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Conformation in Solution of Coenzyme A Thioesters

II. Butyryl-CoA and Arylacetyl-CoA Compounds[1]

JOHN J. MIEYAL[2], KAREN S. BLISARD, AND USMAN A. SIDDIQUI

The Department of Pharmacology, Northwestern University Medical and Dental Schools, Chicago, Illinois 60611

The ^{1}H nuclear magnetic resonance (^{1}H-nmr) spectra of aqueous solutions of butyryl-CoA (Bu-CoA), indoleacetyl-CoA (IA-CoA), and phenylacetyl-CoA (PA-CoA) were examined at various concentrations and temperatures and compared to spectra of acetyl-CoA (Ac-CoA) and benzoyl-CoA (Bz-CoA) in order to determine to what extent, if any, each acyl-CoA compound exists in an intramolecular folded conformation. It was found previously that Ac-CoA exists predominantly in an extended conformation, whereas Bz-CoA is folded (Mieyal *et al.*, *J. Biol. Chem.* **249**, 2633 (1974)). The present study showed: (a) the solution behavior of Bu-CoA was essentially indistinguishable from that of Ac-CoA; thus both of these aliphatic CoA esters probably exist as extended molecules; (b) IA-CoA does form an intramolecular adenyl–indolyl complex, but the folded/unfolded ratio is only about one-half of that for Bz-CoA at physiological temperature; (c) PA-CoA apparently exists predominantly as an unfolded molecule. The diminished tendency of PA-CoA and IA-CoA to fold is probably related to the rotational mobility about the bond adjoining the respective arylmethylene group to the carbonyl moiety of the thioester link. Concentration-independent effects of the phenyl and indolyl ring currents on the ^{1}H-nmr signals of particular pantotheinyl methylene groups in PA-CoA and IA-CoA, respectively, are consistent with this conclusion. The outstanding conformational difference between the closely related Bz-CoA (folded) and PA-CoA (unfolded) molecules may provide insight regarding the nature of the binding sites for these molecules on the specific *N*-acyltransferase enzymes for which they are the best substrates (Webster *et al.*, *J. Biol. Chem.* **251**, 3352 (1976)).

INTRODUCTION

Coenzyme A is a ubiquitous cofactor in biological systems. Different transacylase enzymes located in various mammalian tissues catalyze the transfer of particular acyl moieties from the respective acyl-CoA[3] thioesters to specific acceptors. Most of these enzymic reactions display a narrow specificity with regard to the acyl-CoA substrate, but little is known about its basis (*1*, *2*). A particularly striking example of such substrate specificity was recently observed by Webster *et al.* (*3*), who found that benzoyl-

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[2] Author to whom correspondence and requests for reprints should be directed.

[3] Abbreviations used are: CoA, coenzyme A; CPK, Corey–Pauling–Koltun molecular models; nmr, nuclear magnetic resonance; ppm, parts per million.

CoA is converted to benzoylglycine by one *N*-acyltransferase enzyme, but phenylacetyl-CoA is converted to phenylacetylglutamine by another. The only structural difference between the relatively large benzoyl-CoA and phenylacetyl-CoA molecules is the methylene group between the phenyl ring and the carbonyl moiety of the thioester bond in phenylacetyl-CoA; yet no cross reactivity of these two substrates could be demonstrated although cross inhibition was noted (*3*).

Our previous ^{1}H-nmr studies (*4*) showed that different acyl-CoA compounds may exist in solution in quite different conformations; e.g., acetyl-CoA is predominantly extended, whereas benzoyl-CoA forms a specific intramolecular folded conformation. In light of those results and the differential substrate specificity of the transferases (*vide supra*), we set out to compare the solution behavior of phenylacetyl-CoA and benzoyl-CoA, as well as certain lesser substrates for the two enzymes. This was done in order to test whether the substrate specificity of the enzyme might be reflected by the shapes of the substrate molecules themselves. In this article it is reported that, unlike benzoyl-CoA which is folded (*4*), phenylacetyl-CoA apparently exists predominantly in an extended conformation in solution.

