J. Pharm. Pharmac., 1967, **19**, 827–836 Received October 3, 1967

Micelles as simple models of drug receptors

A. H. BECKETT, G. KIRK AND A. S. VIRJI

Some interactions of optically active *N*-alkyl-*NN*-dimethylalanine hydrobromide (alkyl betaine) micelles, representing model flexible drug receptors, with simple "drug" molecules, represented by a number of optically active amino-acids, choline derivatives and a dipeptide have been examined. Comparisons of these "drug-receptor" interactions have been made using a refractive index technique and an explanation of the difference in the degree of adsorption of different "drug" molecules at identical micelle surfaces is advanced.

STEREOSELECTIVE adsorbents (Beckett & Anderson, 1957, 1959, 1960) in which active sites or "footprints" are formed during their preparation may be regarded as elementary models of rigid drug receptors incapable of adaptation to meet the requirements of a substrate (Beckett & Youssef, 1963). Koshland (1958) on the other hand has stressed the importance of flexibility in drug-receptor interactions. We now report some interactions of model flexible drug receptors with "drug" molecules represented by a number of pairs of enantiomorphic amino-acids and related compounds. This work emphasizes the importance of steric factors between the amino- and carboxyl-groups, changes in the size of the cationic head and stereochemical features.

Experimental

DIFFERENTIAL REFRACTIVE INDEX MEASUREMENTS

Materials. *N*-Alkyl-*NN*-dimethylalanine hydrobromides (Beckett, Kirk & Virji, 1967). L-Alanyl-L-alanine (Sigma Chemical Company), and α-amino-acids (Koch-Lights). Dimethylalanine hydrochlorides and dimethylvaline hydrochlorides (Bowman & Stroud, 1950). α- and β-Methylacetylcholine iodides (Beckett, Harper & Clitherow, 1963).

Apparatus. A Hilger Rayleigh Interference Refractometer for liquids (Model M.154) fitted with constant temperature water jacket and tungsten lamp. Cells had path lengths of 1 and 10 cm.

Preparation of betaine-"drug" solutions. Filtered solutions of betaines of the highest required concentration were prepared using double distilled water; from these, dilutions were made as required. All these solutions were mixed with similarly prepared "drug" solutions to give solutions containing betaine and "drug" in the required molar concentrations.

Measurements of differential refractive index. All measurements were made at 20 ± 1°. The zero of the instrument was checked before and at the end of each experimental run. For each measurement, when using a 1 cm cell, a 20 min period was allowed for equilibration of cell and contents; when a 10 cm cell was used, 35 min was allowed. All measurements were made on duplicate solutions and an average of 3 to 4 readings was taken on each.

From the Department of Pharmacy, Chelsea College of Science and Technology (University of London), Manresa Road, London, S.W.3.

CONSTRUCTION OF MOLECULAR MODELS

Catalin models of the "drug" molecules and a portion of the betaine micelle were constructed in the light of the following considerations.

1. The long-chain alkyl group on the betaine nitrogen atom will be orientated away from the aqueous phase. Its conformation will be such that non-bonded interactions are at a minimum and the closest possible "fit" with the long-chain alkyl group of a neighbouring betaine molecule is obtained.

2. The charged carboxyl group and nitrogen atom of the betaine molecule will be orientated towards the aqueous phase. Maximum stability will result when the nitrogen atom of one betaine molecule lies in the closest possible proximity to the carboxyl group of a neighbouring betaine molecule. Carboxyl groups by reason of electrostatic repulsion will be as far as possible from each other on the micelle surface.

3. The methyl group on the betaine asymmetric carbon atom will be orientated in the same direction as the long-chain alkyl group and the hydrogen atom on the same carbon atom will be in the micelle-water interface. The choice of this conformation is supported by critical micelle concentrations (CMC) of *N*-alkyl-*NN*-dimethylglycines and the corresponding *N*-alkyl-*NN*-dimethylalanines. An increase in the length of the long-chain alkyl group of a glycyl betaine by one methylene group results in a decrease in CMC. A decrease in CMC is also observed between a glycyl betaine and its corresponding alanyl betaine (Beckett & others, 1967). It is therefore logical to assume that the decrease in the CMC of the alanyl betaine is due to the methyl group being orientated in the same direction as the long-chain alkyl group.

