Prediction of Protein Relative Solvent Accessibility With a Two-Stage SVM Approach

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ABSTRACT **Information on relative solvent accessibility (RSA) of amino acid residues in proteins provides valuable clues to the prediction of protein structure and function. A two-stage approach with support vector machines (SVMs) is proposed, where an SVM predictor is introduced to the output of the single-stage SVM approach to take into account the contextual relationships among solvent accessibilities for the prediction. By using the position-specific scoring matrices (PSSMs) generated by PSI-BLAST, the two-stage SVM approach achieves accuracies up to 90.4% and 90.2% on the Manesh data set of 215 protein structures and the RS126 data set of 126 nonhomologous globular proteins, respectively, which are better than the highest published scores on both data sets to date. A Web server for protein RSA prediction using a two-stage SVM method has been developed and is available (http://birc.ntu.edu.sg/~pas0186457/rsa.html). Proteins 2005;59: 30–37.** © 2005 Wiley-Liss, Inc.

Key words: protein structure prediction; solvent accessibility; support vector machines; PSI-BLAST

INTRODUCTION

The knowledge of protein structures is valuable for understanding mechanisms of diseases of living organisms and for facilitating discovery of new drugs. Protein structure can be experimentally determined by NMR spectroscopy and X-ray crystallography techniques or by molecular dynamics simulations. However, the experimental approaches are marred by long experimental time, prone to difficulties, expensive, and therefore limited to small proteins.[1] Bioinformatics approaches have recently being sought to predict relative solvent accessibility (RSA) to help elucidate the relationship between protein sequence and structure, and thereby predict the three-dimensional (3D) structure of proteins.[2,3] The studies of solvent accessibility have shown that the hydrophobic free energies of proteins are directly related to the accessible surface area of both polar and nonpolar groups of amino acid in proteins.[4] Chan and Dill[5] discovered that the burial of core residues is a strong driving force in protein folding. Furthermore, the RSA prediction gives insight into the organization of 3D structure: The position of protein hydration sites playing an important part in a protein's function can be predicted based on solvent accessibility,[6] and information about solvent accessibility has improved the prediction of protein subcellular location, as the distribution of solvent accessibilities is correlated with its subcellular environments.[7] One of the objectives in RSA prediction is to classify a pattern of residues in amino acid sequences to a pattern of RSA types: buried (*B*) and exposed (*E*) residues.

Many different techniques have been proposed for RSA prediction, which broadly fall into the following categories: (1) Bayesian, (2) neural networks, and (3) information theoretical approaches. The Bayesian methods provide a framework to take into account local interactions among amino acid residues, by extracting the information from single sequences or multiple sequence alignments to obtain posterior probabilities for RSA prediction.[8] Neural networks use residues in a local neighborhood, as inputs, to predict the RSA of a residue at a particular location by finding an arbitrary, nonlinear mapping.[9–12] The information theoretical approaches use mutual information between the sequences of amino acids and solvent accessibility values derived from a single amino acid residue, or pairs of residues, in a neighborhood for RSA prediction.[13] Recently, variants of these approaches with increased prediction accuracies have been proposed: Gianese et al.[14] predicted the RSA of a residue based on probability profiles computed on amino acid residues in the neighborhood; Adamczak et al.[15] proposed using neural networks–based regression to find continuous approximations to RSA values.

Despite the existence of many approaches, the current success rates of existing techniques for RSA prediction are insufficient; further improvement of the accuracy is necessary. Most existing techniques for RSA prediction are single-stage approaches in the sense that the solvent accessibility type is directly predicted from amino acid sequences or profiles derived from them, except the PHDacc method[3] using an averaging filter at outputs of the first neural network and the Jnet method[30] combining two multi-layer perceptron (MLP) networks. They suffer from the limited size of the local neighborhood used in the prediction; the sequential relationships among the solvent accessibilities of residues are not taken into account. In

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TABLE I. List of 30 Proteins Used for Training the Single-Stage and Two-Stage SVM Approaches

1aba	1abr	1bdo	1beo	1bib	1bmf	1bnc	1btm	1btn	1cem
1ceo	1cew	1cfy	1chd	1chk	1cyx	1dea	1del	1dkz	1dos
1fua	1gai	1gpl	1gsa	1gtm	1hav	2ilb	2sns	3grs	3mdd

this article, we propose a two-stage approach to RSA prediction by using a second predictor, a support vector machine (SVM) classifier, introduced at the end of a single-stage RSA prediction scheme. The aim of the second stage is to take into account the influence on the RSA of a residue by the RSAs of its neighbors.

SVMs were earlier shown to perform well in multiple areas of biological analysis,[16] including RSA prediction,[17,18] that have strong foundations in statistical learning theory; as shown by Vapnik,[19,20] SVMs implement a classifier that is capable of minimizing structural risk. Furthermore, SVMs offer several associated computational advantages, such as the lack of local minima and a solution completely encompassed by the set of support vectors. In addition, SVMs scale well for large-scale problems, which makes them particularly attractive for predicting structures of large protein sequences.[16] Also, the generalization capability of SVMs is well suited for the prediction of RSAs of novel amino acid sequences. All previous SVM approaches to RSA prediction have been single-stage approaches.

