Immunochemical and Protein-Chemical Studies of Class I Fructose 1,6-Diphosphate Aldolases from Staphylococci

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The class I fructose 1,6-diphosphate aldolases of five staphylococcal strains were purified and characterized. All of these enzymes could be renatured to active structures after exposure to a temperature of 90°C. By using antisera against the enzymes, we measured the immunological cross-reactions by microcomplement fixation and activity inhibition. The amino acid compositions of the purified proteins were determined and compared. Our results provide further insight into the phylogenetical relationships among the staphylococcal species studied. *Staphylococcus capitis*, *Staphylococcus epidermidis*, *Staphylococcus warneri*, and *Staphylococcus haemolyticus* form a group of highly related species. *Staphylococcus aureus* was shown to be closely related to this group, whereas all of the other staphylococcal species showed distant relationships. *Staphylococcus sciuri* subsp. *sciuri* and *S. sciuri* subsp. *lentus* occupy a rather isolated position within the genus *Staphylococcus* and are related to each other at the species level.

The fructose 1,6-diphosphate (FDP) aldolases which are usually found in bacteria and fungi are class II enzymes (31). These enzymes need a divalent metal ion for activity and are inhibited by chelating agents. In contrast, class I aldolases have been found in most staphylococci (9) and a few other bacteria (13, 20, 21, 35). Class I aldolases can be irreversibly inhibited by dihydroxyacetone phosphate in the presence of sodium borohydride (12). The class I FDP aldolases from staphylococci and peptococci appear to be similar in enzymatic kinetics and molecular weight. The presence of class I aldolases in staphylococci is a useful property for distinguishing these organisms from other members of the family *Micrococcaceae* (9). Both class II aldolase activity and class I aldolase activity have been found in *Staphylococcus hyicus* and *Staphylococcus intermedius* strains, but only a class II enzyme occurs in *Staphylococcus caseolyticus* (6). This difference provides a simple method for classifying these staphylococci within the genus. Furthermore, the phylogenetic relationships of staphylococci have been determined by deoxyribonucleic acid (DNA)-DNA (15, 17, 24, 29, 32) and DNA-ribonucleic acid (RNA) homology (14) studies, as well as by comparing 16S ribosomal RNAs (23) and immunological cross-reactions of catalases (30, 32). In the present study we used the following two essentially different methods to reveal the phylo-

genetic relationships among certain staphylococci: (i) immunological cross-reactivity and (ii) homology of amino acid compositions. One gene product was used for both methods (namely, the class I aldolases).

MATERIALS AND METHODS

Cultivation and preparation of crude extracts. The strains used in this study are the type strains of the species examined and were obtained from the American Type Culture Collection (ATCC), Rockville, Md., the Czechoslovak Culture Collection (CCM), Brno, Czechoslovakia, the National Collection of Type Cultures (NCTC), London, United Kingdom, and the Deutsche Sammlung von Mikroorganismen (DSM), Göttingen, Federal Republic of Germany. All staphylococci were cultured at 37°C in a medium containing 1% glucose, 0.5% yeast extract, 0.8% NaCl, and 0.05 M sodium phosphate buffer (pH 7.5). For enzyme preparations, the cultures were grown in aerated 20-liter flasks until the end of the logarithmic phase. The cells were harvested by centrifugation, and the pellets were washed with water and then stored at –20°C (about 50 g [wet weight]). Crude extracts were prepared as previously described (8).

Aldolase purification. The purification method used was modified from the method described previously (8). All steps were carried out at 4°C. Solid ammonium sulfate was added stepwise at different pH values (Table 1). After each step, the precipitate was removed by centrifugation at 48,000 × g for 30 min. The precipitate from the last step was collected and dissolved in 0.06 M tris(hydroxymethyl)aminomethane hydrochloride buffer (pH 7.5) (standard buffer). The pH of the solution was adjusted to pH 4.3 with acetic acid, and the preparation was stirred for 2 h and clarified by centrifugation. The resulting supernatant

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TABLE 1. Purification of the class I aldolase from *S. epidermidis* ATCC 14990^T