As a result of the above restrictions, one of the *N*-methyl groups and the carboxyl group of a betaine molecule lie in close proximity at the surface of the micelle while the other *N*-methyl group points away from the carboxyl group leaving a *cavity* which is partially overlapped by the hydrogen atom on the betaine asymmetric carbon atom.

4. The conformation of "drug" molecules will be such as to allow the closest possible "fit" at the betaine micelle surface to bring together charges of opposite character, and the fit will be such that the "drug" carboxyl group and nitrogen atom are directed towards the aqueous phase.

Results and discussion

INTERACTIONS OF ALANINE ENANTIOMORPHS WITH D-BETAINES

Differential refractive index measurements show that solutions containing D-betaine and D-alanine have higher refractive indices than the corresponding solutions of D-betaine and L-alanine. Refractive index is a function of molecular density and polarizability. The difference in interaction of the enantiomorphs with an asymmetric betaine micelle are

considered to arise from differences in density in the packing of the molecules rather than differences in polarizability: the reasons are as follows.

(*a*) When the concentration of a betaine is increased from a value below its CMC to a value above this there is an increase in the slope of the refractive index–concentration curve. Thus when betaine molecules aggregate there is an increase in refractive index. This is more likely to be a density effect than a polarizability effect because the interaction of ionized groups on the surface of the micelle would be expected to make the electron cloud less polarizable than in the discrete betaine molecules.

(*b*) A betaine solution above its CMC in the presence of an amino-acid gives a higher refractive index than the sum of the refractive indices of the separate components in the appropriate concentrations. It is reasonable to predict that the amino-acid adsorbed at the highly polar surface of the micelle would increase the density but reduce polarizability.

(*c*) The fact that stereoselectivity is shown by the betaine micelle for the amino-acid enantiomorphs indicates that the amino-acids must come into close contact with the micelle surface. Any explanation of the refractive index results based upon differences in organization of water molecules rather than interaction of the betaine surface with the amino-acid molecules would not account for the differences obtained with enantiomorphs.

It would appear to follow that a D-betaine-D-alanine complex has a smaller volume to mass ratio than the corresponding D-betaine-L-alanine complex. Thus at the surface of a D-betaine micelle, D-alanine is better adsorbed than is L-alanine. Examination of molecular models supports this conclusion. The hydrogen atom attached to the asymmetric carbon atom of D-alanine can "fit" into the *cavity* between one of the *N*-methyl groups and the carboxyl group of a betaine molecule at the surface of a D-betaine micelle (see Fig. 1 (a) R = Me). With L-alanine the hydrogen atom is now on the opposite side of the molecule and comes into contact with the other *N*-methyl group of the D-betaine molecule, thus preventing

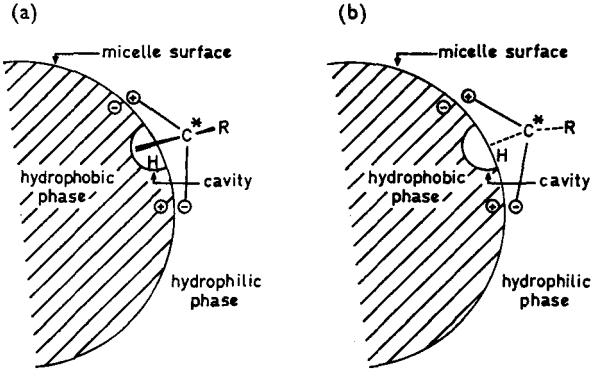
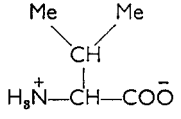


FIG. 1. Diagrammatic representation of amino-acid-betaine interactions. (a) D-Betaine/D-drug. (b) D-Betaine/L-drug. Shaded portion is an equatorial section through two polar groups of a portion of the betaine micelle. C* is the drug asymmetric carbon atom, with thick lines representing bonds above the plane of the paper and dotted lines below.