By using a two-stage SVM approach based on the position-specific scoring matrices (PSSMs) generated by PSI-BLAST, substantial improvements of prediction accuracies up to 7.6% and 4.0% were achieved on the Manesh[13] and the RS126[3] data sets, respectively, compared to previously reported accuracies.[14,18]

MATERIALS AND METHODS

Data Set 1 (RS126)

The set of 126 nonhomologous globular protein chains used in the experiment of Rost and Sander[3] and referred to as the RS126 set was used to evaluate the accuracy of the prediction. Many current-generation RSA prediction methods have been developed and tested on this data set, which is available at http://gibk21.bse.kyutech.ac.jp/rvp-net/all-data.tar.gz. The two-stage SVM approach was implemented with the position-specific scoring matrices (PSSMs) generated by PSI-BLAST, and tested on the data set using a 7-fold cross-validation to estimate the prediction accuracy. With 7-fold cross-validation, approximately one-seventh of the data set was left out while training and, after training, that one-seventh of the data set was used for testing. In order to avoid the selection of extremely biased partitions, the RS126 set was divided into subsets of same size and composition of each type of RSA.

Data Set 2 (Manesh)

The second data set, generated by Manesh,[13] consisted of 215 nonhomologous protein chains and is referred to as the Manesh data set. The dataset contains proteins with less than 25% homology and is available online (http://gibk21.bse.kyutech.ac.jp/rvp-net/all-data.tar.gz). The NETASA prediction method[12] was developed and tested on this data set. A set of 30 proteins containing 7545 residues was selected for training (see Table I). The remaining 185 proteins with 43,137 residues were used for testing. We adopted these training and testing sets in order to provide an objective comparison of the prediction accuracy of the two-stage SVM approach with the results of the NETASA method[12] and the probability profile approach of Gianese et al.[14] The two-stage SVM predicted the RSA types based on the PSSMs generated by PSI-BLAST. The PSI-BLAST profiles contained probabilities of residues, taking into account the significance of each sequence and distant homologues.[21]

RSA and Prediction Accuracy Assessment

RSA percentage (%) of an amino acid residue is defined as the ratio of the solvent-accessible surface area of the residue observed in the 3D structure to that observed in an extended tripeptide (Gly-X-Gly or Ala-X-Ala) conformation. The value of RSA lies between 0% and 100%, with 0% corresponding to a fully buried type and 100% to the fully exposed type. The type of the solvent accessibility of an amino acid residue is considered buried (B) if the RSA value of the residue is smaller than a specified threshold c%, or an exposed (E) otherwise. We demonstrate our approach with a range of thresholds of RSA: 0%, 5%, 9%, 10%, 16%, 20%, 25%, and 50%. The residue solvent-accessible surface areas of the RS126 set were computed with the Dictionary of Protein Secondary Structure (DSSP) program.[22] The Analytical Surface Calculation (ASC) program,[23] with the van der Waals radii of the atoms,[4] was used to compute the residue solvent-accessible surface areas for the Manesh data set. The Ala-X-Ala oligopeptide in an extended conformation instead of Gly-X-Gly is used to calculate RSA in the Manesh data set. The definitions of RSA and programs used to compute it are consistent with those used by other authors, whose methods are compared against the proposed approach.

The prediction accuracy is measured by the percentage of correctly predicted types of solvent accessibility of residues[3]; the sensitivity score indicates the proportion of exposed (E) residues that are correctly predicted as E; the specificity measures the proportion of buried (B) residues that are correctly predicted as B. By changing the thresholds of RSA definition of the prediction, we get a range of sensitivities and specificities, which leads to receiver operation characteristics (ROCs) that plot sensitivity versus 1− specificity. The ROC curves offer comparisons among different prediction methods irrespective of the threshold for determination of solvent accessibility type.

Single-Stage SVM Approach

In this section, we describe how a sequence of RSA types is predicted from an amino acid sequence by using an SVM classifier. Let us denote the amino acid sequence by $\mathbf{r} = (r_1, r_2,\ldots, r_n)$, where $r_i \in \Omega_R$ and Ω_R is the set of 20 amino acid residues, and the corresponding solvent accessibility sequence by $\mathbf{a} = (a_1, a_2,\ldots, a_n)$, where $a_i \in \Omega_A$; n is the length of the sequence. The prediction of the sequence of RSA types, $\mathbf{a}$, from an amino acid sequence, $\mathbf{r}$, is the problem of finding the optimal mapping from the space of Ω_R^n to the space of Ω_A^n.

