548		
2,5-Dimethoxy-amphetamine *	75	−2.544		
3,4-Dimethoxy-amphetamine	55.5	−2.374	−3.03	−2.15
2,3-Dimethoxy-amphetamine	32	−2.043		
2,3,5-Trimethoxy-amphetamine *	54	−2.338	−1.90	1.55
2,3,6-Trimethoxy-amphetamine *	59.5	−2.408		
2,4,5-Trimethoxy-amphetamine *	134	−2.904	−4.30	−4.75
2,3,4-Trimethoxy-amphetamine	34.5	−2.087		
2,4,6-Trimethoxy-amphetamine	152.5	−2.974	−4.38	−4.65
2,4,5-Trimethoxy-amphetamine	57.5	−2.396	−3.51	−3.75

* Oxalate salt.

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