Step	Vol (ml)	Total activity (U) ^a	Total protein (mg) ^b	U/mg	Purification (-fold)	Recovery (%)
Crude extract	306	612	5,202	0.12	0	100
(NH ₄)SO ₄ precipitation steps						
40% (pH 7.5)	335 ^c	502	3,015	0.17	1.4	82
60% (pH 7.5)	356 ^c	496	2,680	0.19	1.6	81
80% (pH 7.5)	380 ^c	462	1,440	0.32	2.7	75.5
100% (pH 5.0)	94 ^d	430	940	0.46	3.8	70.3
Acid treatment						
pH 4.3	184 ^c	429	353	1.2	10	70.1
pH 3.5	30 ^d	425	246	1.73	14.4	69.4
Diethylaminoethyl-Sephadex A-25 chromatography	130	320	71.2	4.5	37.5	52.3
Sephadex G-100 chromatography	17.4	310	21.4	14.5	120.7	51.0

^a One unit was the amount that oxidized 1 μ mol of reduced nicotinamide adenine dinucleotide per min.

^b Protein was determined by the method of Lowry et al. (22).

^c Supernatant.

^d Precipitate.

was adjusted to pH 3.5 with HCl. After 4 h, the precipitated protein was collected by centrifugation at $80,000 \times g$ for 1 h. The resulting sediment was dissolved in 0.6 M tris(hydroxymethyl)aminomethane hydrochloride buffer (pH 7.5) and dialyzed against standard buffer.

The dialysate was applied to a diethylaminoethyl-Sephadex A-25 column (2.5 by 25 cm), which was equilibrated in standard buffer, and then washed consecutively with 100 ml of standard buffer and 100 ml of standard buffer containing 0.1 M NaCl. Elution was carried out with a linear gradient prepared from 200 ml of 0.1 M NaCl and 200 ml of 0.5 M NaCl in standard buffer. The eluant was monitored at 278 nm for protein and enzymatic activities. The fractions containing activity were collected, and the protein was precipitated by adding 564 g of ammonium sulfate (pH 4.0) per liter. The precipitate was dissolved in a minimal volume of 0.6 M tris(hydroxymethyl)aminomethane hydrochloride buffer (pH 7.5; 2 to 3 ml) and was applied to a Sephadex G-100 (superfine) column (1.6 by 200 cm). Elution was carried out with standard buffer containing 1 M NaCl and was monitored as described above and also by sodium dodecyl sulfate (SDS) slab gel electrophoresis (see below). The homogeneous fractions were pooled and stored at -20°C .

SDS gel electrophoresis. SDS-polyacrylamide slab gel electrophoresis was carried out by the method of Laemmli (18). The molecular weights of the proteins were determined by the method of Weber and Osborne (37), using a 3% stacking gel and a 10% separation gel. Bovine serum albumin (molecular weight, 67,000), ovalbumin (45,000), the β -subunit of the *Escherichia coli* RNA polymerase (39,000), and soybean trypsin inhibitor (21,500) were used as standards.

Aldolase activity assay. Aldolase activity was determined spectrophotometrically by the method of Penhoe et al. (27).

Temperature stability assay. Portions (5 μ g) of purified

enzyme in 200 μ l of standard buffer were incubated in sealed glass tubes for 10 min at 90°C . After slow cooling, the activity remaining was determined by a spectrophotometric assay.

Determination of immunological cross-reactions. Antisera against the staphylococcal aldolases were prepared as described previously (30). Cross-reactions of aldolases with antisera were determined by a micro-

FIG. 1. SDS electrophoresis of purified *S. epidermidis* aldolase. A 40- μ g portion of protein was applied and stained with Coomassie brilliant blue.