First, the values of raw matrices of PSI-BLAST[24] used as inputs to first-stage SVM are obtained from the NR (nonredundant) database (ftp://ftp.ncbi.nih.gov/blast/db/FASTA/). The low-complexity regions, transmembrane regions, and coil–coil segments are then filtered from the NR database by the PFILT program.[21] Finally, the *E*-value threshold of 0.001, 3 iterations, BLOSUM62 matrix, a gap open penalty of 11, and a gap extended penalty of 1 are used for searching the NR sequence database to generate PSSM profiles. These arguments are consistent with those used in other methods.[18,21] Let v_i be a vector representing a 21-dimensional coding of the residue r_i, where 20 elements take the values from PSSM profiles ranging from [0, 1],[18] and the last element is used as the padding space to indicate the end of the sequence; the padding element is set to 1 to indicate the end of the sequence, or 0 otherwise. The SVM, a binary classifier *B*/*E*, is constructed to predict whether the solvent accessibility of a residue at a site belongs to a particular type, *B* or *E*. The input pattern to the predictor at site i consists of a vector $\mathbf{r}_i$ of profiles from a neighborhood: $\mathbf{r}_i = (v_{i-h_1}, v_{i-h_1+1},\ldots, v_{i+h_1})$, where h_1 represents the size of the neighborhood on either side.

The SVM transforms the input vectors to a higher dimension via a kernel function, K^1, and linearly combines to derive the outputs with a weight vector, $\mathbf{w}_1$. The function K^1 and vector $\mathbf{w}_1$ are determined to minimize the error in the prediction during the training phase. Let $\Gamma^1_{\text{train}} = \{(\mathbf{r}_j, q_j) : j = 1,2,\ldots,N\}$ denote the set of all training exemplars, where q_j denotes the desired classification, *B* or *E*, for the input pattern $\mathbf{r}_j$ such that the output of SVM is -1 if the correct RSA type is *B* or $+1$ if the type is *E*. When N is the number of training patterns, the vector $\mathbf{w}_1$ is determined by scalars $\alpha_j, j = 1,2,\ldots, N$ that are found by maximizing the following quadratic function Q_1:

$$Q_1 = \sum_{j=1}^{N} \alpha_j - \frac{1}{2} \sum_{j=1}^{N} \sum_{i=1}^{N} \alpha_j \alpha_i q_j q_i K^1(\mathbf{r}_j, \mathbf{r}_i), \qquad (1)$$

subject to $0 \le \alpha_j \le \gamma^1$ and $\sum_{j=1}^{N} \alpha_j q_j = 0$. $K^1(\mathbf{r}_j,\mathbf{r}_i) = \phi^1(\mathbf{r}_i)\phi^1(\mathbf{r}_j)$ denotes the kernel function, and ϕ^1 represents the mapping function to higher dimension; γ^1 is a positive constant used to decide the trade-off between the training error and the margin of the classifier.[19,20]

The weight vector is then given by $\mathbf{w}_1 = \sum_{j=1}^{N} q_j \alpha_j \phi^1(\mathbf{r}_j)$. Once the parameters α_j are obtained from the above algorithm, the resulting discriminant function, say f_1, is given by

$$f_1(\mathbf{r}_i) = \sum_{j=1}^{N} q_j \alpha_j K^1(\mathbf{r}_j, \mathbf{r}_i) + b_1 = \mathbf{w}_1 \phi^1(\mathbf{r}_i) + b_1, \qquad (2)$$

where the bias b_1 is chosen so that $q_j f_1(\mathbf{r}_j) = 1$ for any j with $0 < \alpha_j < \gamma^1$.

In the single-stage SVM method, the solvent accessibility type α_i corresponding to the residue at site i, r_i, is determined by

$$a_i = \begin{cases} E & \text{if } f_1(\mathbf{r}_i) \ge 0 \\ B & \text{otherwise.} \end{cases} \qquad (3)$$

The function, f_1, discriminates the type of RSA, based on the features or interactions among the residues in the input pattern. With optimal parameters, the SVM attempts to minimize the generalization error in the prediction. If the training and testing patterns are drawn independently and identically according to a probability P_1, then the generalization error, err_{P_1}, is given by

$$\text{err}_{P_1}(f_1) = P f_1\{(\mathbf{r}, q) : \text{sign}[f_1(\mathbf{r})] \ne q; (\mathbf{r}, q) \in \Gamma^1\},$$

where Γ^1 denotes the set of input patterns seen by the SVM during both the training and testing phases. In the following sections, we demonstrate that this error can be minimized by connecting another predictor at the output of the SVM predictor.

Two-Stage SVM Approach

The single-stage approach takes only the interactions among amino acid residues in the neighborhood into the prediction scheme. The RSA type of a residue is also influenced by those in its neighborhood. A second SVM predictor is used in the two-stage approach to predict the RSA type of a residue by using the predictions from the first stage, capturing the sequential relationships among the RSA values in the neighborhood. The architecture of the two-stage SVM prediction approach is illustrated in Figure 1.

