Biophysical Chemistry, 32 (1988) 229–237
Elsevier

BPC 01328

Sequence-dependent emission from stacked forms of ApC and CpA

Evidence for stacked base ('dimer') absorption and left-handed stacked conformation *

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Received 5 December 1987
Revised manuscript received 28 April 1988
Accepted 2 September 1988

Sequence-dependent emission; Excimer emission; Dimer emission; Stacked absorption; Adenylyl-3′,5′-cytidine; Cytidylyl-3′,5′-adenosine

The corrected and normalised emission spectrum, quantum yield and emission anisotropy are reported for partially stacked adenylyl-3′,5′-cytidine (ApC) excited at 266 nm and are compared with cytidylyl-3′,5′-adenosine (CpA). Utilizing characteristics determined independently for adenosine and cytidine-5′-monophosphate (CMP), the concurrent self-consistent resolution of emission spectrum and emission anisotropy has been carried out in two ways; first completely empirically as for CpA and second on the basis of a simple stacking model, with concordant results. The total emission spectrum of ApC is resolved into (i) components characteristic of the two monomers and (ii) a red-shifted complex emission. The complex emission spectrum, which is much stronger than from CpA and is complementary to it in bandshape, can also be satisfactorily described by the same components. The dimeric (A, C) system can exist in at least two luminescent stacked forms, proportions of which are determined by the overall stacked fraction and the population within this fraction of the various stacked conformers. The relation between the ratios of the components and the fractional absorption of the stacked forms indicates that the low-energy component is a (hetero-) dimer emission while the high energy component appears to be a true exciplex (hetero-excimer). Comparison with the circular dichroism and NMR literature shows a satisfactory semi-quantitative correlation with the hypothesis that the low energy (hetero-) dimer emission originates from a left-handed stacked conformation M^{bb}, while the higher energy hetero-excimer originates from the predominant right-handed stacked conformation P^{ba}.

1. Introduction

The dinucleoside phosphates have considerable interest as the simplest polymeric forms of the monomer constituents of nucleic acids and the elucidation of their structures and conformation in aqueous solution has been the object of much effort. It was early recognized that the stacking forces which stabilize the characteristic helical structures of nucleic acids lead to an equilibrium distribution of partially ordered structures in the dinucleoside phosphates and the presence of such ordered structures in solution has been inferred from NMR shifts and UV absorption changes (hypochromicity and CD). The proximity of the bases in some ordered structures leads to exciton interaction in the excited state which is responsible for CD absorption, while in emission characteristic spectra are observed, considerably red-shifted from the monomers. Until very recently [1]

* Presented in part at the Second European Conference on the Spectroscopy of Biological Molecules, Freiburg, F.R.G., 6–10 September 1987.

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Abbreviations: ApC, adenylyl-3′,5′-cytidine; CpA, cytidylyl-3′,5′-adenosine; CMP, cytidine-5′-monophosphate; ORD, optical rotatory dispersion; CD, circular dichroism; Cyd, cytidine; Ado, adenosine; mp$\overline{C}$-$\overline{A}$, 5′-methylphosphate-N^4-dimethylcytidylyl-(3′-5′)-N^6-dimethyladenosine; $\overline{C}$-$\overline{C}$-$\overline{A}$, N^4-dimethylcytidylyl-(3′-5)-N^4-dimethylcytidylyl-(3′-5′)-N^6-dimethyladenosine.

such spectra have commonly been *assumed* to be excimeric in nature (exciplex for dissimilar monomers), i.e. to originate in transitions from a bound excited state to a dissociative ground state. As a consequence of this the excimer state, having no corresponding bound ground state, cannot be detected in absorption and in stacked systems such as we are concerned with the experimental criterion for assignment of an emission as excimeric has been based simply on observation of a red-shifted broad emission spectrum clearly different from monomer emissions yet resulting from absorption in the monomer region.