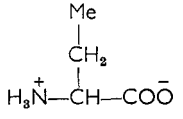
the L-amino-acid from fitting as closely as its enantiomorph at the surface of the micelle (see Fig. 1 (b) R = Me). Refractive index results for other pairs of optically active amino-acids (see Table 1) indicate that D-amino-acids are better adsorbed than their enantiomorphs at the surface of a D-betaine micelle. Examination of molecular models also supports this conclusion.

EFFECT OF INCREASING THE SIZE OF THE "DRUG" α-ALKYL SUBSTITUENT

The difference in refractive index between solutions of D-betaine-D-valine and solutions of D-betaine-L-valine is 127 refractometer units for dodecyl and 131 units for tetradecyl betaine compared with values of 10 and 32 units for the corresponding betaine-alanine solutions (see Table 1). If the difference in volume between a D-betaine-D-valine complex and a D-betaine-L-valine complex is the same as that between a D-betaine-D-alanine complex and a D-betaine-L-alanine complex, then the greater difference in refractive index between betaine-valine complexes could be due to the higher molecular weight of valine compared with that of alanine. Examination of molecular models indicates that a second and probably more important factor is the larger difference in the degree of adsorption of D- and L-valine at a D-betaine micelle surface. In the D-betaine-D-valine complex, the two methyl groups of the isopropyl group of the "drug" molecule can come into close contact with the micelle surface and thus help in binding the "drug" molecule to the micelle surface.



Valine

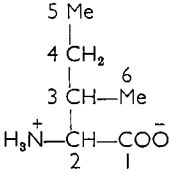


α-Aminobutyric acid

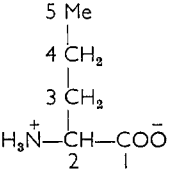
With the D-betaine-L-valine complex the two methyl groups cannot come into contact with the micelle surface. This feature of additional binding of one enantiomorph to the micelle surface is absent in the alanine enantiomorphs. Added support for these conclusions is obtained by examining the adsorption of D- and L-α-aminobutyric acid at the surface of a D-micelle. With these enantiomorphs, the difference in the degree of "fit" at the surface of a micelle would appear to be similar to that obtained with alanine and valine enantiomorphs, but the difference in refractive index between a D-betaine-D-α-aminobutyric acid solution and the corresponding D-betaine-L-α-aminobutyric acid solution is intermediate between the values obtained for alanine and valine enantiomorphs (see Table 1). This may be explained as being due partly to the fact that the molecular weight of α-aminobutyric acid is intermediate between that of alanine and valine and partly to the fact that the terminal methyl group of the ethyl group on the "drug" asymmetric carbon atom of D-α-amino-butyric acid can come into contact with the surface of the micelle thus

assisting in the binding to the surface of a D-betaine micelle. With L-α-aminobutyric acid no such binding is possible.

The difference in the degree of "fit" of the enantiomorphs of isoleucine and of norvaline would appear to be similar to that shown by the isomers of alanine, but the difference in the degree of adsorption in both cases as indicated by refractive index measurements (see Table 1) is less than with alanine isomers. With isoleucine and norvaline, the flexibility of the longer α-alkyl chain allows the terminal methyl group to assist in the binding of both isomers at the micelle surface. With D-isoleucine and D-norvaline, additional binding at the surface of a D-betaine micelle is provided by the 4-methylene group.

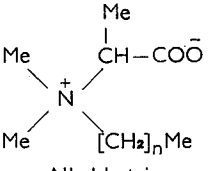


Isoleucine



Norvaline

TABLE 1. INTERACTION OF *N*-ALKYL *NN*-DIMETHYLALANINE HYDROBROMIDES (ALKYL BETAINES) WITH α-AMINO-ACIDS: RESULTS OF DIFFERENTIAL REFRACTIVE INDEX MEASUREMENTS (ΔR)*



Alkyl betaine

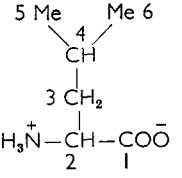
Betaine	Amino-acid	ΔR in scale divisions
Dodecyl (n = 11)	Alanine	10
Tetradecyl- (n = 13)	,,	32
Dodecyl- (n = 11)	Valine	127
Tetradecyl- (n = 13)	,,	131
Dodecyl- (n = 11)	α-Aminobutyric acid	11
Tetradecyl- (n = 13)	,,	82
Dodecyl (n = 11)	Isoleucine	3
Tetradecyl (n = 13)	,,	9
Dodecyl (n = 11)	Norvaline	4
Tetradecyl (n = 13)	,,	5
Dodecyl (n = 11)	Leucine	1
Tetradecyl (n = 13)	,,	4

All solutions contained 0·05 molar concentration of both "drug" and betaine.