The second SVM classifier improves the accuracy of the single-stage RSA prediction schemes by taking into account the sequential relationships among the RSA values of residues into the prediction. The second-stage SVM processes the estimated RSA values at the first stage and minimizes the generalization error by incorporating the contextual information among RSA values. Rost and Sander[3] proposed a simple method to incorporate the sequential relationships of the estimated RSA types, in which an averaging filter is employed to take the average of neighboring outputs of the first neural network at each amino acid residue; then, the solvent accessibility is predicted as the type with the largest average. Two-stage SVM approaches were previously proposed for protein secondary structure prediction.[25,26]

The second-stage SVM processes the output of the discriminant functions of the first stage to enhance the prediction. At the site i, the input to the second SVM is given by a vector $\mathbf{d}_i = (d_{i-h_2}, d_{i-h_2+1},\ldots, d_i,\ldots d_{i+h_2})$, where h_2 is the length of the neighborhood on either side and $d_i = 1/(1 + e^{-f_1(\mathbf{r}_i)})$. The SVM converts the input patterns, usually

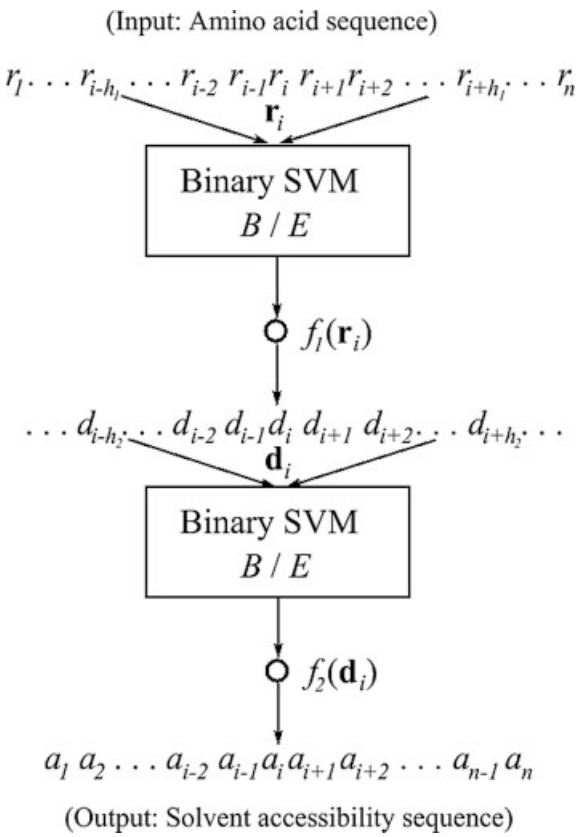

Fig. 1. Two-stage SVM approach for RSA prediction.

linearly inseparable, to a higher dimensional space by using the mapping ϕ^2 with a kernel function $K^2(\mathbf{d}_i,\mathbf{d}_j) = \phi^2(\mathbf{d}_i)\phi^2(\mathbf{d}_j)$.

As in the first stage, the hidden outputs in the higher dimensional space are linearly combined by a weight vector, $\mathbf{w}_2$, to obtain the prediction output. Let the training set of exemplars for the second-stage SVM be $\Gamma^2_{\text{train}} = \{(\mathbf{d}_j,q_j) : j = 1,2,\ldots,N\}$. The kernel function K^2 and vector $\mathbf{w}_2$ are obtained by solving the following convex quadratic programming problem, over all the patterns seen in the training phase:

$$\max_{\beta} \sum_{j=1}^{N} \beta_j - \frac{1}{2}\mathbf{w}_2^T\mathbf{w}_2, \tag{4}$$

such that $0 \le \beta_j \le \gamma^2$ and $\sum_{j=1}^{N} \beta_j q_j = 0$, where $\mathbf{w}_2 = \sum_{j=1}^{N} q_j\beta_j\phi^2(\mathbf{d}_j)$.

The discriminant function, f_2, at the second stage is given by

$$f_2(\mathbf{d}_i) = \sum_{j=1}^{N} q_j\beta_j K^2(\mathbf{d}_j, \mathbf{d}_i) + b_2 = \mathbf{w}_2\phi^2(\mathbf{d}_i) + b_2, \tag{5}$$

where the bias b_2 is chosen so that $q_j f_2(\mathbf{d}_j) = 1$ for any j with $0 < \beta_j < \gamma^2$. The solvent accessibility type a_i corresponding to the residue r_i is given by

TABLE II. Comparison of Performances of Two-Stage SVM Approach With Other Methods in RSA Prediction on the RS126 Data Set With PSSMs Generated by PSI-BLAST

Method/threshold	0%	5%	9%	16%
Rost and Sander[3] (PHDacc)	86.0	—[a]	74.6	75.0
Gianese et al.[14] (PP)	—	—	76.8	75.1
Kim and Park[18] (single-stage SVM)	86.2	79.8	—	77.8
Two-stage SVMs	90.2	83.5	81.3	79.4

[a]Dashes indicate that the corresponding result was not available from the literature.