This position, taken in our earlier papers [2] and in fact in all previous work with nucleic acid systems [3] is consistent with the common understanding of absorption processes in DNA in which the bases absorb independently but with reduced intensity relative to the monomers. The changes in overall absorption between monomers and oligomers are thus dominated by the hypochromic effect, and in fact there has been no direct observation of exciton splitting in nucleic acid systems. However, our work on the emission spectroscopy of CpA has led us to conclude that emission from the stacked fraction has a corresponding ground state absorption associated with it, quite independently of the unstacked monomer absorptions of C and A, and the question then arises as to whether the complex emission originates from hypochromic absorption of stacked C and A or whether the stacked pairs can be regarded as ground-state dimers, so that the corresponding emission is 'excited-dimer' rather than 'excimer' or 'exciplex'. (Although the term 'excimer' originated as a contraction of *exci*ted-di*mer*, it is commonly used in a restricted sense (due to Birks) as an excited dimeric state formed from an excited monomer, and hence having no corresponding ground state so that the reverse transition to ground is dissociative.)

In the present paper we report on the excited state behavior of ApC, the sequence isomer of CpA, for which the degree of stacking in solution probably differs from CpA, while on the basis of X-ray based RNA-type stacking structure the stacking geometry is also anticipated to be different. We find further evidence that the emission from the stacked fraction in dinucleoside phosphates is complex and show that it can be most simply treated as being two-component. Comparison of ApC with CpA shows that both complex emissions are complementary to each other and correlation with the fractional absorptions of the stacked forms indicates that one component, at the lower emission energy, is a (hetero-) dimer while the other seems to be a true exciplex.

If the two emissions originate from different stacking conformations, then CD and NMR work suggests that the low-energy dimer emission comes from a left-handed M^{bb} conformation, while the exciplex originates from the predominant right-handed P^{ba} stack. A single value of an absorption/emission parameter is sufficient to correlate our results for both emissions in ApC and CpA with the CD/NMR population analysis and thus lends support to the hypothesis of different conformational origins.

2. Materials and methods

Experimental procedures were exactly the same as our preceding paper on CpA [1] and so are not described in detail. Background spectra were collected immediately before and after the sample emission runs, and showed only solvent Raman scatter and a very weak apparent emission ~ 340–350 nm which disappeared on applying spectral correction procedures. This indicates the absence of luminescing impurities in the solvent system (triply-distilled water $+10^{-2}$ M phosphate buffer, pH 6.8). The ApC, CMP and adenosine were Calbiochem and Sigma products and the concentration of ApC was 5×10^{-5} M, thus avoiding any problem of self-association.

The final experiment results which ensue are: (i) Digital emission spectra, corrected for the combined spectral response of the emission double monochromator (Baird-Atomic, SF-100) and dry-ice cooled photomultiplier (EMI 6256 S), and presented normalized; (ii) emission quantum yields from numerical integration of the spectra, ratioed to a convenient secondary standard (thymine, $\phi_f = 1.02 \times 10^{-4}$ [4]; (iii) emission anisotropy spectra, at the same resolution as the emission spectra,

corrected for instrumental artifacts by using tryptophan fluorescence as a source of depolarized radiation. Multiple scanning (Nicolet 1072) was used in collecting the adenosine polarization data because of the low quantum yield (see below) and the final results for the monomers were smoothed (Savitzky-Golay, 5 point cubic) before use in curve-resolution and modelling procedures.

3. Results

3.1. Emission spectra

The reported emission spectrum was observed only on exciting at various wavelengths in the absorption band. Some variation in emission profile was observed but weakness of source intensity in the UV below 300 nm together with low quantum yields prevented the acquisition of excitation spectra in which small changes could be considered significant. Consequently this report is restricted to effects observed on exciting at a single wavelength (266 nm), for consistency with previous work on ApA and CpC systems [1,2,5]. The issues this raises are discussed again in the ultimate paragraph of the paper, but for the moment we make the point that the spectral resolutions which we obtain are consistent with previous work and are internally consistent with the anisotropy results and hence are not likely to be due to adventitious impurities. The corrected normalized total emission spectrum of ApC excited at 266 nm and pH 6.8 is shown in fig. 1 and is seen to be quite different from its sequence isomer CpA. The emission spectrum of CMP has been presented previously [5] and is essentially identical with that of Cyd given by Shaar et al. [1]. Similarly the emission spectrum of Ado is virtually identical with AMP (also shown by Shaar et al. [1]). The emission of those 'monomers' is responsible for the peak in the CpA spectrum around 320 nm and we may reasonably anticipate that it is responsible for the shoulder at the same wavelength in ApC. This will be born out by data analysis below. The striking feature of the emission of ApC is the broad peak around 360 nm, which is hardly noticeable in the overall emission of CpA, but there is also a weaker component in the region centered on 440 nm, as in CpA.