TABLE 2. Molecular weights of purified class I aldolases, as determined by SDS slab gel electrophoresis

Strain	Mol wt of class I aldolase	Remaining activity (%) ^a
<i>S. aureus</i> ATCC 12600 ^T	35,000	98
<i>S. epidermidis</i> ATCC 14990 ^T	34,500	95
<i>S. haemolyticus</i> DSM 20263 ^T	34,500	98
<i>S. xylosus</i> DSM 20266 ^T	35,500	50
<i>S. sciuri</i> subsp. <i>sciuri</i> ATCC 29062 ^T	35,000	92

^a After heat inactivation of class I aldolases.

complement fixation method (2), as described previously for catalase antiserum (30, 32). In the microcomplement fixation assay, the antiserum concentration which gives optimal complement fixation with the homologous protein is determined. When a heterologous protein is used, a higher concentration of antiserum is necessary to achieve the same rate of complement fixation observed in the homologous system. The factor by which the antiserum concentration has to be increased is called the index of dissimilarity. The immunological distance (ID) used in this study is defined as 100 times the logarithm of the index of dissimilarity. Inhibition of an aldolase activity by an antiserum was determined by incubating 20 μ l of the aldolase (0.1 to 0.2 U of activity) with 100 μ l of the antiserum. This amount corresponds to a 40-fold excess of antiserum, which affords maximum inhibition of the activity of the homologous enzyme.

Determination and comparison of amino acid compositions. Each protein was dialyzed against 0.1 M pyridine acetate buffer (pH 7.0). A sample (5 to 10 μ g) was dried and then dissolved in a mixture of HCl and trifluoroacetic acid (2:1) containing 0.005% freshly distilled phenol. After hydrolysis at 166°C for 25 and 50 min, the amino acids were determined by using a Durrum model D-500 amino acid analyzer. The value for each amino acid was calculated as described by Tsugita and Scheffler (36). Tryptophan was determined by the method of Pajot (26), and cysteine was determined after performic acid oxidation of the protein (33).

The sequence homology of proteins was calculated by using the amino acid compositions to obtain a divergence matrix by the method of Cornish-Bowden (4), where the divergence index ($S\Delta_n$) is defined as follows: $S\Delta_n = 1/2\sum(n_{iA} - n_{iB})^2$, where n_{iA} is the number of amino acid i in protein A and n_{iB} is the number of the corresponding amino acid in protein B. $S\Delta_n$ divided by one-half the total number of amino acids in proteins A and B gives the composition divergence index ($S\Delta_n/N$), which is used in this study. The coefficient of variation is around 38%. $S\Delta_n/N$ values lower than 0.42 indicate similarity and are significant at a 95% level of confidence. $S\Delta_n/N$ values between 0.42 and 0.93 indicate possible similarity, whereas values higher than 0.93 indicate no similarity.

RESULTS

Purification. The FDP aldolases of the type strains of five staphylococcal species were purified.

Recovery was about twofold higher than that obtained with the method described previously for the isolation of the FDP aldolase from *Staphylococcus aureus* (8). The details of purification of the enzyme from *Staphylococcus epidermidis* are shown in Table 1. The homogeneity of the protein, as determined by SDS gel electrophoresis, is shown in Fig. 1. Elution from the Sephadex G-100 chromatographic column and relative mobilities during SDS gel electrophoresis were used to determine the molecular weights of the purified proteins. The class I aldolases from the staphylococci studied were shown to be monomers with molecular weights between 33,000 and 35,000 (Table 2).

Temperature stability. The five purified class I aldolases renatured to active conformations after incubation at 90°C and slow cooling (Table 2). The addition of 0.004 M β -mercaptoethanol did not affect renaturation.

Immunological cross-reactions. Antisera were prepared against the purified enzymes from the type strains of *S. aureus*, *S. epidermidis*, and *Staphylococcus sciuri* subsp. *sciuri*. Using antiserum against the aldolase from *S. aureus* we analyzed the inhibition of enzyme activity with aldolases from several staphylococci (Table 3). After crude extracts of *S. aureus* were incubated with an excess of anti-*S. aureus* aldolase serum, an 80% decrease in activity was observed. If the crude extracts from other strains were treated in the same way, inhibitory activity was considerably reduced. Even though the amount of inhibition varied in different experiments, the relative values were similar in any one assay. A crude extract from *Micrococcus luteus* having a class II aldolase (9) was used as a negative control for this inhibition assay.