$$a_i = \begin{cases} E & \text{if } f_2(\mathbf{d}_i) \ge 0 \\ B & \text{otherwise.} \end{cases} \tag{6}$$

If the set of input patterns for the second-stage SVM in both training and testing phases is denoted by Γ^2, the generalization error of the two-stage SVM approach, $\text{err}_{P_2}(f_2)$, is given by

$$\text{err}_{P_2}(f_2) = P_2\{(\mathbf{d}, q) : \text{sign}[f_2(\mathbf{d})] \ne q;\ (\mathbf{d}, q) \in \Gamma^2\}.$$

If the input pattern $\mathbf{d}$ corresponds to a site i, then $\mathbf{d} = \mathbf{d}_i = [(1 + e^{-f_1(\mathbf{r}_{i-h_2})})^{-1},\ldots,(1 + e^{-f_1(\mathbf{r}_i)})^{-1},\ldots,(1 + e^{-f_1(\mathbf{r}_{i+h_2})})^{-1}]$; that is, the second stage takes into account the influences of the RSA values of residues in the neighborhood into the prediction. It could be easily conjectured that if the RSA type of a residue depends on those of its neighborhood, $\text{err}_{P_2}(f_2) \le \text{err}_{P_1}(f_1)$, where the equality occurs when $h_2 = 0$.

RESULTS

For SVM classifiers, a window size of 13 amino acid residues $h_1 = 6$ gave optimal results in the [9, 21] range for the first stage, and a window size of width 21; $h_2 = 10$ in the [11, 27] range gave the optimal accuracy for the second stage. The kernels selected were Gaussian functions, $K(\mathbf{x},y) = e^{-\sigma\,|\,x-y\,|^2}$ with the parameters $\sigma = 0.1$, $\gamma^1 = 1.0$ at the first stage, and $\sigma = 0.15$, $\gamma^2 = 1.0$ at the second stage, which were determined empirically for optimal performances in [0.01, 0.5] and [0.1, 2] ranges, respectively. In the literature, the Gaussian kernel has been used in many classification problems.[16] The main reason is that it can result in complex (but smooth) decision function, and therefore has the ability to better fit the data where simple discrimination by using a hyperplane or a low-dimensional polynomial surface is not possible. The use of Gaussian kernel showed the best performance when the dimension of feature space is infinite,[27] and gave better results over the linear and polynomial kernels for RSA prediction.[18] The Gaussian kernels have shown faster convergence than linear kernels for large and complex training sets of RSA problem. The SVM method was implemented using the sequential minimization algorithm,[28] which is simple to implement without needing storage for matrices or to invoke an iterative numerical routine for each subproblem.

Table II shows the performances of different solvent accessibility predictors and two-stage SVM approach on the RS126 set. Two-stage SVMs with PSI-BLAST profiles

TABLE III. Comparison of Performances of Two-Stage SVM Approach in RSA Prediction Based on PSSMs Generated by PSI-BLAST, With Other Methods on the Manesh Data Set

Method/threshold	0%	5%	10%	20%	25%	50%
Ahmad and Gromiha[12] (NETASA)	87.9	74.6	71.2	—	70.3	75.9
Gianese et al.[14] (PP)	89.5	75.7	73.4	—	71.6	76.2
Two-stage SVMs	90.4	82.9	81.0	78.6	78.1	79.1
Giorgi et al.[29] (PredAcc)	85.0	—	—	—	70.7	—
Cuff and Barton[30] (Jnet)	86.6	79.0	—	—	75.0	—
Li and Pan[10]	—	—	—	71.5	—	—
Pollastri et al.[11] (BRNN)	86.5	81.2	—	—	77.2	—
Adamczak et al.[15] (SABLE)	—	76.8	77.5	77.9	77.6	—

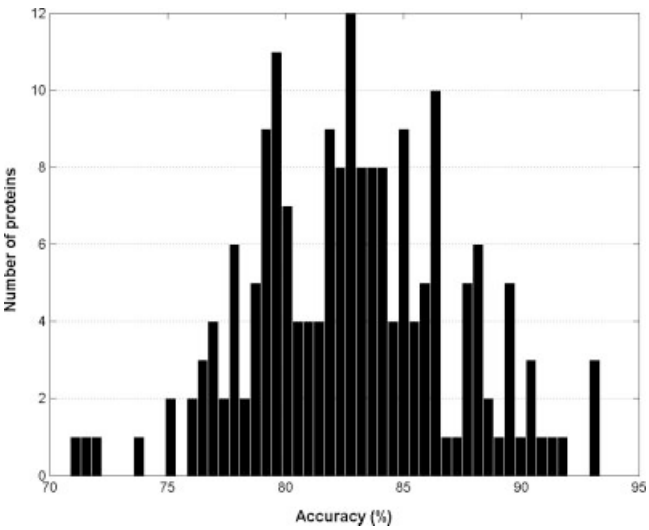

Fig. 2. The distribution of prediction scores obtained by two-stage SVMs for the banchmark 185 proteins of the Manesh data set at a 5% threshold based on PSI-BLAST profiles.