EXPERIMENTAL PROCEDURE

Materials. The sodium salts of butyric acid, indoleacetic acid, and phenylacetic acid were purchased from Aldrich Chemical Company. CoA and acetyl-CoA were obtained from P-L Biochemicals, Inc. Deuterium oxide (99.97% D), methanol-d_4 (99.5% D) and sodium 2,2-dimethyl-2-silapentane sulfonate were obtained from Wilmad Glass Co. Sodium deuteroxide (40% in D_2O) and deuterium chloride (38% in D_2O) were products of Stohler Isotope Chemicals. Acetone, phosphate salts, and other common chemicals were reagent grade. Butyryl-CoA, indoleacetyl-CoA and phenylacetyl-CoA were synthesized and purified as described elsewhere (*3*, *4*). Purity was confirmed by thin-layer chromatography (*4*), ^{1}H-nmr spectroscopy (*vide infra*), and elemental analysis[4] (indoleacetyl-CoA and phenylacetyl-CoA).

[4] Analyses were performed by Galbraith Laboratories, Knoxville, Tenn. Details concerning percentage of error, waters of hydration of the hygroscopic CoA esters, etc. were described earlier (*4*).

Phenylacetyl-CoA. Calculated values are based on a molecular weight of 983.45 which corresponds to the disodium salt with 3 H_2O.

Atom	Calcd (%)	Found (%)
C	37.45	37.15
H	4.31	4.41
N	10.56	10.43
P	9.99	10.03
S	3.41	3.27
Na	4.95	4.91

Indoleacetyl-CoA (calculated values are based on a molecular weight of 996.17 which corresponds to the free acid with 4 H_2O)

Atom	Calcd (%)	Found (%)
C	40.4	39.63
H	4.65	4.40
N	12.21	11.80
P	10.06	10.05
S	3.48	3.47

Methods. The concentrations of solutions of CoA, acetyl-CoA, butyryl-CoA, indoleacetyl-CoA, and phenylacetyl-CoA were determined from the absorbance of diluted aliquots of these solutions at 260 nm according to their respective $\varepsilon^{mM}_{260\ nm}$ values.[5] All solutions for ^{1}H-nmr spectra were in D_2O and the pH was adjusted if necessary with NaOD or DCl. After spectra were recorded, the apparent pH of each solution in D_2O was rechecked with a glass electrode pH meter (Radiometer PHM 26); pD = observed pH + 0.4 (*6*). Acetone was used as the internal reference for all spectra and its signal was set at δ 2.20 ppm. The suitability of acetone for this purpose was established previously (*4*), and it was reconfirmed for this study in the same way. All proton chemical shifts are expressed according to the δ scale in ppm downfield from 0 ppm; values of δ are positive in this direction. ^{1}H-nmr spectra were recorded with 60 MHz Hitachi Perkin–Elmer R20B, 90 MHz Perkin–Elmer R32, and 270 MHz Bruker[6] nmr spectrometers, all equipped for variable temperature operation. Actual temperatures of samples within the spectrometer probes were checked by direct measurement (Microadjustatherm thermometer JM 7600A, Scientific Glass Apparatus, Co.).

RESULTS

^{1}H-nmr spectra of acyl-CoAs: General features. The ^{1}H-nmr spectrum of unsubstituted CoA was described in detail earlier (*4*). The most characteristic spectral change which was shown to be diagnostic of substitution on the S-atom of CoA was a downfield displacement of the signal corresponding to the $-CH_2-S-$group (*4*). This feature was evident also in the spectra of butyryl-CoA, indoleacetyl-CoA and phenylacetyl-CoA, thereby confirming their thioester nature (see Fig. 3). Table 1 gives the assignment of the signals in spectra of these compounds which correspond to the respective acyl moieties and are consistent with the structures of these thioester compounds. In each case the signal for the methylene group adjacent to the carbonyl of the thioester bond occurs downfield from its position in the unsubstituted acid, e.g., the

$$CH_3CH_2CH_2C(=O)-O^-$$

[5] For CoA and acetyl-CoA the National Research Council Standard for the adenosine-5′-phosphate moiety at pH 7 was used to determine concentration, namely, $\varepsilon^{mM}_{260\ nm} = 15.4\ mM^{-1}\ cm^{-1}$. Values for butyryl-CoA, indoleacetyl-CoA, and phenylacetyl-CoA were determined both according to dry weight and from enzymatic release of thiol as described previously (*5*). The average of the two values so obtained was used in each case.