TABLE 3. Inhibition of aldolase activity by serum against *S. aureus* aldolase^a

Strain	Inhibition (%)
<i>S. aureus</i> ATCC 12600 ^T	70 $\pm$ 10
<i>S. epidermidis</i> ATCC 14990 ^T	60 $\pm$ 10
<i>S. warneri</i> ATCC 27836 ^T	60 $\pm$ 10
<i>S. haemolyticus</i> DSM 20263 ^T	60 $\pm$ 10
<i>S. hominis</i> ATCC 27844 ^T	60 $\pm$ 10
<i>S. capitis</i> ATCC 27840 ^T	60 $\pm$ 10
<i>S. xylosus</i> DSM 20266 ^T	30 $\pm$ 10
<i>Staphylococcus saprophyticus</i> CCM 883 ^T	30 $\pm$ 10
<i>S. sciuri</i> subsp. <i>sciuri</i> ATCC 29062 ^T	15 $\pm$ 5
<i>S. intermedius</i> CCM 5739 ^T	5 $\pm$ 5
<i>M. luteus</i> CCM 169 ^T ^b	0

^a Crude extracts were incubated with the antiserum against *S. aureus* aldolase, and the remaining activity was determined. The values differed in different assays, but the relative inhibition values were the same.

^b *M. luteus* contains a class II aldolase.

TABLE 4. Results of microcomplement fixation with antisera against staphylococcal aldolases

Strain	ID with antiserum against aldolase from: ^a		
	<i>S. aureus</i> ATCC 12600 ^T	<i>S. epidermidis</i> ATCC 14990 ^T	<i>S. sciuri</i> subsp. <i>sciuri</i> ATCC 29062 ^T
<i>S. aureus</i> ATCC 12600 ^T	0	57	131
<i>S. epidermidis</i> ATCC 14990 ^T	31	0	111
<i>S. warneri</i> ATCC 27836 ^T	30	28	ND ^b
<i>S. capitis</i> ATCC 27840 ^T	32	21	ND
<i>S. hominis</i> ATCC 27844 ^T	34	25	ND
<i>S. haemolyticus</i> DSM 20263 ^T	36	31	ND
<i>S. hyicus</i> subsp. <i>chromogenes</i> NCTC 1462 ^T	95	ND	ND
<i>S. hyicus</i> subsp. <i>hyicus</i> NCTC 10350 ^T	97	120	ND
<i>S. intermedius</i> CCM 5739 ^T	110	110	ND
<i>Staphylococcus cohnii</i> DSM 20260 ^T	120	119	ND
<i>Staphylococcus saprophyticus</i> CCM 883 ^T	125	110	ND
<i>S. xyloso</i> DSM 20266 ^T	130	113	108
<i>S. sciuri</i> subsp. <i>sciuri</i> ATCC 29062 ^T	135	108	0
<i>Staphylococcus simulans</i> ATCC 27848 ^T	139	111	135
<i>S. sciuri</i> subsp. <i>lentus</i> ATCC 29070 ^T	ND	ND	67

^a The deviation was $\pm 5\%$. For an explanation of ID, see the text.

^b ND, Not determined.

A more exact quantitation of immunological cross-reactions was obtained by using the micro-complement fixation technique (2). Antisera against the aldolases from *S. aureus*, *S. epidermidis*, and *S. sciuri* were tested with crude extracts from several strains (Table 4). We ob-

served no difference in the cross-reactions when either a purified aldolase or a crude extract was used. With antiserum against *S. aureus* aldolase, high degrees of cross-reaction were observed with crude extracts from the following five strains: *Staphylococcus warneri*, *S. epidermidis*,