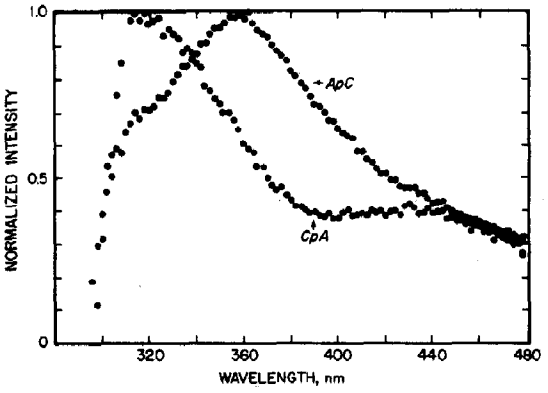

Fig. 1. Corrected normalized emission spectra for ApC and CpA; λ_{ex}266 nm, pH 6.80.

3.2. Polarization of emission; emission anisotropy

The polarization of the total emission from ApC is shown in fig. 2a (upper curve), while the anisotropies of CMP and Ado (not shown) are very similar to those of Cyd and AMP presented by Shaar et al. [1]. The anisotropy of ApC is significantly different from CpA (fig. 2b). The peak in ApC is weaker and sharper due to the more rapid decrease to longer wavelengths. This can be correlated with the more prominent role of the depolarized (see below) 360 nm component in emission. It will be shown below that these distinctive features can be quantitatively accounted for by the multi-component nature of the emission from the partially stacked ApC system, in which the intrinsic anisotropies of the monomers are weighted by their emission bandshapes and fractional contributions, while the overall emission is dominated by a depolarized exciplex fluorescence.

3.3. Quantum yields

The observed quantum yields for ApC and its monomeric constituents are listed in column 2 of table 1. The significant result here is that the emission from ApC is twice as strong as from CpA. As before ϕ(CMP) and ϕ(Ado) in column 2 have been determined for solutions containing

only CMP and Ado respectively, at the same absorbance as the ApC system.

In summary, the observed spectral bandshapes, emission quantum yields and emission anisotropies are distinctively different for the sequence isomers ApC and CpA. However, it will be shown by self-consistent band resolution and modelling that there is an underlying unity in these results.