	$\varepsilon^{mM}_{260\ nm}$, ($mM^{-1}cm^{-1}$)		
Acyl-CoA	Dry weight	Thiol release	Average
Butyryl-CoA	15.8	15.4	15.6
Indoleacetyl-CoA	15.9	16.5	16.2
Phenylacetyl-CoA	14.5	14.6	14.6

[6] The Bruker 270 MHz nmr spectrometer was made available to the author (J.J.M.) by the University of Chicago, Department of Chemistry. This instrument facility is supported in part by Grant GP 33116 from the National Science Foundation and Grant CA 14599 from the National Cancer Institute to the University of Chicago.

signal occurs at δ 2.13 ppm, whereas the

$$CH_3CH_2CH_2\overset{\overset{O}{\|}}{C}-S-CoA$$

signal appears at δ 2.52 ppm (Table 1). This behavior is also indicative of the thioester link.

TABLE 1

^{1}H-nmr SPECTRA[a] OF ACYL-CoAs

Compound	Moiety	Signal appearance	Relative area	Chemical shift (δ ppm)
Butyryl-CoA	$CH_3CH_2CH_2\overset{\overset{O}{\|}}{C}-$	Triplet	2	2.52
	$CH_3CH_2CH_2\overset{\overset{O}{\|}}{C}-$	Multiplet	2	1.57
	$CH_3CH_2CH_2\overset{\overset{O}{\|}}{C}-$	Triplet (masked)[b]	3	~0.9
Indoleacetyl-CoA	Indole-$CH_2\overset{\overset{O}{\|}}{C}-$	Singlet	2	3.84
	Indole	Multiplet[c]	5	7.11[c]
Phenylacetyl-CoA	Phenyl-$CH_2\overset{\overset{O}{\|}}{C}-$	Singlet	2	3.82
	Phenyl	Broad singlet	5	7.23

[a] All spectra were of solutions of ~0.1 *M*, pH_{obs} ~6.0. Spectra were recorded at 60, 90, and 270 MHz in order to resolve most of the overlapping resonances of either the acyl moiety or the CoA moiety (see Fig. 3). All δ values are reported at 5°C.

[b] The middle peak of the triplet remained masked by the singlet signal of the $-\overset{\overset{CH_3}{/}}{C}-\underset{\underset{CH_3}{\backslash}}{}$ group of the CoA moiety, even at 270 MHz.

[c] The sharpest portion of the multiplet signal for the indole moiety probably corresponds to the proton attached to the pyrrole moiety of the indole ring since it would not be strongly coupled to any neighboring protons (*7*). The multiplet spans the region 6.8–7.4 ppm, the sharpest peak being at δ 7.11 ppm.

Evidence for intermolecular and intramolecular interactions. The major difference in the spectrum of the CoA moiety of benzoyl-CoA relative to that of acetyl-CoA was the upfield displacement of the (C-1′)-*H*, (C-2)-*H* and (C-8)-*H* signals of the adenosyl moiety (*4*). These differences in signal position were independent of concentration and indicative of a specific intramolecular ring stacking interaction involving the benzoyl and adenyl moieties. To test whether phenylacetyl-CoA or indoleacetyl-CoA also

exists in such folded conformations, we examined the concentration dependence of the adenosyl signals in spectra of these compounds relative to that of the same signals in spectra of acetyl-CoA. This relationship is shown in Fig. 1 for the (C-2)-*H* signal which is the most sensitive indicator; these data were accumulated at low temperature in order to maximize any intermolecular or intramolecular ring stacking interactions. The straight line drawn through the data points at the top of Fig. 1A shows an increasing upfield displacement of the signal with increasing concentration and it is indicative of intermolecular interaction of the adenyl moieties of both acetyl-CoA and butyryl-CoA.