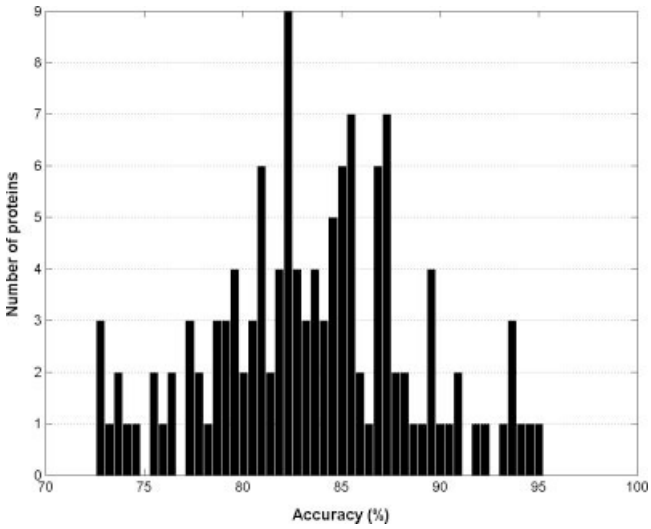

Fig. 3. The distribution of prediction scores obtained by two-stage SVMs for the banchmark RS126 data set at a 5% threshold based on PSI-BLAST profiles.

achieved accuracies of 90.2%, 83.5%, 81.3%, and 79.4% at thresholds of 0%, 5%, 9%, and 16%, respectively, which are the highest scores on the RS126 set to date. Compared to the newest method of Kim and Park,[18] using single-stage SVM, the two-stage SVM method significantly obtained 4.0%, 3.7%, and 1.6% higher prediction accuracies at 0%, 5%, and 16% thresholds, respectively. On the RS126 data set, the accuracies were improved by 4.5% and 4.3% at thresholds of 9% and 16%, respectively, compared to the results of the probability profiles approach of Gianese et al.[14] The prediction accuracy of two-stage SVMs outperformed the results by the multilayer perceptron networks of PHDacc method proposed by Rost and Sander[3] at all thresholds.

Table III shows the performance of the two-stage SVM approach on the Manesh data set based on PSI-BLAST profiles and comparison with other solvent accessibility predictors. The best performance was shown by the cascade of two SVMs. On the Manesh data set, the accuracies were significantly improved by 2.5%, 8.3%, 9.8%, 7.8%, and 3.2% for 0%, 5%, 10%, 25%, and 50% thresholds, respectively, compared to the results of NETASA method.[12] Comparing two-stage SVMs to the probability profiles method,[14] substantial gains of 0.9% to 7.6% of prediction accuracy were observed for different thresholds.

Figures 2 and 3 present the distributions of prediction scores obtained by two-stage SVMs for the benchmark Manesh and RS126 data sets with a 5% threshold based on PSI-BLAST profiles. The ROC curves on the Manesh and RS126 data sets for single-stage and two-stage SVM approaches at different thresholds are illustrated in Figures 4 and 5. As shown, the prediction accuracy of two-stage SVMs outperformed the single-stage SVM methods for RSA prediction at all thresholds.

For RSA prediction, the accuracy of two-stage SVMs using PSI-BLAST profiles is significantly higher than results obtained by using multiple sequence alignments. For example, the accuracy of two-stage SVM method on the RS126 data set was only 78.6% at a threshold of 5% based on multiple sequence alignments. As mentioned,[21] PSI-BLAST profiles contain more information on homologous protein structures than do multiple sequence alignments. Additionally, improvements of accuracies are observed when larger sequences or more homologous profiles are used in training. As shown in Table IV, by using a set of 205 proteins instead of 30 proteins for training, the prediction accuracies of 10 sequences, obtained from the

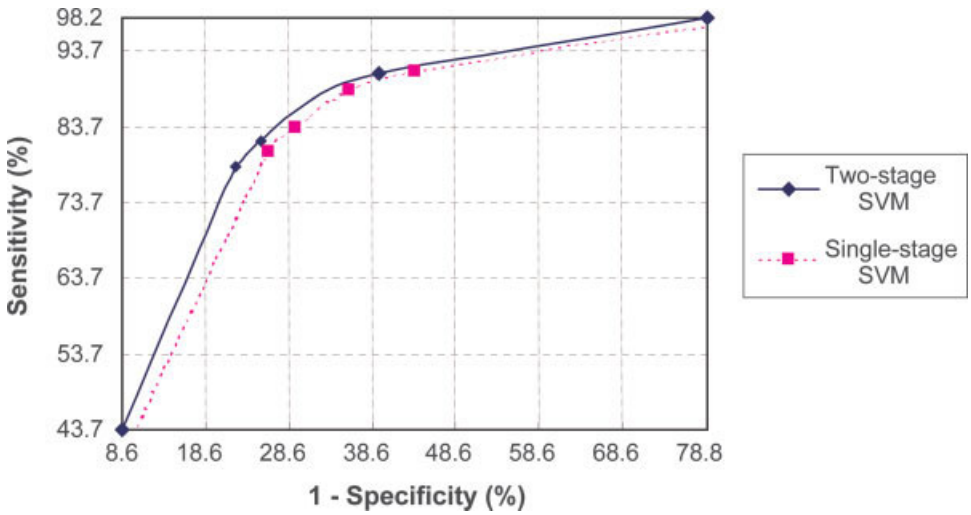

Fig. 4. The ROC curves on the Manesh data set for single-stage and two-stage SVM approaches for RSA prediction.