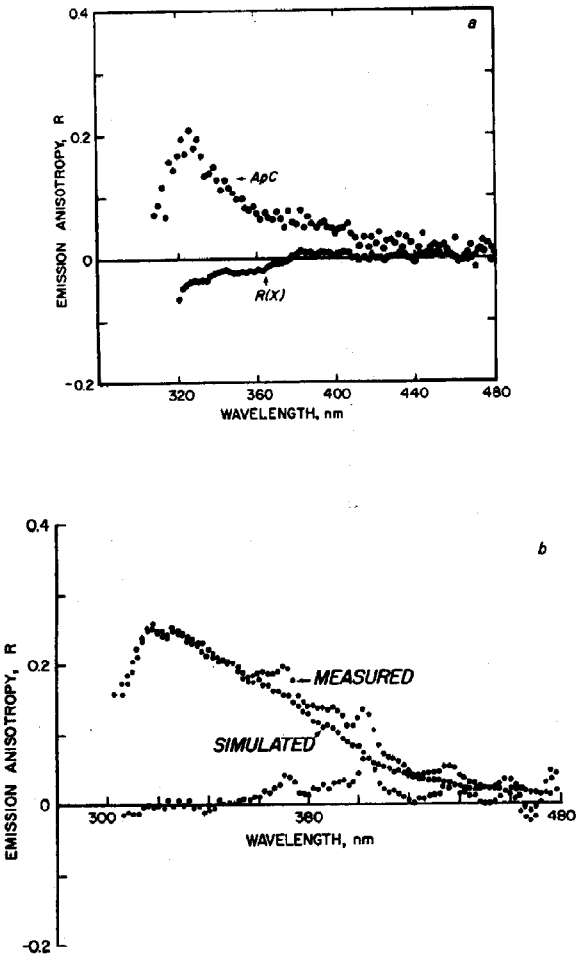

Fig. 2. (a) Upper curve, emission anisotropy for ApC, experimental and simulated data superimposed. Lower curve; emission anisotropy of complex emission $R(X)$ which results from data treatment of text. (b) Upper curve, emission anisotropy for CpA, experimental and simulated data superimposed; lower curve, emission anisotropy of complex emission, $R(X)$. Data from ref. 1.

Table 1

Fractionation of the overall quantum yield of ApC emission, based on the spectral resolution of fig. 3

	ϕ [a,e]	f_{em} [b]	ϕ' [c,e]	f_{abs} [d]
ApC	16.7			
CMP	10.9	0.22	3.67	0.34
Ado	5.0	0.16	2.67	0.53
Complex (X)		0.62	10.35	0.13

[a] Observed total quantum yield.
[b] Fraction of emitted photons integrated over all wavelengths.
[c] Apparent quantum yield of this species in the ApC system.
[d] Fraction of absorbed photons (see text).
[e] $\times 10^{-5}$.

4. Discussion

4.1. Emission and resolution

The general picture obtained from inspection of the emission spectra and anisotropy spectra (figs. 1 and 2) is that the emission from partially stacked ApC is multicomponent and consists of 'monomer' emissions from CMP and Ado, together with a complex emission. The former are polarized, consistent with their picosecond lifetimes, while the complex emission appears to be largely depolarized. It is obviously of interest to resolve the observed total emission spectrum into physically significant components, for comparison with CpA.

Resolution of the overall emission from ApC has been carried out in two ways. The first is an entirely empirical procedure, as was carried out for CpA and is described in detail in ref. 1. The corrected normalized emission spectra of CMP and Ado have been taken as 'library' spectra and assigned wavelength-independent weighting factors W_C and W_A; these same factors are then required to give a fit to the anisotropy spectrum with respect to peak location, peak height and general shape. Initial estimates were obtained by assuming the emission anisotropy of the complex, $R^{\lambda}(X)$, to be zero (rotationally depolarized) at various points across the spectrum, and also by assuming the emission spectrum of the complex,

$f_{em}^{\lambda}(X)$, to be zero at certain wavelengths and *an iterative procedure was developed for simultaneously fitting both the emission spectrum and the anisotropy spectrum.* It rapidly became apparent that only a narrow range of factors could fit both the emission spectrum of ApC and the anisotropy spectrum, and when this was done the ratio of these factors, W_C/W_A, was almost the same as that found to give a fit to the very different emission and anisotropy spectra of CpA. This can be understood if the C and A components of the unstacked fraction of ApC behave identically with those of the unstacked fraction of CpA, almost as if they are isolated monomers (because of their picosecond lifetimes). This has led us to develop the second method, a model-based resolution procedure. In this, a dinucleoside phosphate is modelled as a two state system, stacked/unstacked, depending on the presence or absence of base–base interactions. Naturally, there can be several conformers existing in each state and it is particularly important to note that this allows the possibility of multi-component emission from the stacked fraction if each conformer has a distinct geometry and transition probability. From this model, the relative contributions of C and A should be independent of sequence, i.e. the same for ApC and CpA, as has been observed empirically. Furthermore, provided monomer emission originates only from the open, unstacked fraction then W_C/W_A can, ab initio, be set equal to $[f_{abs}(\text{CMP}) \cdot \phi(\text{CMP})]/[f_{abs}(\text{Ado}) \cdot \phi(\text{Ado})]$, where the fractional absorptions, f_{abs}, are derived from molar absorbances and the quantum efficiencies, ϕ, are reported in this paper. Such a procedure is computationally simpler and more rapid than arbitrarily searching for a best fit and we find it leads to the same result. The quality of the fit to the anisotropy spectrum obtained by this nonadjustable procedure is shown in fig. 2a where the upper anisotropy curve is a superposition of experimental and simulated points. A consequence of this modelling procedure is that the anisotropy of the complex emission $R^{\lambda}(X)$ is obtained directly without any further assumptions (lower curve of fig. 2) and can be seen to be close to zero over a large part of the spectrum, as expected.