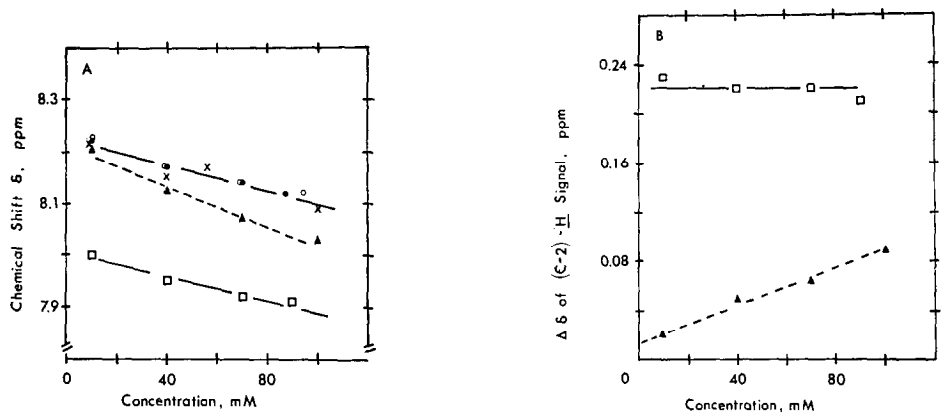

FIG. 1. (A) Variation of the chemical shift δ of the (C-2)-*H* signal with concentration. Acetyl-CoA: (o) diluted with D_2O; (●) diluted with K-phosphate, $pH_{obs} = 6.0$ in D_2O; the ionic strength of this buffer was equivalent to that of the most concentrated solution of acetyl-CoA, 0.087 *M*, $\mu = 0.25$ *M* (assuming acetyl-CoA dianion at pH 6). Butyryl-CoA (×), diluted with K phosphate; phenylacetyl CoA (▲), diluted with D_2O; indoleacetyl-CoA (□), diluted with D_2O. All spectra were 90 MHz, recorded at $5 \pm 1°C$; pH_{obs} of all solutions $= 6.0 \pm 0.2$. (B) Differences in chemical shift $\Delta\delta$ of the (C-2)-*H* signals between spectra of acetyl-CoA and the arylacetyl-CoAs. $\Delta\delta = \delta^{acetyl-CoA}_{(C-2)-H}$ minus $\delta^{arylacetyl-CoA}_{(C-2)-H}$. Values were calculated from the data of (A).

Such results are consistent with the tendency of purines to self-associate (*8*). Dilution of acetyl-CoA in either the presence or the absence of a buffer for strict control of ionic strength and pH gives identical results (i.e., downfield displacement of the (C-2)-*H* signal); thus, these factors are precluded as contributors to the changes observed. The fact that the data for butyryl-CoA appear to fall on the same line suggests that a bulky acyl moiety alone at the other end of the molecule is not a sufficient condition to alter the behavior in solution of the adenyl moiety. Conversely, since the positions of the ^{1}H-nmr signals corresponding to the butyryl moiety (except for that of the methylene group adjacent to the thioester carbonyl moiety, *vide supra*) are identical in the spectra of butyryl-CoA and butyric acid (data not shown), this means that the adenyl ring current has no effect on these groups. Therefore, it may be concluded that the adenyl and butyryl moieties do not closely approach one another; i.e., the butyryl-CoA molecule is predominantly in an extended conformation.

It seems that two types of interaction may occur in solutions of indoleacetyl-CoA. The line drawn through the data points for indoleacetyl-CoA (bottom, Fig. 1A) is