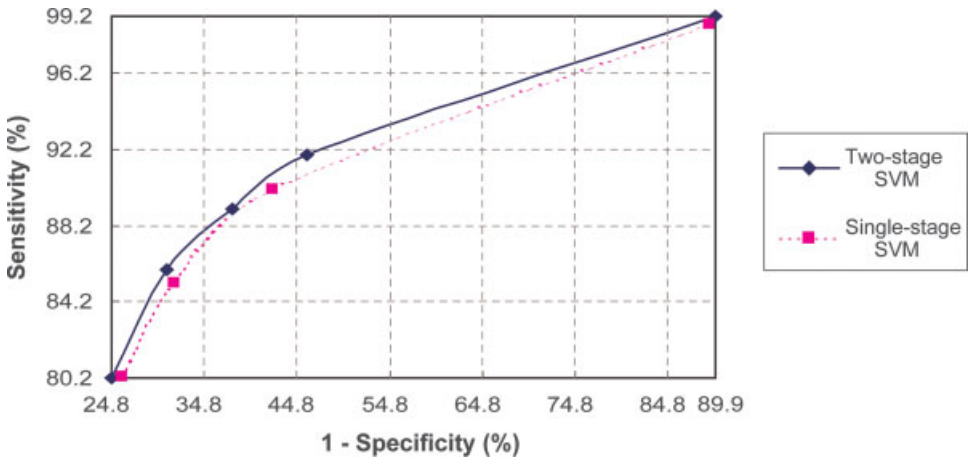

Fig. 5. The ROC curves on the RS126 data set for single-stage and two-stage SVM approaches for RSA prediction.

TABLE IV. Comparison of Performances of Two-Stage SVM Approach on 10 Proteins Based on PSI-BLAST Profiles With Two Different Training Sets of 30 and 205 Proteins at a 5% Threshold

Training set	1lts	1nba	1afw	3cox	2wsy	7rsa	1amm	1mai	1knb	1kte
30 proteins	70.9	71.4	71.8	73.6	75.2	91.1	92.0	93.3	93.3	93.3
205 proteins	72.6	71.8	71.8	73.9	76.9	91.0	93.1	94.1	93.3	93.3

tails of the histogram in Figure 2 (1lts, 1nba, 1afw, 3cox, 2wsy, 7rsa, 1amm, 1mai, 1knb, 1kte) were improved at a threshold of 5%. These observations suggest that the performance of two-stage SVM method based on PSI-BLAST profiles for a novel amino acid sequence suffers if it lacks in the homologous structures in the training set. For a completely new protein whose homologous proteins are not used in training, the two-stage SVM method predicts its solvent accessibilities with a low accuracy. To our knowledge, Rost and Sander[3] and Adamczak et al.[15] concluded that the overall performance of any method based on evolutionary profiles suffers when very remote or no homologues are included.

Table V lists the properties of 20 amino acids and their average occurrence and probabilities for exposure and error in RSA prediction on the Manesh data set at a 25% threshold. Nelson and Cox,[31] based on the polarity or tendency to interact with water of R group at biological pH, grouped 20 amino acids into 5 main classes. According to the statistical data, amino acids Ala, Val, Leu, Ile, Phe, and Cys were easy to predict, while Gly, Pro, Trp, Thr, Arg, and His were difficult to predict by two-stage SVMs. As shown, the two-stage SVM method frequently predicted A, V, L, I, M, F, W, Y, and C to be buried, and G, P, S, T, N, Q, K, R, H, D, and E to be exposed. The statistical data confirm that the nonpolar R groups (hydrophobic) tend to

TABLE V. Properties of 20 Amino Acids: Average Occurrences, Probabilities of Exposures, and the Error in RSA Prediction on the Manesh Data Set at a 25% Threshold