The resolution of the emission spectrum of ApC resulting from the above procedures is shown in fig. 3 from which we see that it is dominated by a broad asymmetric 'excimer-like' component peaking at 360 nm, apparently quite different from the CpA complex which peaks at ~ 440 nm. The agreement between the empirical weighting factors for ApC and CpA and the 'a priori' model factors lends support to the model and our use of it later (see below) for interpreting the relation between absorption by the stacked fraction and multi-component emissions.

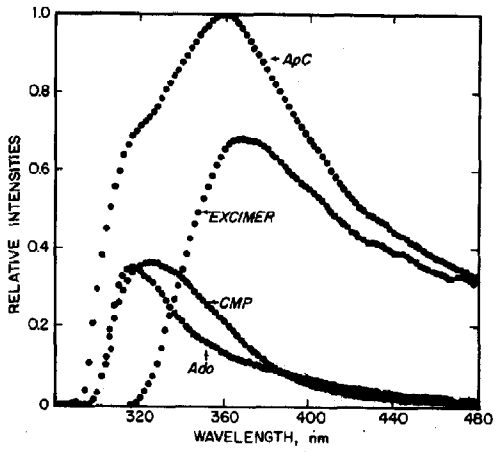

Fig. 3. Measured total emission spectrum of ApC and its resolution into monomer components (Ado and CMP) and complex component (labelled 'excimer').

4.2. Quantum yields

Integrating the resolved spectra of fig. 3 and taking account of the Gaussian resolution of fig. 5 allows us to assign fractional emissions to the C, A and complex components, column 3 of table 1. These values can then be used to partition the overall quantum yield, ϕ(ApC) into apparent quantum yields, ϕ', of the components (column 4, table 1). If we now follow the model of independent behavior of the unstacked and stacked fractions of ApC then the fractional absorptions of C and A in ApC can be obtained, for example, from $\phi'(C)/\phi(C) = f_{abs}(C)$, column 5. As in the CpA case, $f_{abs}(C) + f_{abs}(A) \neq 1.0$ and thus obtain the fractional absorbance of the stacked fraction in ApC, $f_{abs}(X) = 0.13$, which is correlated with the

Table 2

Parameters of stacked ApC and CpA

	ApC	CpA
Fractional absorption, $f_{abs}(X)$	0.13	0.33
Fractional total emission of X, $f_{em}(X)$	0.62	0.38
Fractional emission of X_1, $f_{em}(X_1)$	0.28	0.29
Fractional emission of X_2, $f_{em}(X_2)$	0.34	0.09

complex emission. As pointed out previously [1] this raised the possibility that X* may be an excited dimer and not an exciplex. However, the situation is not quite as simple as this because decreased fractional absorption by X in ApC compared to CpA (0.13 vs. 0.33) is accompanied by increased X* emission (table 2). Clarification follows when the complex nature of the emission spectrum of X is realized and discussion of this point is taken up after the next section.