parallel but displaced from the acetyl-CoA, butyryl-CoA line, i.e., the top line. (Analogous behavior was observed previously (*4*) for benzoyl-CoA relative to acetyl-CoA, except that the line for benzoyl-CoA would be displaced further from the acetyl-CoA line than is the indoleacetyl-CoA line (*vide infra*)). Thus, the difference between the values of the two lines appears in Fig. 1B (top, horizontal line) as a concentration-independent relationship. The negative slope of the bottom line in Fig. 1A, parallel to the top line, is consistent with concentration-dependent intermolecular adenyl–adenyl complex formation[7] in solutions of indoleacetyl-CoA. The concentration-independent upfield displacement of the (C-2)-*H* signal of indoleacetyl-CoA relative to that of acetyl-CoA or butyryl-CoA (Fig. 1B, top) is indicative of intramolecular indolyl–adenyl complex formation. The latter conclusion is also supported by the observation that concentration-independent upfield displacements also occur for the (C-1′)-*H* and (C-8)-*H* signals of the adenosyl moiety as well as for the indolyl signal of indoleacetyl-CoA relative to the same signals in spectra of mixtures of indoleacetate and CoA. As observed earlier for benzoyl-CoA (*4*), this indoleacetyl-CoA intramolecular complex also is disrupted by elevated temperature and by methanol, a solvent of low polarity (data not shown). For indoleacetyl-CoA, the relative $\Delta\delta$ values (i.e., δ acetyl-CoA minus δ indoleacetyl-CoA) at low temperature for the adenosyl signals (C-2)-*H* (0.22 ppm), (C-8)-*H* (0.09 ppm), and (C-1′)-*H* (0.09 ppm), roughly parallel the changes observed for benzoyl-CoA (0.27, 0.12, and 0.15 ppm, respectively (*4*)), but are smaller in magnitude. The $\Delta\delta$ values for indoleacetyl-CoA are decreased to ~60% at 35°C, e.g., $\Delta\delta$ (C-2)-*H* = 0.13. These data may be used to estimate the percentage of indoleacetyl-CoA molecules in the folded conformation at physiological temperature relative to benzoyl-CoA (see Discussion).

The behavior of phenylacetyl-CoA in aqueous solution appears to be different from acetyl-CoA and butyryl-CoA on one hand and from indoleacetyl-CoA on the other. The dashed line drawn through the data points for phenylacetyl-CoA (Fig. 1A, middle) appears to have a greater slope than the other two lines. This result suggests either increased adenyl–adenyl interaction, or an additional mode(s) of intermolecular interaction or both possibilities. The near-zero- y-intercept of the $\Delta\delta$ versus concentration plot (Fig. 1B, bottom) further suggests that the additional mode of intermolecular interaction could be phenyl–adenyl complex formation occurring at the expense of intramolecular phenyl–adenyl interaction. It is clear that the substitution of a methylene group between the phenyl ring and the thioester bond has virtually abolished the type of intramolecular ring stacking interaction characterized for benzoyl-CoA, i.e., $\Delta\delta$ for (C-2)-*H* for benzoyl-CoA was 0.27 ppm (*4*) whereas $\Delta\delta$ for phenylacetyl-CoA is <0.02 (extrapolated y-intercept, Fig. 1B, bottom). Therefore, the predominant effect that the methylene group may have is to provide much greater rotational freedom at the site of possible adenyl–phenyl interaction. Figure 2A shows the probable favored conformation of benzoyl-CoA which was deduced earlier (*4*). Figure 2B shows the

[7] Although other intermolecular complexes are conceivable for indoleacetyl-CoA, such as indolyl–adenyl, indolyl–indolyl, double indolyl–adenyl interaction etc., the adenyl–adenyl complex seems most likely because the line for indoleacetyl-CoA parallels that for acetyl-CoA which has no other aromatic ring besides the adenyl moiety. The same conclusion seems valid for intermolecular interaction of benzoyl-CoA molecules, since analogous data obtained previously for benzoyl-CoA also parallelled the data for acetyl-CoA (*4*).