Amino acid		Occurrence (%)	Exposure (%)	Error in RSA prediction (%)
Nonpolar R group (hydrophobic)				
Gly	G	7.5	55.8	27.1
Ala	A	7.7	39.7	19.0
Val	V	6.8	16.7	16.2
Leu	L	8.8	14.1	15.7
Ile	I	5.7	12.1	14.8
Met	M	2.2	20.8	21.7
Pro	P	4.5	64.8	27.5
Aromatic R group (hydrophobic)				
Phe	F	4.1	10.5	16.5
Trp	W	1.4	12.3	25.1
Tyr	Y	3.8	18.8	24.8
Polar, uncharged R group (hydrophilic)				
Ser	S	5.9	63.7	24.7
Thr	T	5.6	53.2	25.6
Cys	C	1.6	12.5	15.1
Asn	N	4.6	74.4	25.0
Gln	Q	3.9	79.4	24.6
Positively R charged (hydrophilic)				
Lys	K	6.1	84.5	19.8
Arg	R	4.8	72.5	28.3
His	H	2.2	51.2	30.5
Negatively R charged (hydrophilic)				
Asp	D	6.3	80.9	23.2
Glu	E	6.4	84.7	21.4

be buried (i.e., in the interior of a protein) and the polar R groups (hydrophilic) tend to be on the surface (exposed), except for G, P, and C.[32] This is because two Cys are readily oxidized to form a disulfide bond, and disulfide-linked residues are hydrophobic. Chen et al.[32] also explained the reasons that Pro and Gly tend to be exposed from their structures. The results from Table V suggest that the amino acid residues that tend to be buried (A, V, L, I, M, F, W, Y, C) are predicted with higher accuracies than exposed ones (G, P, S, T, N, Q, K, R, H, D, E).

As shown in Tables II and III, predictions were best for buried residues (e.g., 90.2% and 90.4% of the completely buried sites were correctly predicted at a threshold of 0% on RS126 and Manesh data sets, respectively). The two-stage SVM method achieved the highest prediction accuracy for the extreme case of fully buried types because the accessibility of completely buried residues is best conserved in 3D homologous structures.[3] Residues in α-helix and β-strand structure segments were predicted better than ones in coil segments (e.g., 80.7%, 82.2%, and 77.5% residues were correctly predicted in α-helix, β-strand, and coil segments, respectively) on the Manesh data set at a 25% threshold.

We also estimated the effect of the growing size of NR databases used to generate position scoring matrices by PSI-BLAST on the accuracy of two-stage SVM method. Two NR databases were used: one as of December 22, 2003, with 1,581,064 sequences, and a newer version as of April 7, 2004, with 2,745,128 sequences. The different results of two-stage SVMs on two NR databases were not significant (see Table VI).

TABLE VI. Comparison of Performances of Two-Stage SVM Approach on the Manesh Data Set Based on PSSMs Generated by PSI-BLAST With Two Different NR Databases

Database/threshold	0%	5%	10%	20%	25%	50%
1,581,064 NR	90.2	82.8	80.9	78.6	78.1	79.0
2,745,128 NR	90.4	82.9	81.0	78.6	78.1	79.1

A Web server for protein relative solvent accessibility prediction using two-stage SVM method has been developed and is available (http://birc.ntu.edu.sg/~pas0186457/rsa.html). A set of 30 proteins containing 7545 residues (see Table I) was selected for training two-stage SVM method presented on the Web server.

DISCUSSION AND CONCLUSION

The existing bioinformatics techniques for RSA prediction are mostly single-stage approaches that predict the RSA types of residues based on only the information available in amino acid sequences. We demonstrated a two-stage approach, by using SVMs, that utilizes the output predicted by single-stage prediction schemes and improves the accuracy of RSA prediction. In this way, the influences on the RSA value of a residue by those of its neighbors are accounted for. This is because the solvent accessibility at a particular position of the sequence depends on the structures of the rest of the sequence (i.e., it accounts for the fact that the buried or exposed type consists of at least two consecutive residues). Therefore, another layer of SVM classifier incorporating the contextual relationship among the solvent accessibility characteristics makes the prediction more realistic in terms of predicted mean lengths of solvent accessibility elements. The analysis of prediction results from single-stage and two-stage SVM methods showed that the second-stage SVM ultimately cleans the output prediction of the first stage SVM, mostly by removing isolated buried or exposed residues.

SVMs are more suitable for prediction of RSA values because they minimize the generalization error in the prediction. We showed that the generalization error made in the first stage is further minimized by the second stage of the two-stage approach. The SVM is an optimal classifier for the second stage in terms of the margin of separation; it attempts to minimize not only the empirical risk of known sequences but also the actual risk for unknown sequences. Two stages of SVMs are sufficient to find an optimal classifier for RSA prediction as the second stage SVM attempts to minimize the generalization error of the first stage by solving the optimization problem at the second stage.

Recently, Kim and Park[18] suggested using the information of the PSSMs generated by PSI-BLAST as inputs to SVMs for RSA prediction. By combining PSI-BLAST profiles, the present approach achieved better results than the

methods using information from single sequences and multiple sequence alignments. Compared to the method of Kim and Park, our method showed a considerable improvement in the accuracy of prediction. By incorporating the state-of-the-art methods based on PSI-BLAST profiles and SVMs in a two-stage approach, we are able to report the best accuracies to date for RSA prediction on the tested data sets. The RSA elements of residues predicted by our approach could facilitate the prediction of the structure and function of amino acid sequences.

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