4.3. Emission spectrum of the complex; comparison with CpA

The emission spectrum of the stacked fraction of ApC which emerges from these self-consistent treatments (labelled 'excimer' in fig. 3) is quite unsymmetric and very broad, extending from 33000 cm^{-1} to beyond 21000 cm^{-1} (limitations of the present apparatus prevent us from obtaining reliable data at lower wave numbers at present). The only obvious similarity with the corresponding complex emission from CpA is that the peak of the ApC lies close to the poorly resolved minor peak of CpA. In the case of CpA, an attempt to resolve the band envelope into Gaussian components was reasonably successful and we have followed the same procedures here, viz. starting by fitting the first major feature at ~ 360 nm, then the shoulder at ~ 450 nm. As a result of this some surprising similarities with the CpA resolution emerge (fig. 4). First, the envelope cannot be fitted by two Gaussians: a minimum of three are required. Second, the Gaussians which give the best fit lie at practically the same position as for CpA. The origin of the very different bandshapes of ApC and CpA is then simply accounted for by the relative intensities (magnitudes) of these three Gaussians. However, caution must be exercised in interpreting such results. In itself this sort of analysis does not mean that the Gaussians necessarily represent three physically distinct emissions, though this is a possibility. It is noticeable that the Gaussians located at ~ 350 nm and ~ 385 nm bear essentially the same integrated ratio in both ApC and CpA. Hence the *simplest* multicomponent description of the complex emission is in terms of two components, the lower energy one at ~ 445 nm being described by a single Gaussian and the higher energy one at ~ 365 nm being described by a sum of two Gaussians. But the possibility that the three Gaussians correspond to three real emissions, two of which bear a fixed relationship to each other, exists and

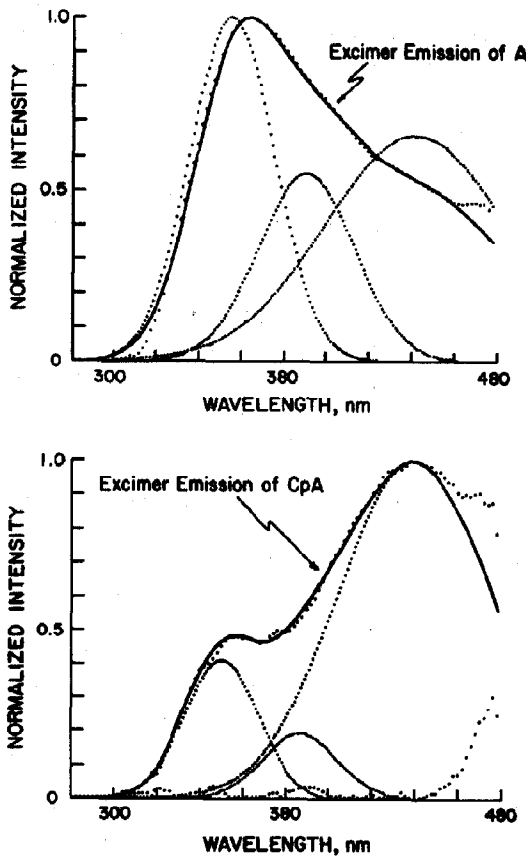

Fig. 4. Resolution of 'excimer' emissions of ApC and CpA into Gaussian components. Full line is calculated band envelope in each case. CpA data taken from ref. 1.

cannot be eliminated on the evidence presently available. Resolution of this problem will require the input of other independent experimental evidence such as may be obtained from high resolution lifetime measurements at appropriate wavelengths. This approach is being actively pursued but for the present we continue the discussion in terms of the minimum hypothesis.

4.4. Origin and nature of the complex emissions from ApC and CpA; applicability of the stacking model

We have previously suggested that the multicomponent nature of the complex emission from CpA may find its origin in the existence of several (two or three) different base stacking arrangements in the ground state, and have associated the phenomenon with absorption by the stacked fraction. Whilst still generally applicable to ApC, this point of view must be modified to take account of both the similarities and differences between the results for these sequence isomers. First, the close similarity of the components suggests that we are observing the same excited states in ApC and CpA. However, the relation between the fractional absorption due to the stacked fraction, $f_{abs}(X)$, and the distribution of emission components is so different for CpA and ApC as to force a re-evaluation of the assignment of the nature of these emissions. Referring to the lowest energy (Gaussian) component as X_1 and the (non-Gaussian) higher-energy component as X_2, table 2 shows that X_1 is 45% of total X when $f_{abs}(X)$ is 13% (in ApC) and increases to 77% when $f_{abs}(X)$ increases to 33% (in CpA). This is qualitatively consistent with the assignment of X_1 as an 'excited-dimer'.