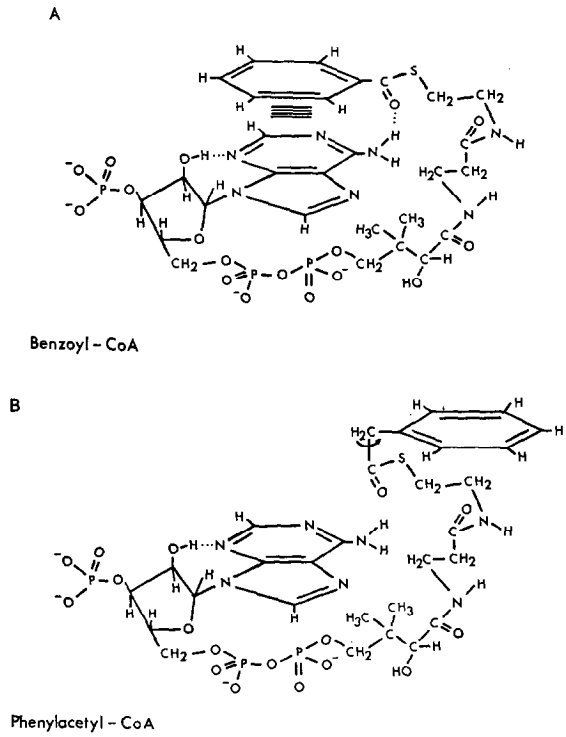

FIG. 2. (A) Folded conformation of benzoyl-CoA. Taken from Ref. (*3*); phosphate moieties are arbitrarily depicted as fully charged. (B) Model of rotational mobility of the phenyl moiety of the phenylacetyl-CoA molecule. The rest of the molecule is arbitrarily drawn in the same arrangement as that for benzoyl-CoA in order to facilitate comparison.

phenylacetyl-CoA molecule drawn for comparison in the same conformation, but depicted with free rotation about the phenylmethylene bond. Evidence for such rotation was observed in the methylene regions of the 270-MHz spectra of phenylacetyl-CoA and indoleacetyl-CoA (Fig. 3). The dashed vertical lines correspond to the positions of the side-chain methylene signals in spectra of acetyl-CoA, butyryl-CoA, benzoyl-CoA and *o*-, *m*-, and *p*-hydroxybenzoyl-CoAs; with the exception of signal *c*, the same is true for unsubstituted CoA. The upfield displacements, most notable for signals *b* and *e*, are concentration independent and can be ascribed to the effects of the ring currents of the phenyl moiety and of the indolyl moiety on those particular methylene groups when the phenyl or indole rings are rotated over that region of the molecule. Manipulation of CPK molecular models confirmed that those two methylene groups may be positioned closest to the phenyl or indole rings of the respective aryl-CoA molecules when these rings are rotated about the arylmethylene bond.

DISCUSSION

Estimation of percentage in complex. On the basis of previous ^{1}H-nmr studies, we concluded that CoA and acetyl-CoA exist predominantly as extended molecules in

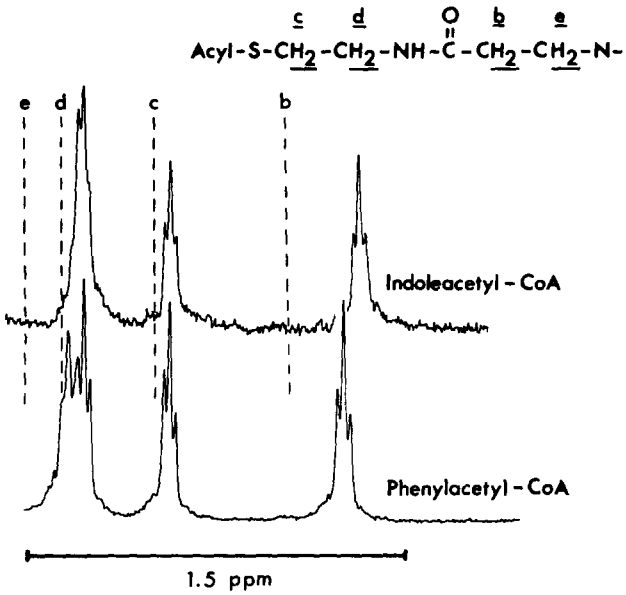

FIG. 3. Methylene region of 270-MHz ^{1}H-nmr spectra of arylacetyl-CoAs. Solutions in D_2O, ~0.05 *M*, $pH_{obs} \simeq 6$, 19°C. The small letters above the structural diagram and above the dotted lines show the signal assignment that was used in the complete assignment of the acetyl-CoA spectrum reported earlier (*4*). In all cases, the corresponding signals in the spectra of indoleacetyl-CoA and phenylacetyl-CoA are observed to the right of the dotted lines (upfield). In contrast to their complete separation in 270-MHz spectra of all of the other acyl-CoA compounds, the triplet signals for the *d* and *e* methylene groups overlap in these spectra. These results are indicative of a differential effect of the respective aromatic ring currents (phenyl and indolyl) on these particular methylene groups (see text).