Cell path length 1 cm.

ΔR = Refractive index of D-betaine -D-"drug" solution minus the refractive index of D-betaine: L-"drug" solution.

Differential refractive index measurements (see Table 1) indicate little or no difference in the degree of adsorption of D- and L-leucine at a D-betaine micelle surface. This conclusion is supported by examination of molecular models.

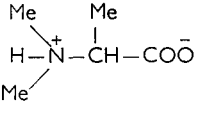


Leucine

In both D- and L-leucine the 5- and 6-methyl groups can assist in binding the "drug" molecule to the micelle surface. The D-isomer receives additional binding from the 4-methine group, but this appears to be the only difference in the adsorption of D- and L-leucine at a D-micelle surface.

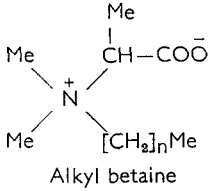
EFFECT OF INCREASING THE SIZE OF THE DRUG CATIONIC HEAD

Solutions containing D-betaine-D-dimethylalanine have a higher refractive index than the corresponding solutions of D-betaine-L-dimethylalanine, the difference in arbitrary units being 4 for dodecyl betaine and 12 for tetradecyl betaine (see Table 2). The corresponding values for D- and L-alanine were respectively 10 and 32.



Dimethylalanine

TABLE 2. INTERACTIONS OF *N*-SUBSTITUTED-α-AMINO-ACIDS WITH *N*-ALKYL *NN*-DIMETHYLALANINE HYDROBROMIDES: RESULTS OF DIFFERENTIAL REFRACTIVE INDEX MEASUREMENTS (ΔR)



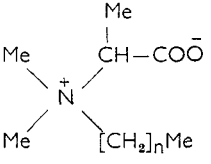
Alkyl betaine

Betaine	Amino-acid derivative	ΔR in scale divisions
Dodecyl- (n = 11)	Dimethylalanine HCl	4
Tetradecyl- (n = 13)	,, ,,	12
Dodecyl- (n = 11)	Dimethylvaline HCl	21
Tetradecyl- (n = 13)	,, ,,	37
Dodecyl (n = 11)	Dimethylalanine methiodide	0
Tetradecyl (n = 13)	,, ,,	0

See footnote to Table 1.

The molecular weight of alanine is less than that of dimethylalanine. A larger difference in refractive index would be expected between solutions of D-betaine-D-dimethylalanine and the corresponding solutions of D-betaine-L-dimethylalanine provided the degree of "fit" of these molecules at D-betaine micelle surfaces is the same as that of alanine enantiomorphs. Since however the differences are smaller with the dimethylalanine enantiomorphs, it is concluded that the "fit" of dimethylalanine is not so close as that obtained with alanine at identical betaine micelle surfaces. Solutions of D-betaine-L-alanine have higher refractive indices than the corresponding solutions of D-betaine-L-dimethylalanine, the difference with the tetradecyl betaine being 60 units (see Table 3). These results indicate that

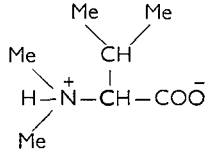
TABLE 3. INTERACTION OF α-AMINO-ACIDS AND *NN*-DIMETHYL DERIVATIVES WITH ALKYL BETAINES: RESULTS OF DIFFERENTIAL REFRACTIVE INDEX MEASUREMENTS (ΔR)



Contents of left hand cell	Contents of right hand cell	ΔR
D-(+)-Betaine (n = 13): L-(+)-alanine	D-(+)-Betaine (n = 13): L-(+)-dimethylalanine	60
D-(+)-Betaine (n = 13): L-(+)-valine	D-(+)-Betaine (n = 13): L-(+)-dimethylvaline	20
L-(−)-Betaine (n = 13): D-(-)-valine	L-(−)-Betaine (n = 13): D-(−)-dimethylvaline	20