TABLE 5. Amino acid compositions of class I aldolases

Amino acid	Molar ratio in aldolase of: ^a				
	<i>S. aureus</i> ATCC 12600 ^T	<i>S. epidermidis</i> ATCC 14990 ^T	<i>S. xyloso</i> DSM 20266 ^T	<i>S. haemolyticus</i> DSM 20263 ^T	<i>S. sciuri</i> subsp. <i>sciuri</i> ATCC 29062 ^T
Asx	44	48	49	40	40
Thr	10	12	13	14	12
Ser	20	21	21	24	20
Glx	45	41	44	43	55
Pro	10	10	10	11	9
Gly	23	21	24	23	25
Ala	28	25	21	28	25
Val	22	19	22	26	19
Met	7	8	7	7	6
Ile	14	15	20	14	20
Leu	31	29	28	30	26
Tyr	9	9	8	9	10
Phe	9	9	11	11	11
His	5	6	6	4	3
Lys	28	28	26	27	25
Arg	11	10	10	11	10
Cys ^b	2	2	2	2	2
Trp ^c	0	0	ND ^d	ND	ND

^a The results are expressed as the molar ratio of each amino acid residue after acid hydrolysis. The total numbers of amino acid residues in the aldolases were as follows: *S. aureus* ATCC 12600^T, 318; *S. epidermidis* ATCC 14990^T, 313; *S. xyloso* DSM 20266^T, 322; *S. haemolyticus* DSM 20263^T, 311; and *S. sciuri* subsp. *sciuri* ATCC 29062^T, 315. The molecular weights of the aldolases as determined by SDS gel electrophoresis were as follows: *S. aureus* ATCC 12600^T, 35,000; *S. epidermidis* ATCC 14990^T, 34,500; *S. xyloso* DSM 20266^T, 35,500; *S. haemolyticus* DSM 20263^T, 34,500; and *S. sciuri* subsp. *sciuri* ATCC 29062^T, 35,000.

^b Cysteine was determined after performic acid oxidation (33).

^c Tryptophan was determined by the method of Pajot (26).

^d ND, Not determined.

TABLE 6. Divergence matrix of the Δ_n/N values for the class I aldolases

Strain	Δ_n/N values ^a					
	<i>S. aureus</i>	<i>S. epidermidis</i>	<i>S. xylosus</i>	<i>S. sciuri</i> subsp. <i>sciuri</i>	<i>S. haemolyticus</i>	<i>P. aerogenes</i>
<i>S. aureus</i> ATCC 12600 ^T	0	0.106	0.222	0.273	0.121	0.548
<i>S. epidermidis</i> ATCC 14990 ^T	0.106	0	0.128	0.420	0.252	0.694
<i>S. xylosus</i> DSM 20266 ^T	0.222	0.128	0	0.303	0.325	0.856
<i>S. sciuri</i> subsp. <i>sciuri</i> ATCC 29062 ^T	0.273	0.420	0.303	0	0.363	0.541
<i>S. haemolyticus</i> DSM 20263 ^T	0.121	0.252	0.325	0.363	0	0.697
<i>P. aerogenes</i> ATCC 14963 ^b	0.548	0.694	0.856	0.541	0.697	0

^a The values were calculated from amino acid compositions by using the method of Cornish-Bowden (4). Values of <0.42 indicate that the aldolases are related, values between 0.42 and 0.93 are inconclusive, and values of >0.93 indicate that the aldolases are not related.

^b Data from reference 19.

Staphylococcus capitis, *Staphylococcus hominis*, and *Staphylococcus haemolyticus*. In these experiments the IDs varied from 30 to 36. *S. hyicus* subsp. *hyicus* and *S. hyicus* subsp. *chromogenes* had higher IDs (95 to 97). The IDs for the other crude extracts of staphylococci examined were more than 100. When an *S. epidermidis* aldolase antiserum was used, we obtained IDs similar to those obtained with the *S. aureus* aldolase antiserum. Five strains (strains of *S. capitis*, *S. hominis*, *S. warneri*, *S. haemolyticus* and, to a lesser extent, *S. aureus*) showed low IDs. Again, values obtained with other staphylococci were quite high (>100) and did not allow a clear differentiation of these strains. Antiserum against the *S. sciuri* subsp. *sciuri* aldolase gave significant cross-reactions with the crude extract of *S. sciuri* subsp. *lentus* (ID, 67). The IDs for all other *staphylococcus* strains were more than 100.