Table 3

Survey of fractional extent of stacking, f_s, for ApC and CpA (neutral aqueous solution, 20 ° C)

ApC	CpA	Method	Reference
0.48	0.49	ORD/CD	Brahms et al. [6]
0.39	0.26	ORD/CD	Davis and Tinoco [7]
0.38	0.24	NMR	Lee et al. [8]
			Ezra et al. [9]
	0.78	UV hypochrom.	Watts and Tinoco [10]
0.45	0.42	UV hypochrom.	Frechet et al. [11]
0.48	0.32	CD	Bobruskin et al. [12]

However, the relation of X_2 to $f_{abs}(X)$ is the inverse of this; as $f_{abs}(X)$ decrease, X_2 emission as a fraction of total X increases from 23% to 55%. This suggests to us that the X_2 component(s) may be 'exciplex' in nature, originating from that part or conformation of the stacked fraction which has the geometry giving rise to hypochromic intensity interaction in the bases, while the X_1 component arises by excitation of a dimeric ground state with a presumably different stacking geometry.

On this basis our results can be understood if both ApC and CpA each exist in two stacking arrangements, S_1 and S_2, of which S_1 leads to 'excited-dimer' emission X_1, while S_2 leads to 'exciplex' emission X_2. This model is summarized in fig. 5.

The differences in the luminescence behavior of ApC and CpA are thus seen to depend on several factors. First and most obvious is the overall extent of stacking, as determined by UV hypochromicity effects and CD measurements of base interaction, and indirectly by NMR. It is well known that results in the area constitute a problem in themselves, as reference to table 3 shows.

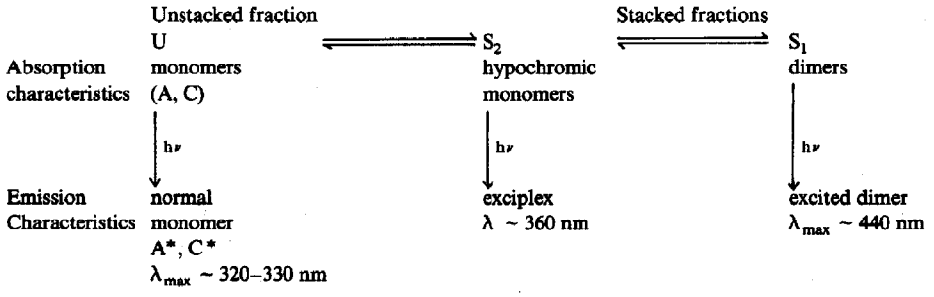

Fig. 5. Stacking equilibria, absorption processes, and complex emissions of dinucleoside phosphates.

In particular the values reported for CpA cover a considerable range and the reasons for this are not obvious. If there were only one base-stacked conformation having a significant probability of emission then the stacking fraction would be a sufficient measure of the difference between ApC and CpA. But even the variable results of table 3 show this to be inadequate because the ratio $f_s(\text{ApC})/f_s(\text{CpA})$ is in general < 1.6 while the integrated complex emissions are in the ratio of 4:1. If the stacked fractions are multi-component, as seems necessary to explain our results, then the fractional populations of the components S_1 and S_2 are required. Furthermore, if these conformations undergo different types of excitation process, they must in general be ascribed different excitation probabilities, $\epsilon(S_1)$ and $\epsilon(S_2)$. Lastly, the emission probabilities of S_1 and S_2 will also differ from each other. Obviously such a multiplicity of parameters cannot be evaluated from the present information, but it is possible to show that there exists a simplification in which the intrinsic absorption and emission parameters of the stacked components of ApC appear to have the same ratios as the stacked components of CpA. (This is consistent with their assignment as identical states, see above.) To demonstrate this we have first to consider the stereochemistry of the stacked bases of dinucleoside phosphates.