solution. Subsequently, several other ^{1}H- and ^{13}C-nmr studies confirmed that conclusion (*9–11*). In contrast we found that benzoyl-CoA assumes an intramolecular folded conformation. The close correspondence between the observed values of $\Delta\delta$ and those predicted from a molecular model of the folded conformation led us to conclude that benzoyl-CoA exists almost completely in that conformation at $\leqslant$10°C (*4*). If phenylacetyl-CoA existed in a folded conformation wherein the mutual orientation of the adenyl and phenyl rings were equivalent to that of benzoyl-CoA, then the effect of the phenyl ring current on the adenosyl signals would be essentially the same as that for benzoyl-CoA. We may therefore estimate the percentage of phenylacetyl-CoA molecules in such a conformation from the ratio of $\Delta\delta$ values for phenylacetyl-CoA and benzoyl-CoA at low temperature:

$$\left(\frac{\delta^{\text{acetyl-CoA}}_{\text{(C-2)-}H} \text{ minus } \delta^{\text{phenylacetyl-CoA}}_{\text{(C-2)-}H}}{\delta^{\text{acetyl-CoA}}_{\text{(C-2)-}H} \text{ minus } \delta^{\text{benzoyl-CoA}}_{\text{(C-2)-}H}} \times 100\right) = \frac{<0.02^*}{0.27} \times 100,$$

where the asterisk (*) indicates the *y*-intercept of the phenylacetyl-CoA line (Fig. 1B, bottom).

Thus, <7% of the phenylacetyl-CoA molecules are folded at low temperature, and this value would be even less at 35°C.

In the case of indoleacetyl-CoA, there is evidence of significant intramolecular ring stacking (Figs. 1A and B); however, the ring current of indole cannot be considered equivalent to that of the phenyl ring. On theoretical grounds it is predicted that each heteroatom in an aromatic ring will decrease the size of the ring current relative to benzene (*12*). It has also been found that $\Delta\delta$ values for interactions involving multiple ring systems may be treated as the sum of the contribution of the rings considered separately (*13*). In the case of indole, we might consider the entire ring current as the sum of the benzene component and the pyrrole component. A ring current of about 40% that of benzene has been estimated for pyrrole (*14*). Thus, the ring current of indole may be considered to be approximately 1.4 times that of benzene.[8] If it is assumed that the indolyl–adenyl orientation in the indoleacetyl-CoA folded conformation is similar to the benzoyl–adenyl orientation in folded benzoyl-CoA, we may estimate the percentage of indole acetyl-CoA molecules that are folded at low temperature:

$$\left(\frac{\Delta\delta^{\text{indoleacetyl-CoA}}_{\text{(C-2)-}H} \div 1.4}{\Delta\delta^{\text{benzoyl-CoA}}_{\text{(C-2)-}H}} \times 100\right) = \left(\frac{(0.22 \div 1.4)}{0.27} \times 100\right) \simeq 60\%.$$

Thus, at low temperature ~60% of the indoleacetyl-CoA molecules are folded, and at 35°C ($\Delta\delta = 0.13$ for (C-2)-*H* of indoleacetyl-CoA), less than 35% are folded.