Determination of amino acid compositions and calculation of sequence homology. After hydrolysis of the purified proteins by a new hydrolysis method (36), the amino acid compositions were determined (Table 5). The method which we used generally gives a higher recovery of amino acids than the conventional method (25). All proteins showed similar compositions. The only major difference was the high content of glutamic acid residues in the *S. sciuri* enzyme. The samples were hydrolyzed and analyzed in parallel in order to obtain similar standard deviations for all compositions. The sequence homology of the class I aldolases was calculated from the amino acid compositions of the enzymes by using two independent methods (4, 11). The two methods gave comparable results. The values determined by the method of Cornish-Bowden (4) are shown in Table 6. The class I aldolase

from *Peptococcus aerogenes* (19, 20) was also included as a control. The Δ_n/N values for the staphylococcal aldolases are within the optimal range of this method (Δ_n/N , ≤ 0.42), which allows a clear order of similarity in sequence homology. Δ_n/N values of 0.42 to 0.93 indicate possible similarity of proteins, but do not allow conclusions as to any specific order of relatedness (4). A peptococcal aldolase having an Δ_n/N value between 0.541 and 0.856 is obviously related to these proteins even if the Δ_n/N values are out of the range of high significance (4). When the class I aldolase from *S. aureus* was compared with aldolases from several other organisms (Table 7), the only protein that was

TABLE 7. Δ_n/N values for aldolases from several organisms compared with the class I aldolase from *S. aureus*

Source of aldolase	Δ_n/N compared with <i>S. aureus</i> ATCC 12600 ^T aldolase ^a
<i>S. aureus</i> ATCC 12600 ^T	0
<i>P. aerogenes</i> ATCC 14963 ^b	0.548
Rabbit muscle ^c	1.194
<i>E. coli</i> Crooke strain (class I) ^d	1.976
<i>E. coli</i> Crooke strain (class II) ^e	0.810
Yeast (class II) ^f	0.825

^a The values were calculated from amino acid compositions according to the method of Cornish-Bowden (4). Values of <0.42 indicate that the aldolases are related, values between 0.42 and 0.93 are inconclusive, and values of >0.93 indicate that the aldolases are not related.

^b Data from reference 19.

^c Data from reference 12.

^d Data from reference 35.

^e Data from reference 1.

^f Data from reference 10.

FIG. 2. Dendrogram of several staphylococci derived from the IDs of their class I aldolases. The values were determined by the microcomplement fixation method.

highly related was the one from *P. aerogenes* ($S\Delta_n/N$, 0.548). The class I aldolase from *E. coli* (35) was not related ($S\Delta_n/N$, 1.976), whereas the class II enzyme from the same organism (1) was moderately related ($S\Delta_n/N$, 0.81) as was the class II aldolase from yeast ($S\Delta_n/N$, 0.825) (10). The rabbit muscle aldolase (12) did not show any similarity ($S\Delta_n/N$, 1.194).

DISCUSSION

Previously (8, 9), it was shown that the class I FDP aldolases from staphylococci appear to be very similar in biochemical properties. After specific labeling of the aldolases with [^{14}C]dihydroxyacetone phosphate in crude extracts, the enzymes appeared to be monomeric proteins with molecular weights ranging from 31,000 to 36,000, as determined by SDS gel electrophoresis (6). These results could be confirmed by using purified proteins.

As shown previously for the *S. aureus* aldolase (8), all purified enzymes used in this study tolerated incubation at a high temperature. The capability to renature to an active form after exposure to a high temperature is a common property of staphylococcal class I FDP aldolases.