In searching for an understanding of the existence of two complex emissions from CpA we [1] realized that the low energy barriers to rotation about the *N*-glycoside bond implied that consideration of possible base-stacked conformers in dinucleoside phosphates should not be limited to the familiar RNA/DNA type structures valid for infinite chains. In particular the 'front' and 'back' faces of the bases are stereochemically inequivalent and this leads to the possibility of four distinct types of stacked conformations of any base pair which we described as front-front, front-back, back-front and back-back. Since then we have rather belatedly become aware that the same concept had been realized earlier by Lysov et al. [13] who had set up standard definitions for 'a' and 'b' sides of the bases. When in solution the two possible directions of rotation of stacked bases relative to each other are considered (right-handed, positive rotation P, and left-handed, negative rotation M), this leads to eight types of base-stacking arrangement. Extensive work by this group has included computation of energies of conformers of ApA, ApC, CpA and CpC by the method of atom-atom potentials [13,14], consideration of the transfer of such results to a room temperature aqueous solution situation and iterative comparison with CD and NMR parameters [12] leading to the establishment of the major closed (stacked) conformers, their geometry and relative populations. We find that the conclusions of this group can provide an explanation for our results. In brief, Bobruskin et al. find that the main closed conformer in both ApC and CpA is P^{ba}, a right-handed form. In addition, both contain small amounts of left-handed forms, ApC having M^{ab} and M^{bb} while CpA has M^{bb}. If ApC and CpA have only two luminescent forms in common, suggested to be P^{ba} and M^{bb}, then values can be found in the population results consistent with the relative intensities of our X_1 and X_2 emissions. Thus, for ApC one possible population ratio is $[M^{bb}]/[P^{ba}] = 11\%/63\% = 0.175$. Experimentally we find the ratios of emission intensities to be $X_1/X_2 = 0.82$ (table 2). Accordingly the radiation parameters (absorption coefficients and quantum emission efficiencies) relating these two ratios are encompassed by the factor 4.70. For CpA, $[M^{bb}]/[P^{ba}] = 40\%/60\% = 0.67$ and with the same radiation parameters an emission intensity ratio X_1/X_2 would be expected to be $0.67 \times 4.70 = 3.15$. Experimentally we find $X_1/X_2 = 3.22$, in substantial agreement.

Thus the drastic change in the X_1/X_2 intensity ratio between ApC and CpA as well as the spectral similarity of the emission profiles finds a ready explanation in the existence of two stacked forms, one of them left-handed, which are the same (or very similar) in ApC and CpA, and whose populations are given by the theoretical, CD, and NMR work of Bobruskin et al. [12].

Further support for the interpretation suggested here is found in the work of Doornbus et al. [15] on the CD and NMR of mpC-A and C-C-A, who find evidence for at least two stacked states, namely a classical right-handed helix and a left-handed anti-parallel one. The left-handed

component is minor in the dimer but actually becomes predominant in the trimer.

In conclusion, this present work has produced evidence that the emission spectra of the stacked fractions of ApC and CpA are multi-component, that the components are associated with right-handed and left-handed stacking geometries, and there are relations suggesting that one of the components may be an 'excited-dimer' and the other(s) may be 'exciplex' in nature. This latter point in turn suggests that there may be different excitation spectra for the components, hidden under the broad uninformative absorption spectra of ApC and CpA. All the work reported here and by Shaar et al. [1] has been carried out under continuous excitation at 266 nm with $\Delta\lambda 6$ nm so as to favor neither A nor C absorption. To search for excitation spectra distinguishing 'exciplex' and excited-dimer' transitions will require a high level of instrumental discrimination including short pulse-width narrow band-width excitation together with time-resolved, polarized, and wavelength-selective detection. Experiments will be carried out to determine the applicability of the model of fig. 5 to these and other stacked sequences when appropriate synchrotron or laser facilities are available.

Addendum

Preliminary time-resolved experiments carried out recently with the ACO synchrotron beam at LURE, Orsay (France) have yielded partially resolved excitation spectra, consistent with the model of fig. 5 (Ding-guo Hu, Malcolm Daniels, Jean-Pierre Ballin and Paul Vigny, work in progress). A full account will be published in due course.

Acknowledgements

This work was supported by NIH grant GM30474. Thanks are also due to Professors D.B. Davies (University of London) and Cornelius Altona (University of Leiden) for their very helpful comments on the interpretation of NMR studies and recent results on NA trimers and dimers.

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