Inspection of Fig. 2 and of molecular models of benzoyl-CoA, phenylacetyl-CoA and indoleacetyl-CoA revealed that the insertion of the additional methylene group between the phenyl ring and the thioester bond served to widen the interior cavity of the folded models of the arylacetyl-CoA molecules or to increase the vertical and/or horizontal distances between the two aromatic rings or both, relative to the model of benzoyl-CoA. Widening the cavity would tend to decrease hydrophobic interactions; lengthening the ring–ring distance would diminish the π–π interaction as well as the proposed hydrogen-bonding interaction depicted in Fig. 2A. All of these possible effects along with the increased mobility of the phenyl ring may explain the lack of intramolecular association for phenylacetyl-CoA. In the case of indoleacetyl-CoA, the large indole ring may partially compensate for the increased interring distance as well as partially refill the hydrophobic cavity. The larger ring might also be less mobile, restricting its motion to a smaller area. However, it must be considered also that a folded conformation of the indoleacetyl-CoA molecule entirely different from that of benzoyl-CoA may represent its average favored spatial arrangement.

Studies of correlations between structure and function of CoA and substituted CoAs have generally employed altered forms of the CoA moiety. For example, dephos-

[8] A rough correlation to this estimate of the indole ring current may be derived from Fig. 3, if several assumptions are made; for example, (a) the displacements of signals *b* and *e* for indoleacetyl-CoA and phenylacetyl-CoA are a measure of the indolyl and phenyl ring current effects, respectively. (b) The indolyl and phenyl moieties spend about the same amount of time rotated over the *b* and *e* methylene groups of the respective CoA esters. This assumption must be tempered by the observation that the indolyl moiety spends more "time" in intramolecular complex with the adenyl moiety than does the phenyl moiety of phenylacetyl-CoA (see Discussion). The estimate of the ratio of ring currents indole/phenyl may be calculated from Fig. 3 as follows:

$$\frac{\Delta\delta^{b}_{\text{indoleacetyl-CoA}}}{\Delta\delta^{b}_{\text{phenylacetyl-CoA}}} = \frac{\Delta\delta^{e}_{\text{indoleacetyl-CoA}}}{\Delta\delta^{e}_{\text{phenylacetyl-CoA}}} = 1.26,$$

where $\Delta\delta$ in each case refers to the difference between the dotted line to the left (representing acetyl-CoA) and the actual position of the signal in the appropriate spectrum.

pho-CoA, desamino-CoA, acetyl-pantetheine, etc. have been employed as substitutes for the respective compounds containing the intact CoA moiety in tests of their relative activity as substrates and/or modifiers of enzymes (*1*).

In the particular case of enzyme specificity pertinent to the present study, the separate glycine and glutamine *N*-acyltransferase enzymes of mammalian liver mitochondria displayed the greatest discrimination between two acyl-CoA substrates for which the CoA moiety was unaltered and the acyl moiety differed by only a methylene group (*3*), i.e., benzoyl-CoA and phenylacetyl-CoA, respectively. Compounds with more extensive differences in the molecular structure of the acyl moiety (indoleacetyl-CoA and butyryl-CoA) were not good substrates for either enzyme. It is remarkable that the benzoyl-CoA and phenylacetyl-CoA molecules also differ markedly with respect to their behavior in solution, the former favoring a compact conformation, the latter an extended conformation.

We might speculate that these particular conformations may also be favored by the respective enzymes, and that could be the basis for their differential reactivity as substrates. Evaluation of this hypothesis will depend upon detailed nmr studies that will reflect the molecular events that occur on the enzyme when both the acyl-CoA and amino acid molecules are present. Whether a solution conformation of a substrate would be retained or altered when it is in complex with an enzyme would depend upon the architecture of the substrate binding site on that enzyme and upon the relative energies of stabilization of the enzyme-substrate complex and the favored conformation of the free substrate. There are, of course, other factors that might contribute to or govern the substrate specificity of these enzymes irrespective of the conformation of the bound CoA ester substrate. For example, if the mechanism of catalysis by these enzymes involved intermediate formation of an acyl-enzyme, then the exact nature of the acyl moiety of the CoA ester might affect the ease of formation of the acyl-enzyme intermediate and/or the ease of attack by the specific amino acid acceptor (glycine or glutamine). Final conclusions must await the elucidation of the detailed molecular mechanisms of action of these enzymes. Our current studies have accomplished the necessary first steps, namely, purification of the separate enzymes (*3*) and characterization of the conformations of the unbound substrates ((*4*) and present).

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