All solutions contained 0·05 molar concentration of both "drug" and betaine.
Cell path length 1 cm.

the D-betaine-L-dimethylalanine complex is less dense than the corresponding D-betaine-L-alanine complex. Molecular models show that the two *N*-methyl groups of dimethylalanine prevent a close "fit" between the positively charged nitrogen of the "drug" molecule and the negatively charged carboxyl group at the surface of the betaine micelle. As a result, neither the D- nor the L-forms of dimethylalanine can "fit" closely at the micelle surface and hence there is little difference in the volumes of the drug-betaine complexes. A similar explanation may account for the small differences in refractive index between solutions of D-betaine-D-dimethylvaline and solutions of D-betaine-L-dimethylvaline (see Table 2). The differences shown are higher than those obtained for the corresponding



Dimethylvaline

betaine-dimethylalanine solutions and lower than the values for betaine-valine solutions (see Table 1). The view that neither of the dimethylvaline enantiomers fit closely is supported by refractive index measurements which show that a solution of L-betaine-D-valine has a higher refractive index than the corresponding solution of L-betaine-L-dimethylvaline (see Table 3). The L-betaine-D-valine complex is therefore more dense than the corresponding L-betaine-D-dimethylvaline complex.

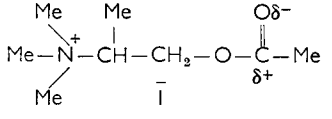
In the case of the quaternary dimethylalanine methiodide enantiomers the refractive index of a solution of D-betaine-D-isomer is the same as that of a corresponding solution of D-betaine-L-isomer (see Table 2).

A number of contributing factors may be involved in the failure of enantiomorphs of quaternary derivatives to show any difference in adsorption at the surface of the micelle.

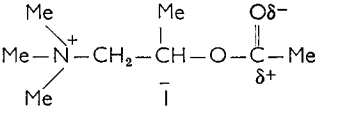
1. The increase in bulk of the group on the nitrogen will prevent it from coming close to the carboxyl group of the betaine micelle. Molecular models show no difference for either enantiomer in the degree of "fit" at the betaine micelle surface.

2. Quaternary amines can form ionic bonds with anions, but there is no possibility of such bonds being reinforced by hydrogen bonding. Apart from quaternary compounds, cations of all amines are known to form hydrogen bonds and ionic bonds simultaneously with the anions of carboxylic acids. The resulting bond has double the strength of a purely ionic bond and has greatly increased permanence (Albert, 1965). With dimethylalanine methiodide the strength of bonding between the postively charged nitrogen of the "drug" molecule and the negatively charged carboxyl group at the betaine micelle surface will be much less, for example, than that for alanine. Furthermore any bond which does form will be less permanent.

No difference can likewise be observed in refractive index between the enantiomorphs of α- and β-methylacetylcholine iodides in the presence of D-betaine micelles, and this may be explained in similar terms. An additional factor for the choline derivatives may be that the very weak negative charge on the oxygen of the acetyl carbonyl group may not be sufficient, in the absence of other reinforcing factors, to bind this end of the molecule to a positively charged nitrogen atom on the micelle surface.



α-Methylacetylcholine iodide



β-Methylacetylcholine iodide

INTERACTIONS OF A DIPEPTIDE WITH BETAINE MICELLES

Solutions containing L-alanyl-L-alanine and a D-betaine each present in 0·05 molar concentration have a lower refractive index than corresponding solutions of L-alanyl-L-alanine and L-betaine, the difference in arbitrary

units being 15 for dodecyl and 28 for tetradecyl betaine with a cell of path length 1 cm. These results indicate that the dipeptide "fits" better at the surface of an L-betaine than at the surface of a D-betaine micelle. Molecular models of the betaine molecule were constructed as previously described. The conformation of the "drug" molecule was arranged in such a manner that the $-\overset{+}{N}H_3$, the — CONH and the $-\overset{-}{COO}$ groups were all orientated in the same general direction, so that all three groups pointed in the direction of the aqueous phase when the peptide was adsorbed at the micelle surface. The two methyl groups were orientated in the same general direction, but for steric reasons pointed away from each other. As a result of these restrictions the two hydrogen atoms on the asymmetric carbon atoms are orientated almost parallel to each other and the $-\overset{-}{COO}$ and $-\overset{+}{N}H_3$ groups point towards each other. Fig. 2 represents that conformation of L-alanyl-L-alanine most stable at the betaine surface.