Inhibition experiments with several staphylococcal aldolases and antiserum against the *S. aureus* enzyme showed that these aldolases differ in cross-reactivity. More quantitative values for the cross-reactions were obtained by using the microcomplement fixation method. The results were used to derive a dendrogram (Fig. 2). *S. capitis*, *S. epidermidis*, *S. hominis*, *S. war-*

neri, and *S. haemolyticus* form a group of highly related species. *S. aureus* was shown to be closely related to this group, whereas all other *staphylococcus* strains examined showed distant immunological relationships. The ID of 67 between the FDP aldolases of *S. sciuri* subsp. *sciuri* and *S. sciuri* subsp. *lentus* implies a relationship at the species level, as proposed by Kloos (16). If these results are compared with the data from DNA-DNA hybridization studies (7, 17, 24, 29, 32) and with the immunological relationships among staphylococcal catalases (30, 32), there is a clear concordance.

A comparison of the amino acid compositions of the class I aldolases from *S. aureus*, *S. epidermidis*, *Staphylococcus xylosus*, *S. haemolyticus*, and *S. sciuri* was used to derive a second dendrogram (Fig. 3). In contrast to the microcomplement fixation method, all of the values obtained are within the range of high confidence of this method ($S\Delta_n/N$, <0.42). Figure 3 differs from Fig. 2 in the positions of *S. xylosus* and *S. haemolyticus* relative to *S. aureus* and *S. epidermidis*, but the results are in good agreement with the data from an analysis of 16S ribosomal RNAs (23) (Fig. 4).

In the case of the class I aldolases, one gene product and the same enzyme preparation or lysate were compared by using two essentially distinct methods. The microcomplement fixation test gives an indirect measurement of antibody binding to the surface of the enzyme, which is known to be a rather rapidly evolving part of a protein (3, 5, 28). Although calculation of sequence homology from amino acid composition is empirically derived, it is a direct measurement of the amount of similarity over the whole protein. Analysis of the staphylococcal 16S ribosomal RNAs (23) also suggests a close

FIG. 3. Dendrogram of several staphylococci and *P. aerogenes* derived from the divergence matrix of amino acid compositions. $S\Delta_n/N$ values were determined by the method of Cornish-Bowden (4).

FIG. 4. Dendrogram of several staphylococci determined from a comparison of 16S ribosomal RNAs. The data are taken from a study by Ludwig et al. (23). S_{AB} , Similarity coefficient.

relationship between *S. aureus* and *S. epidermidis* and even more similarity between *S. haemolyticus* and *S. xylosus* (Fig. 4). These results, however, differ from the results of DNA-DNA homology studies, which show that *S. haemolyticus* is closely related to *S. epidermidis*. Ludwig et al. (23) explained this difference by the occurrence of a highly conserved DNA sequence of the 16S ribosomal RNA cistrons that might not reflect the overall rate of change in the base sequences.

A comparison of amino acid compositions provides a good criterion for determining the phylogenetic relationships among different organisms (4, 11). By choosing proteins of a highly conserved structure, it should be possible to compare even rather distantly related organisms. Tests for immunological cross-reactions among aldolases seemed to produce limited resolution of some relationships in the present

FIG. 5. Projection of the three-dimensional relationships among *S. aureus* (A), *S. epidermidis* (E), *S. xylosus* (X), *S. haemolyticus* (H), *S. sciuri* subsp. *sciuri* (S), and *P. aerogenes* (P). The distances are proportional to the binary values for S_{AB}/N (see Table 7).

study, although cross-reactions occurring with several proteins reflected a similarity in protein sequences (3, 28). The obvious advantage of the immunological method is that it also reflects similarities in the tertiary structures of proteins (5).

A dendrogram does not always show the distances of binary values (34). The binary rates in Table 6 show a greater distance between *S. haemolyticus* and *S. xylosus* (S_{AB}/N , 0.325) than between *S. aureus* and *S. sciuri* (S_{AB}/N , 0.273). This finding is not visible in the dendrogram (Fig. 3), but such relationships may only be expressed by a three-dimensional figure. A projection of the three-dimensional relationship, as determined from the S_{AB}/N values, is shown in Fig. 5; here the discrepancy mentioned above can be clarified. *S. sciuri* subsp. *sciuri* still has an isolated position, whereas all other staphylococci form a homogeneous cluster.

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