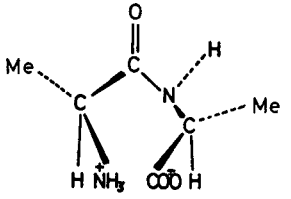


FIG. 2. L-Alanyl-L-alanine.

In fitting the dipeptide model to that of a portion of the betaine micelle it is found that when the carboxyl group of the "drug" molecule is brought close to the positively charged nitrogen of a betaine molecule, then the $-\overset{+}{N}H_3$ group of the dipeptide can bind only to the carboxyl group of a

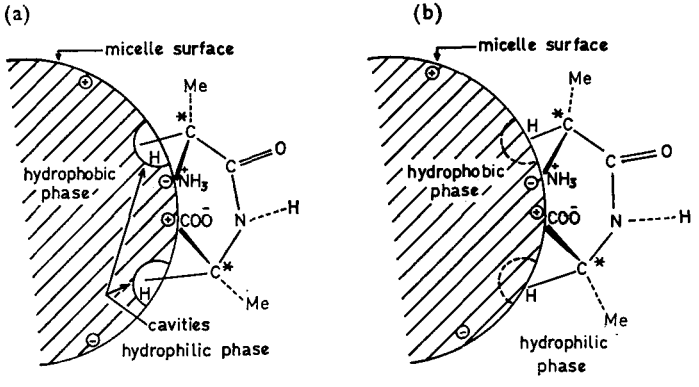


FIG. 3. Diagrammatic representation of dipeptide-betaine interactions. (a) L-Alanyl-L-alanine/L-betaine. (b) L-Alanyl-L-alanine/D-betaine. Shaded portion is an equatorial section through polar groups of a portion of betaine micelle. C* is a drug asymmetric carbon atom, with thick, broken, and unbroken lines for bonds above, below, and in the plane of the paper respectively.

neighbouring betaine molecule in the micelle. With L-alanyl-L-alanine and L-betaine the hydrogens attached to the "drug" asymmetric carbon atoms are able to "fit" into two cavities at the surface of the micelle [see Fig. 3 (b)]. Each of these cavities lies between one of the *N*-methyl groups and the carboxyl group of a betaine molecule. When models representing a portion of the D-betaine micelle surface are used it is found that the two hydrogens on the asymmetric carbon atoms of the drug molecule prevent a close "fit" with the micelle surface by coming into contact with the other *N*-methyl groups on the two betaine molecules involved in the adsorption of the drug molecule [see Fig. 3 (b)].

References

Albert, A. (1965). *Selective Toxicity*, pp. 129, London: Methuen.
Beckett, A. H. & Anderson, P. (1957). *Nature, Lond.*, **179**, 1074–1075.
Beckett, A. H. & Anderson, P. (1959). *J. Pharm. Pharmac.*, **11**, *Suppl.*, 258*T*–260*T*.
Beckett, A. H. & Anderson, P. (1960). *Ibid.*, **12**, *Suppl.* 228*T*–236*T*.
Beckett, A. H., Harper, N. J. & Clitherow, J. W. (1963). *Ibid.*, **15**, 349–361.
Beckett, A. H. & Youssef, H. Z. (1963). *Ibid.*, **15**, *Suppl.* 253*T*–266*T*.
Beckett, A. H., Kirk, G. & Virji, A. S. (1967). *Ibid.*, **19**, *Suppl.*, 71S–75S.
Bowman, R. E. & Stroud, H. H. (1950). *J. chem. Soc.*, 1343.
Koshland, D. E. (1958). *Proc. natn. Ass. Sci.*, **44**, 98.